CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF ESSENTIAL OILS FROM SELECTED NIGERIAN MEDICINAL PLANTS

BY

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DEDICATION

This research work is dedicated to the Almighty God for the life He has given me because it is only the living that can carryout a research. He stood by me from the beginning to the end.

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ABSTRACT

Oxidative stress and microbial infections are major threats to humans. Toxicity, side effects and resistance to the existing drugs necessitate the need for alternatives. Essential Oils (EOs) of plants, which are complex mixtures of terpenes and terpenoids, have demonstrated multiple pharmacological activities. However, there is dearth of information on the EOs of most plants used as antioxidant and in the treatment of microbial infections. Therefore, this study was designed to determine the chemical constituents and biological activities of EOs from selected medicinal plants in Nigeria.

Tecoma stans (TS) (FHI 112524), *Plumeria acuminata* (PA) (FHI 112495), *Plumeria rubra* (PR) (FHI 112567), *Delonix regia* (DRe) (FHI 112523), *Gliricidia sepium* (GS) (FHI 112496), *Duranta repens* (DR) (FHI 112525), *Zanthoxylum zanthoxyloides* (ZZ) (FHI 112982), *Ceiba pentandra* (CP) (FHI 112962) and *Annona muricata* (AM) (FHI 112526) were collected within University of Ibadan and authenticated at Forest Research Institute of Nigeria. Different plant parts (leaves, stem, stembark, fruits, twig, seeds, flowers, heartwood, root) were air-dried, pulverised and subjected to hydrodistillation. The EOs were analysed for their chemical constituents using gas chromatography-mass spectrometry. Antioxidant properties and cytotoxicity of the oils were respectively determined using 2,2-diphenyl-1-picryhydrazyl radical-scavenging and brine shrimp lethality assays, with reference to the standards. Antimicrobial activities were also determined using agar-diffusion method against selected fungi (*Candida albicans*, *Aspergillus niger*, *Fusarium solani*) and bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Leclericia adecarboxylata*, *Morganella morganii*, *Citrobacter freundii*, *Klebsiella pneumoniae*) using Ketoconazole and Gentamycin as reference drugs. Data were analysed using descriptive statistics.

The yields of the EOs ranged from 0.06 to 0.62 % (w/w). Dominant terpenes in the EOs were linalool (11.4%, stem), α-terpineol (11.7%, seed) in TS; linalool (16.1%, leaf), β-eudesmol (43.0%, root) in PA; spathulenol (34.7%), carryophyllene (17.8%) in PR stem; phytol (12.7%) in DRe root; humulene epoxide II (17.5%) in GS stem; geranyl acetone (4.3%) in DR leaf. Others were α-pinene (38.5%, stembark), caryophyllene oxide (27.0%, leaf) in ZZ; β-elemene (18.5%, stem) α-eudesmol (21.1%, heartwood), β-caryophyllene (28.7%, stembark) in CP; E-caryophyllene (28.2%) in AM leaf. Prominent non-terpenes were pentadecanal (51.2%) in PR leaf; (9Z)-docosenamide (51.6%) in DRe twig; palmitic acid (55.7%, root), styrene (52.5%, fruit) in DR. Thus, the chemical constituents were established for *Tecoma stans*, *Delonix regia* and *Ceiba pentandra* essential oils. The EOs of TS (seed, stem), DR (fruit, stem) and AM (leaf) displayed higher antioxidant activities (IC50 6.44 - 43.22 μg/mL) than control (IC50: BHA- 45.11 μg/mL, α-tocopherol-81.58μg/mL). Cytotoxicities of all the EOs were high (LC50 5.8992-89.8190 ppm) compared with standard values. The EOs exhibited antifungal activities (7.8±0.4 - 22.1±0.1 mm) compared with Ketoconazole (10.3±0.4 – 21.0±1.4 mm). However, only EOs of PA exhibited antibacterial activities (7.9±0.0 - 12.1±0.1 mm) comparable with Gentamycin (8.0±0.0 - 12.5±0.1 mm).

The essential oils of selected plants had antioxidant properties, were cytotoxic and possessed antimicrobial activities. Essential oils of *Tecoma stans* (stem, seed), *Duranta repens* (fruit, stem) and *Annona muricata* (leaf) could be alternative sources of antioxidants, while *Plumeria acuminata* (leaf, root, flower) oils could act as a natural antimicrobial agent.

Keywords: Antioxidant, Antimicrobial, Linalool, Caryophyllene, *Tecoma stans*, *Plumeria acuminata*.

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TABLE OF CONTENTS

	Page
Title	i
Certification	ii
Dedication	iii
Acknowledgements	iv
Abstract	vi
Table of Content	vii
List of Tables	xi
List of Figures	xiii
List of Plates	xv
List of Appendices	xvi

CHAPTER ONE

INTRODUCTION

1.1	Background of the study	1
1.2	Problem Statement	3
1.3	Justification for Study	3
1.4	Aim of Research	4
1.5	Objectives of Research	4

CHAPTER TWO

LITERATURE REVIEW

2.1	Plants Investigated	5
2.1.1	*Tecoma stans* (Bignoniaceae)	5
2.1.2	Genus *Plumeria* (Apocynaceae)	9
2.1.3	*Duranta repens* (Verbenaceae)	14
2.1.4	*Zanthoxylum zanthoxyloides* (Rutaceae)	18
2.1.5	*Ceiba pentandra* (Bombacaceae)	22
2.1.6	*Gliricidia sepium* (Fabaceae)	26
2.1.7	*Delonix regia* (Fabaceae)	30
2.1.8	*Annona muricata* (Annonaceae)	34
2.2	Essential Oils	38
2.3	Factors Affecting Major Properties of Essential Oils	38
2.4	Chemical Constituents of Essential Oils	40

2.4.1 Terpenes 40
2.4.1.1 Classification of terpenes 41
2.5 Biosynthesis of Terpenes 44
2.5.1 Mevalonic pathway 44
2.5.2 Deoxyxylulose phosphate (DOXP) pathway/ Methylerythritol phosphate (MEP) Pathway 45
2.6 Intramolecular Rearrangement of Terpenes 47
2.7 Non-terpene Essential Oils Components 49
2.8 Extraction of Essential Oils 52
2.8.1 Expression 52
2.8.2 Solvent extraction 53
2.8.3 Carbondioxide extraction 53
2.8.4 Enfleurage 54
2.8.5 Hydrodistillation 54
2.8.6 Steam distillation 55
2.8.7 Solid phase micro-extraction 55
2.8.8 Simultaneous distillation extraction 55
2.8.9 Microwaves distillation 56
2.8.10 Headspace sampling 56
2.8.11 Microwave irradiation process 56
2.9 Extraction Procedure Effect on the Quality of Essential Oils 56
2.10 Analyses of Essential Oils 57
2.10.1 Thin layer chromatography (TLC) 57
2.10.2 Liquid column chromatography (LCC) 58
2.10.3 High performance liquid chromatography (HPLC) 58
2.10.4 Gas chromatography (GC) 58
2.10.5 Enantioselective capillary gas chromatography (eGC) 59
2.10.6 Gas chromatography-Mass spectroscopy analysis 59
2.11 Uses of Essential Oils 60
2.12 Antimicrobial Activities of Essential Oils 61
2.13 Antioxidant Activities of Essential Oils 62

2.14 Biological Assay 63
2.14.1 Antioxidant assay 63
2.14.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay 64
2.14.2Antimicrobial assay 66
2.14.2.1 Antimicrobial agents 66
2.14.2.2Characteristics of Microorganisms 67
2.14.3 Brine shrimp lethality bioassay 68

CHAPTER THREE
MATERIALS AND METHODS
3.1 Collection of Plant Materials 70
3.2 Extraction of Essential Oils 70
3.3 Gas Chromatography-Mass Spectrometry (GC-MS) 74
3.4 Antimicrobial Assay 75
3.4.1 Test organisms 75
3.4.2 Sample preparation 75
3.4.3 Preparation of the bacteria medium 75
3.4.4 Antibacterial assay procedure 76
3.4.5 Preparation of the fungi medium 76
3.4.6 Antifungal assay procedure 76
3.5 Antioxidant Assay 77
3.6 Brine Shrimp Lethality Assay 77
3.6.1 Dilution procedure 78

CHAPTER FOUR
RESULTS AND DISCUSSION
4.1 Extraction Results 79
4.2 *Tecoma stans* Essential Oils Chemical Constituents 82
4.3 *Plumeria acuminata* (white flower) Essential Oils Chemical Constituents 90
4.4 *Plumeria rubra* (Pink flower) Essential Oils Chemical Constituents 97
4.5 *Delonix regia* Essential Oils Chemical Constituents 108
4.6 *Duranta repens* Essential Oils Chemical Constituents 115

4.7 *Zanthoxylum zanthoxyloides* Essential Oils Chemical Constituents 123
4.8 *Ceiba pentandra* Essential Oils Chemical Constituents 131
4.9 *Gliricidia sepium* Essential Oils Chemical Constituents 137
4.10 *Annona muricata* Essential Oils Chemical Constituents 144
4.11 Antioxidant Activity 150
4.12 Brine Shrimp Lethality Test Results 154
4.13 Antimicrobial Activity 158
4.13.1 Antimicrobial activity of *Tecoma stans* 158
4.13.2 Antimicrobial activity of *Plumeria acuminata* 160
4.13.3 Antimicrobial activity of *Plumeria rubra* 165
4.13.4 Antimicrobial activity of *Delonix regia* 167
4.13.5 Antimicrobial activity of *Duranta repens* 169
4.13.6 Antimicrobial activity of *Zanthoxylum zanthxyloides* 171
4.13.7 Antimicrobial activity of *Ceiba pentandra* 174
4.13.8 Antimicrobial activity of *Gliricidia sepium* 176
4.13.9 Antimicrobial activity of *Annona muricata* 179

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary 181
5.2 Conclusion 183
5.3 Recommendations 183
5.4 Contributions to knowledge 183
References 184

LIST OF TABLES

3.1 List of Plant Used 71

4.1 Physical Properties of the Essential Oils 81

4.2 Chemical Constituents of *Tecoma stans* Essential Oils 86

4.3 Classes of Components Identified in *T. stans* Essential Oils 89

4.4 Chemical Constituents of *Plumeria acuminata* Essential Oils 93

4.5 Chemical Compositions of *Plumeria rubra* Essential Oils 102

4.6 Comparison of Classes of Components Identified in *P. acuminata* and *P. rubra* Essential Oils 107

4.7 Chemical Constituents of *Delonix regia* Essential Oils 112

4.8 Classes of Components Identified in *D. regia* Essential Oils 114

4.9 Chemical Composition of *Duranta repens* Essential Oils 119

4.10 Classes of Components Identified in *D. repens* Essential Oils 122

4.11 Chemical Constituents of *Zanthoxylum* zanthoxyloides Essential Oils 127

4.12 Classes of Components Identified in *Z. zanthoxyloides* Essential Oils 129

4. 13 Comparison of Dominant Constituents in Present and Previous Studies 130

4.14 Chemical Constituents of *Ceiba pentandra* Essential Oils 134

4.15 Classes of Components Identified in *C. pentandra* Essential Oils 136

4.16 Chemical Compositions of *Gliricidia sepium* Essential Oils 140

4.17 Classes of Components Identified in *G. sepium* Essential Oils 143

4.18 Chemical Compositions of *Annona muricata* Essential Oils 146

4.19 Classes of Components Identified in *A. muricata* Essential Oils 149

4.20 IC_{50} Values indicating free radical-scavenging activity of the Essential oils 152

4.21 Brine Shrimp Lethality Assay Result 157

4.22 Antimicrobial Activity of *T. stans* 159

4.23 Antibacterial Activity of *P. acuminata* 163

4.24 Antifungal Activity of *P. acuminata* 164

4.25 Antimicrobial Activity of *P. rubra* 166

4.26 Antimicrobial Activity of *D. regia* 168

4.27 Antimicrobial Activity of *D. repens* 170

4.28 Antimicrobial Activity of *Z. zanthoxyloides* 173

4.29 Antimicrobial Activity of *C. pentandra* 175
4.30 Antimicrobial Activity of *G. sepium* 177
4.31 Antimicrobial Activity of *A. muricata* 180

LIST OF FIGURES

2.1	Alkaloids in *T. stans*	8
2.2	Compounds isolated in *P. acuminate* and *P. rubra*	13
2.3	Compounds in *D. repens*	17
2.4	Structure of compounds in *Z,* zanthoxyloides	21
2.5	Phytochemicals in *C. pentandra*	25
2.6	Compounds from *G. Sepium* that were active against *Artemia salina* larvae	29
2.7	Phytochemicals Isolated in *D. regia*	33
2.8	Alkaloids in *A. muricata*	37
2.9	Essential oils components	39
2.10	Examples of Terpene	42
2.11	Terpenes Biosynthesis	46
2.12	Intramolecular reaction of Terpenes	48
2.13	Oxidative Cleavage of Carotenoid	51
2.14	Inhibition of DPPH	65
4.1	Constituents of *T. stans* Essential oils	85
4.2	Sesquiterpenes in *P. acuminata* essential oils	92
4.3	Sesquiterpenoids identified in *P. rubra* essential oils	101
4.4	Constituents of *D. regia* volatile oils	111
4.5	Most abundant constituents in *D. repens* volatile oils	118
4.6	Non-volatile constituents in *Z. zanthoxyloides* essential oils	126
4.7	*C. pentandra* essential oil components	133
4.8	Sesquiterpene oxides in the *G. sepium* oils	139

LIST OF PLATES

2.1	*Tecoma stans* plant	6
2.2	*Plumeria acuminate* plant	10
2.3:	*Plumeria rubra* Plant	11
2.4	*Duranta repens* tree	15
2.5	*Zanthoxylum zanthoxyloides* tree	19
2.6	*Ceiba pentandra* tree	23
2.7	*Gliricidia sepium* plant	27
2.8	*Delonix regia* plant	31
2.9	*Annona muricata* tree	35
4.1	Antibacterial activity of *P. acuminata* leaf essential oil (labelled 2) in the pilot study	161
4.2	Inhibitory zones of *P. acuminata* essential oils against *Candida albicans* in the pilot study	162
4.3	Zones of Inhibition of *G. sepium* essential oil against *F. solani*	177

LIST OF APPENDICES

1	Gas Chromatogram of *Tecoma stans* Leaf Essential oil	218
2	Gas Chromatogram of *Tecoma stans* Flower Essential oil	219
3	Gas Chromatogram of *Tecoma stans* Seed Essential oil	220
4	Gas Chromatogram of *Tecoma stans* Stem Essential oil	221
5	Gas Chromatogram of *P. acuminata* Leaf Essential oil	222
6	Gas Chromatogram of *P. acuminata* Root Essential oil	223
7	Gas Chromatogram of *P. acuminata* Flower Essential oil	224
8	Gas Chromatogram of *Plumeri rubra* Leaf Essential oil	225
9	Gas Chromatogram of *Plumeri rubra* Stem Essential oil	226
10	Gas Chromatogram of *Plumeri rubra* Flower Essential oil	227
11	Gas Chromatogram of *Plumeri rubra* Root Essential oil	228
12	Gas Chromatogram of *Delonix regia* Twig Essential oil	229
13	Gas Chromatogram of *Delonix regia* Flower Essential oil	230
14	Gas Chromatogram of *Delonix regia* Root Essential oil	231
15	Gas Chromatogram of *Duranta repens* Leaf Essential oil	232
16	Gas Chromatogram of *Duranta repens* Stem Essential oil	233
17	Gas Chromatogram of *Duranta repens* Fruit Essential oil	234
18	Gas Chromatogram of *Duranta repens* Root Essential oil	235
19	Gas Chromatogram of *Z. zanthoxyloides* Leaf Essential oil	236
20	Gas Chromatogram of *Z. zanthoxyloides* Stem bark Essential oil	237
21	Gas Chromatogram of *Ceiba. pentandra* Stem bark Essential oil	238
22	Gas Chromatogram of *Ceiba. pentandra* Heartwood Essential oil	239
23	Gas Chromatogram of *Gliricidia sepium* Leaf Essential oil	240
24	Gas Chromatogram of *Gliricidia sepium* Stem Essential oil	241
25	Gas Chromatogram of *A. muricata* Leaf Essential oil	242
26	Gas Chromatogram of *A. muricata* Branches Essential oil	243
27	Gas Chromatogram of *A. muricata* Stem bark Essential oil	244
28	Antioxidant activity of *Tecoma stans* leaf essential oil	245
29	Antioxidant activity of *Tecoma stans* stem essential oil	246
30	Antioxidant activity of *Tecoma stans* seed essential oil	247

31	Antioxidant activity of *Tecoma stans* flower essential oil	248
32	Antioxidant activity of *Plumeria acuminata* leaf essential oil	249
33	Antioxidant activity of *Plumeria acuminata* flower essential oil	250
34	Antioxidant activity of *Plumeria acuminata* root essential oil	251
35	Antioxidant activity of *Plumeria rubra* leaf essential oil	252
36	Antioxidant activity of *Plumeria rubra* flower essential oil	253
37	Antioxidant activity of *Plumeria rubra* stem essential oil	254
38	Antioxidant activity of *Plumeria rubra* root essential oil	255
39	Antioxidant activity of *Delonix regia* twig essential oil	256
40	Antioxidant activity of *Delonix regia* flowers essential oil	257
41	Antioxidant activity of *Delonix regia* root essential oil	258
43	Antioxidant activity of *Duranta repens* leaf essential oil	259
44	Antioxidant activity of *Duranta repens* fruits essential oil	260
45	Antioxidant activity of *Duranta repens* stem essential oil	261
46	Antioxidant activity of *Duranta repens* root essential oil	262
47	Antioxidant activity of *Zanthoxylum zanthoxyloides* leaf essential oil	263
48	Antioxidant activity of *Zanthoxylum zanthoxyloides* stem bark essential oil	264
47	Antioxidant activity of *Ceiba pentandra* stem bark essential oil	263
48	Antioxidant activity of *Ceiba pentandra* heartwood essential oil	264
49	Antioxidant activity of *Gliricidia sepium* leaf essential oil	265
50	Antioxidant activity of *Gliricidia sepium* stem essential oil	266
51	Antioxidant activity of *Annona muricata* leaf essential oil	267
52	Antioxidant activity of *Annona muricata* branches essential oil	268
53	Antioxidant activity of *Annona muricata* stem bark essential oil	269
54	Antioxidant activity of α-tocopherol	270
55	Antioxidant activity of Butylatedhydroxyanisole	271
56	Antimicrobial activities of *Tecoma stans* essential oils	272
57	Antibacterial activities of *Plumeria acuminata* essential oils	275
58	Antifungal activities of *Plumeria acuminata* essential oils	280
59	Antimicrobial activities of *Plumeria rubra* essential oils	284
60	Antimicrobial activities of *Delonix regia* essential oils	290

61	Antimicrobial activities of *Duranta repens* essential oils	294
62	Antimicrobial activities of *Zanthoxylum zanthoxyloides* essential oils	300
63	Antimicrobial activities of *Ceiba pentandra* essential oils	303
64	Antimicrobial activities of *Gliricidia sepium* essential oils	306
65	Antimicrobial activities of *Annona muricata* essential oils	309

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Plants have been in use as a source of medicine before the advent of literacy. The medicinal properties of plants are due to the presence of secondary metabolites known as phytochemicals. Although, these chemicals are not really for the growth of the plants producing them but are synthesized by plants for self-defense against external factors like pests, insects, herbivores, UV light and other environmental threats (Egbuna *et al*., 2018). Many have prominent effects on the animal systems while some others possess therapeutic properties which have been utilized in human health care (Vaishnav and Demain, 2010). These chemicals can also be found in other living organisms but the major source is plant. These phytochemicals have been a source of inspiration for many synthetic drugs. Infact, most of the modern medicine are synthesized to mimic the phytochemicals.

From the statistics given by World Health Organization, three-quarters of the earth population depend on medicinal plants for their major care (Okigbo and Ramesh, 2008) because of their therapeutic efficacy and low cost (Cragg *et al*., 1997). The investigation of chemical and biological properties An example is Paditaxel, a synthesized compound from Taxol isolated from *Taxus brevifolia* bark, used in the treatment of many cancer including pancrease cancer, ovarian cancer, breast cancer, cervical cancer and lung cancer (Rungsung *et al*., 2015). Artemisinin, a sesquiterepene lactone isolated from *Artemisia annua* (commonly called Chinese wormwood) (Brown, 2010) is another example. It was discovered by Tu You as a powerful antimalaria drug. Although, the malaria parasite, *Plasmodium falciparum* became resistant to the use of the drug alone, World Health Organization recommends its use with other antimalaria drugs for effective treatment (Graham *et al*., 2010). Other bioactive components from plants that are useful in modern

therapeutics include aspirin, an analgesic from salicin that was first isolated from *Salix alba* in 1899 (Ahluwalia and Chopra, 2008), quinine used as antimalarial and antipyretic from cinchona species bark (Achan *et al*., 2011), cocaine a local anesthetic from *Erythroxylum coca* leaf (Calatayud and González, 2003) to mention few.

Secondary metabolites are also produced by bacteria and fungi. Many bacterial secondary metabolites may be toxic to human. An example is botulinum toxin, a secondary metabolite synthesised by *Clostridium botulinum* which has both positive and negative effects on man. The most known fungal secondary metabolite is penicillin from mold penicillium. Penicillin was the first antibacterial filtrate discovered by Alexander Fleming in 1928 that reduced the number of death in World War II by over hundred thousand. Its discovery had led to other antibiotics like erythromycin, chloramphenicol, vancomycin, chlortetracycline, cephalosporin and streptomycin were produced which are still in use till today (Tan and Tatsumura, 2015).

Over 2 million secondary metabolites from plants (i.e phytochemicals) are known and they are classified based on the diversity in their structure, function and biosynthesis. Some of the phytochemicals are alkaloids, glycosides, flavonoids, saponins, steroids, phenolics, tannins and terpenes. Among the phytochemicals essential oils which are terpenes in combined form have been considered attractive due to the diversity of their bioactivities like antiseptic, anti-inflammatory, spasmolytic, sedative, analgesic, anesthetic, anticancer, antiviral, antibacterial and antifungal (Hussein *et al*., 2015; Russo *et al*., 2015). Previous works revealed that essential oils with their constituents possess many functional properties and thus exert their actions in humans and other organisms like bacteria, fungi, viruses and insects. As a result of these, essential oils find applications in many industries. Various essential oils' constituents are used as flavor and fragrant agents in the food industry. Their strong antioxidant and antimicrobial properties help to prolong food shelf-life when used as food additives. They are also incorporated into the food parkaging materials to control food spoilage by hindering oxidation reactions from external factors or microbial attacks (Saeed *et al*., 2022). In addition, essential oils are used in cosmestics, perfumes and related household products because of their bioactivity and pleasant odour (Sarkic and Stappen, 2018). Apart from their medicinal value, they are also used in fine chemistry reactions as

intermediates (Chamorro *et al*., 2012). Furthermore, because essential oils are sources of many terpenoids and phenylpropanoids, they are useful chemical reagents in the synthesis of novel products with higher added value thereby replacing the existing ones which are of petroleum origin (Kouznetsov 2019).

Most interesting property of essential oils is the synergistic effect of their components which provides optimisation of their bioactivity. Essential oils are complex combinations of compounds which demonstrate higher activities than their isolated compounds due to the combined activities of other minor constituents. Therefore, essential oils research is a promising field that will assist in overcoming the setback arising from using synthentic drugs as the only means of treating diseases (Yap *et al*., 2014)

1.2 Problem Statement

Most of the health challenges of human today are majorly associated with oxidative deterioration and microbial infection which are being taken care of by synthetic drugs. The continuos use of the synthetic antimicrobial drugs has led to alarming rise in the drug resistance by the microorganisms. Also, the problems of side effects and toxicity linked with the use of synthetic antioxidants have call for scientific intervention (Uzombah, 2022). As a result, there is a need for an alternative source which could replace or used alongside with the existing drugs for optimum therapeutic efficacy.

1.3 Justification for Study

In order to overcome the challenges arising from the use of synthetic drugs, there is need to search for new antioxidants and antimicrobial agents precisely, from plants since they have shown optimum medicinal efficacy with no report of resistance, side effects and high toxicity when used in traditional medicine (Saalu, 2016). Therefore, there are a lot of ongoing researches on the investigation of antioxidant and antimicrobial activities of phytochemicals from medicinal plants. Literature review showed that essential oils among plant phytochemicals possess very good antimicrobial and antioxidant properties (Cowan 1999; Zahid *et al*., 2010; Bicas *et al* 2011; Gyesi *et al*., 2009) and there are many medicinal plants bearing essential oils that are yet to be investigated.

1.4 Aim of Research

The aim of this research is to examine the chemical constituents and biological activities of essential oils from different parts of nine medicinal plants (*Tecoma stans*, *Plumeria acuminata, Plumeria rubra*, *Duranta repens*, *Delonix regia*, *zanthoxylum zanthoxyloids*, *Ceiba pentandra, Gliricidia sepium* and *Annona muricata*), if they could be an alternative source of antioxidant and antimicrobial agents. The plants were randomly selected from seven families based on their ethnomedicinal uses and the pharmacological activities of other extracts from the plants,

1.5 Objectives of Research

The major objectives are to:

(i) extract essential oil from different parts of nine medicinal plants using hydrodistillation in all glass Clevenger-type apparatus,

(ii) qualitatively and quantitatively characterise the constituents of the essential oils by means of gas chromatography coupled with mass spectrometry technique,

(iii) investigate the antibacterial as well as antifungal activity of the volatile oils against some bacteria and fungi isolates using agar-diffusion method,

(iv) evaluate the antioxidant potential of the volatile oils by DPPH free radical-scavenging assay.

(v) assess the cytotoxic potential of the essential oils by means of Brine shrimps lethality assay.

CHAPTER TWO

LITERATURE REVIEW

2.1 Plants Investigated

2.1.1 *Tecoma stans* (Bignoniaceae)

Tecoma stans (L.) Kunth commonly known as the yellow trumpet tree belongs to Bignoniaceae family (family of flowering plants). It is locally called Awun in yoruba land. Nearly all the plants in this family are woody, but few are subwoody, either as vines or as subshrubs and distributed at large in various parts of the world but mostly in the tropical regions. Many species of Bignoniaceae have some uses either commercially or ethnobotanically, but most common among them are those planted as ornamentals like *Tecoma* species (George *et al*., 2005). The genus *Tecoma* consists of 14 species of shrubs. Twelve among them are from the America, while the remaining two species are found in Africa. The generic name was obtained from the Nahuatl word *tecomaxochitl*, used by the indigenous people of Mexico for plants that have tubular flowers (Quattrocchi, 2000).

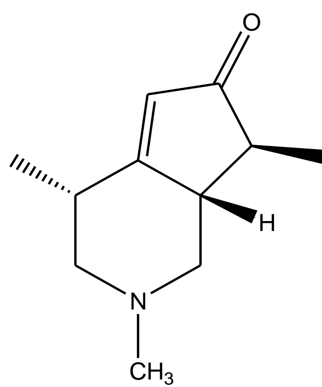
Yellow trumpet tree is an ornamental plant. The plant is upright, branched, and slightly hairy or virtually smooth. It grows upto 2.5 to 10 meters high, with dark ridged bark (Plate 2.1). The leaves are opposite, odd-pinnate, and about 20 cm long with 5 to 7 leaflets. The yellow trumpet-shaped flowers with faint scent appear in dense, terminal clusters. The calyx is green, having length of 5 to 7 mm, and 5-toothed. Ovary is narrow and cylindrical about 3 mm long. The seed is 2.2 to 2.7 cm in length and 3 to 7 mm broad. The plant is drought-tolerant and sprouts well in temperate regions. Bees, butterflies, and hummingbirds are attracted by the flowers. Yellow trumpet bush can be found throughout the neotropical America, including the Caribbean, the Bahamas, Florida, Mexico, South America, Northern Argentina, and introduced to other regions like Philippines, Hawaii, India and Africa (Kampati *et al*., 2018).

Plate 2.1: ***Tecoma stans*** **plant (Saunders road, University of Ibadan, 7° 0.2 N/3° 28.8 E)**

The leaf, root and flowers are used in traditional medicine. *Tecoma stans* leaf infusion are reported to cure diabetes and stomach pains when taken orally (Aguilar-Santamaria *et al*., 2009). The leaf and root decoction are used to treat syphilis and intestinal worms. Extracts from the roots exhibited a powerful diuretic and vermifuge activity. Flowers and leaves have some medicinal values in cancer therapy (Roman-Ramos *et al*., 1991; Anburaj *et al*., 2016). *Tecoma* leaves are also used traditionally in Mexico (Lozoya-Meckes and Mellado-Campos 1985; Costantino *et al.,* 2003) and in Latin America (Susano, 1981) to control diabetes, as cattle feed and in the synthesis of nanoparticles (Patriota *et al*., 2016). *T. stans* gives helpful shade, especially in gardens, where it is planted as an ornamental plant with its many yellow trumpet-shaped flowers. The plant also provides wood useful in the construction of buildings as well as firewood and charcoal.

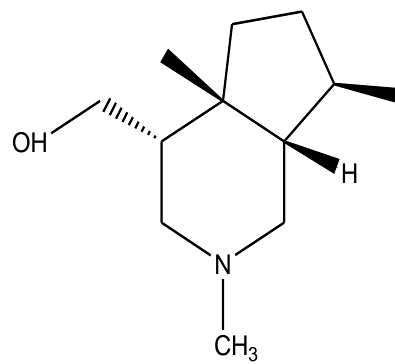
Preliminary phytochemical screening of petroleum ether, aqueous, ethanol, chloroform, and acetone flower extracts of *Tecoma stans* and six other members of the family showed the existence of varying degrees of phytochemicals (Solomon *et al.*, 2013). Extracts of *Tecoma stans* contained phenols, flavonoids, terpenoids, steroids, quinone, phytosterol, tannins, proteins, carbohydrates, saponins, glycosides, coumarines and quinines (Bhat 2019). Some of the isolated compounds from *T. stans* are tecomine **[2.1]** and tecostamine **[2.2]** from the leaf which were accountable for the hypoglycaemic property of the extracts of the plant (Aguilar-Santamaria *et al*., 2009), β-carotene and zeaxanthin from the flower (Anburaj *et al*., 2016).

The flowers, branches and leaves extracts of *T stans*, displayed considerable action against a variety of bacteria and also have moderate antioxidant activity. The alkaloid tecomine also possess significant antibacterial property (Alma *et al* 2009, Bhat 2019; Salem *et al*., 2013). Furthermore, the leaves extracts possess analgesic and antispasmodic potentials (Anburaj *et al*., 2016). Central nervous system (CNS) depressant potential of different extracts (using chloroform, methanol and water) of the flower was also investigated on albino mice (18-25 g) of both sexes. The methanol extract exhibited the highest dose-dependent CNS depressant activity (Kameshwaran *et al*., 2012). Cipriani *et al*., (2014) gave account of the phytotoxicity of *T. stans* leaves extract on the germination and root development of Handroanthus achraceus (cham) Mattos (ipe), Lautuca sativa L. (lettuce)



Tecomine

2.1



Tecostamine

2.2

Figure 2.1: Alkaloids in *T. stans*

and Medicago sativa L. (alfalfa). Wound healing, cytotoxicity, antiinflammatory, antioxidant, anticancer, and antiproliferative activities of the plant extracts have been investigated (Zhu *et al*., 2008; Alma *et al*., 2009; Thirumal *et al*., 2012; Marzouk *et al*., 2016; Bindu and John, 2018; Kampati *et al*., 2018; Bhat 2019).

2.1.2 Genus *Plumeria* (Apocynaceae)

Plumeria is a genus under the family Apocynaceae and it consists of 11 species. They are usually cultivated in both subtropical and tropical areas of the world and are well known for their beautiful and fragrant flowers. Among the genus are *Plumeria acuminata* and *Plumeria rubra*.

Plumeria acuminata commonly known as white frangipani is indigenous to Central America, South America, the Caribbean and Mexico. In traditional medicine, the plant has long record of use. Other scientific names are *P. alba* Blanco and *P. acutifolia* Poir. *P. acuminata* is a small, deciduous tree, which grows up to 3-7 m high, has smooth and shining stem, succulent with abundant sticky milky latex (Plate 2.2). The leaves are large, green, about 40-50 cm long, alternate, stipules not present or rarely present and cluster at the end of the branches. The sweet-smelling flowers of about 5-6 cm long are bisexual, with whitish upper portion and yellowish inner part. The wood is soft and yellowish-white. The branches are thick, swollen with many leaves at the tips. The fruits are linear with ellipsoid or oblong follicles and brownish black in colour. The seeds also are oblong (Shinde *et al*., 2014).

P. rubra L. is a small tree or spreading shrub and grows to a height of 2-8 m. The stem is thick and succulent, sausage-like blunt branches covered with a thin grey bark. The bark oozes white latex when broken, which can irritate the skin and mucus membrane. The leaves are large, green, 30-50 cm long, alternate, crowded at the terminal end part of the twigs. The flowers have strong fragrance, five petals and appear at the end of branches throughout the summer. The colours range from pink to red, having shades of yellow in the centre, intially tubular before opening out, diameter 5-7.5 cm and rarely proceeded to the level of producing seed of about 20-60 wings (Shinde *et al*., 2014).

The major difference between *P. acuminata* and *P. rubra* is the colour of their flowers.

Plate 2.2: *Plumeria acuminate* **plant (Saunders road, University of Ibadan, 7° 0.2 N/3° 28.8 E)**

Plate 2.3: ***Plumeria rubra*** **Plant (https://www.masterclass.com/articles/plumeria-plant-care-guide)**

Steroids, terpenoids, flavonoids, saponins, tannins, glycosides, sugars, fats and oils were phytochemicals in the different extracts of *P. acuminata* leaf studied by Gupta *et al*., (2016). The leaf extract contains lupeol acetate, lupeol carboxylic acid, stigmast-7-enol **[2.3]**, and ursolic acid, the root contains fulvoplumierin **[2.4]**, plumericin, isoplumericin **[2.5]**, β-dihydroplumericin and β-dihydroplumericinic acid, and the bark contains plumierid, a bitter glucoside (Gupta *et al*., 2016). The latex consists of caoutchouc, resins and calcium salts of plumieric acid. The essential oil consists of citronellol, primary alcohols, farnesol, geraniol, phenylethylalcohol, ketone and aldehyde (Shinde, 2014).

The parts of *P. acuminata* used in traditional medicine include leaves, flowers, stem and root bark. The bark decoction is used to counteract pains in the toothgum and also used as an emmenagogue, a febrifuge and a purgative. It is also taken as cooling tea for stroke prevention. The mixture of the latex and coconut oil helps to treat arthritis, rheumatism and chronic itchy skin. The juice is used as rubefacient in rheumatic pain (Tembare *et al*., 2012). Leaves decoction is applied on the feet to remove eruption and cracks while the leaf concoction is used as remedy for asthma (Devi *et al*., 2017). The crushed bark is used in the treatment of tumours while the latex is used as remedy for herpes, scabies and ulcer. The bark is as well used as diuretic, cardiotonic, purgative and hypotensive (Zaheer *et al*., 2010). Pharmacological activities of *P. acuminata* documented were anti-inflammatory, antioxidant, antimicrobial (Gupta *et al*., 2008), toxicity (Gomathi *et al*., 2012) and antimutagenic (Amelia *et al*., 1996).

Phytochemical screening of *Plumeria rubra* flowers and leaf showed the presence of phenolic compounds, saponins, steroids, flavonoids, tannins, alkaloids, terpenoids and glycosides (Egwaikhide *et al*., 2009). Traditional uses of *P. rubra* include treatment of ulcer, leprosy, itching, veneral sores, urinary discharges, tumours, rheumatic pains, tooth ache, diabetes mellitus and to stop bleeding. Pharmacological activities of *P. rubra* from literature include anti-inflammatory and antihelmintic (Kumar *et al.,* 2009), antioxidant and hypolipidemic (Merinda *et al.,* 2010), antipyretic and antinociceptive (Vimlesh *et al*., 2012), antiulcer (Mistra *et al*., 2012), analgesic (Kumar *et al*., 2009), abortifacient effect (Dabhadkar and Varsha, 2012) and protective action of the ethanol leaf extract on the

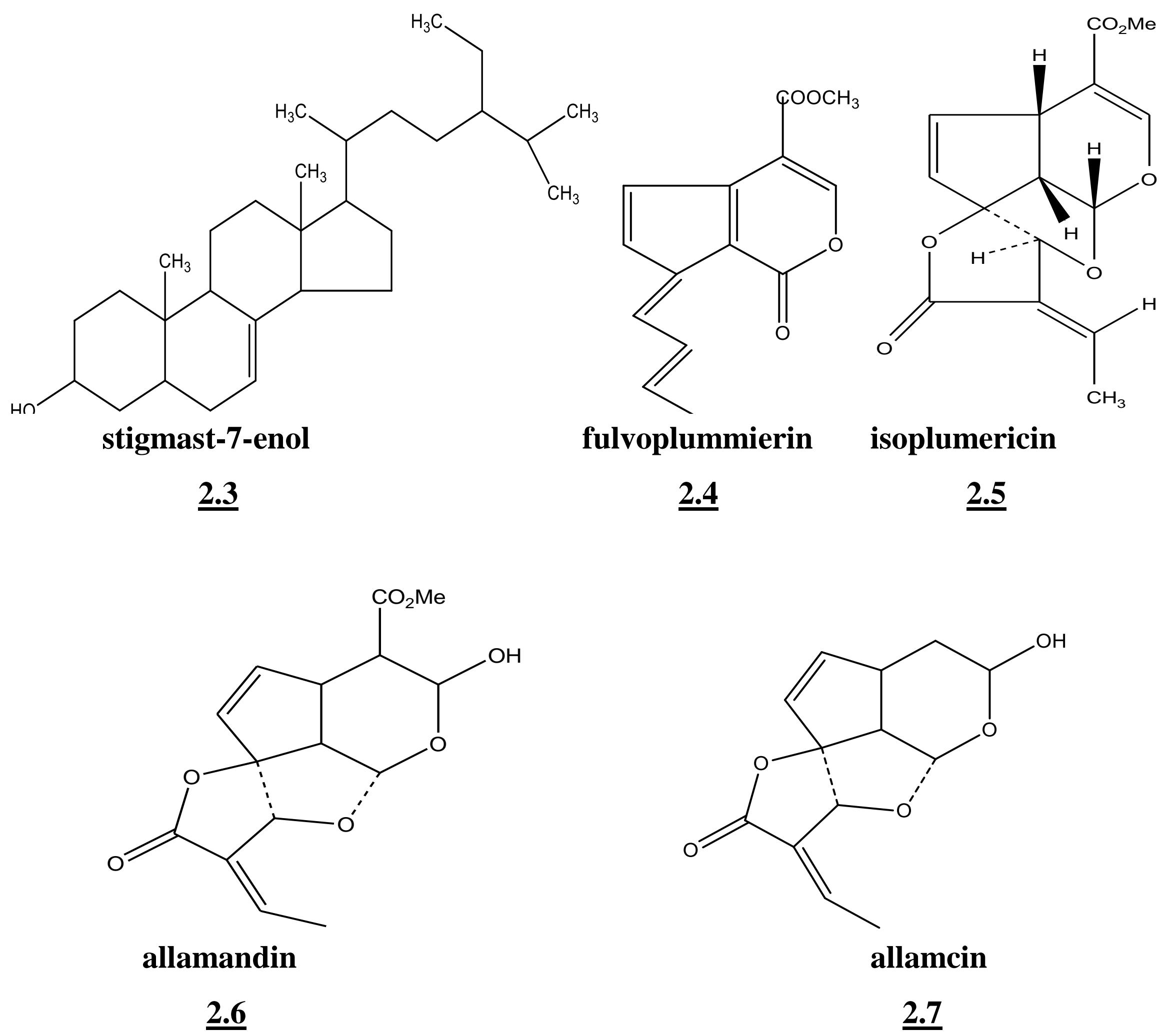


Figure 2.2 Compounds isolated in *P. acuminata* and *P. rubra*:

haemopoietic system (Rekha and Jayakar 2011). Inhibitory effects of organic extracts from the plant against human pathogenic bacteria and four fungi have been reported (Jarin *et al*., 2008). The bark extract exhibited cytotoxicity activity against numerous in vitro human cancer cell lines: lung, fibrosarcoma, melanoma, breast and colon. In addition, the leaves are used in the curing of leprosy, inflammations and ulcer. Four iridoids from the stem bark namely plumeridoids A, B, C and epiplumeridoid C demonstrated antialgal, antifungal and antibacterial activities (Kuigoua *et al*., 2010). 2,5-dimethoxy-*p*-benzoquinone and three iridoids: fulvoplumierin **[2.4]**, allamandin **[2.6]** and allamcin **[2.7]** were active compounds isolated from the trichloromethane and petroleum ether of the plant's bark extracts, with cytotoxic potential. Volatile oils of *P. rubra* flower are used in aromatherapy and perfumery (Shaida *et al*., 2008). Other isolated compounds include irroides, β-β-sitosterol-β-D-glucoside, rubrinol glucoside, lupeol nanoate, plumieride coumarate, lupeol heptanoate, fulvoplumierin, 2,5-dimethoxy-p-benzoquinone, flavan-3-ol glycoside as well as (2R,3S)-3,4'-dihydroxy-7,3',5'-trimethoxyflavan-5-O-β-D-glucopyranoside (Matthias *et al*., 1991; Shinde *et al.,* 2014)

2.1.3 *Duranta repens* (Verbenaceae)

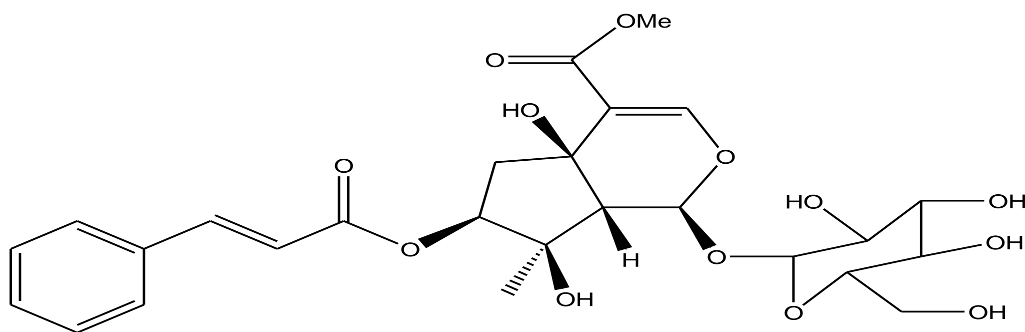
Duranta repens Linn. (Syn. *Duranta plumier* Jacq., *Duranta erecta* Linn.) belongs to the family Verbenaceae. It is commonly called golden dewdrop or pigeon berry and locally known as peregun (Yoruba). The plant is a shrub or small tree usually up to 6 m high (Plate 2.3). The leaves are light green, elliptic to ovate and opposite, with the length of 7.5 cm and width of 3.5 cm. The light gray bark becomes rough and fissured when old. The colour of the flowers is either lavender or light-blue. The flowers cluster at the terminal of the branches, flourishing almost throughout the year. The fruits are small in size and they are either yellow or orange berry with numerous seeds inside (Huxley, 1992). The plant is native of Southern America, Central America, Mexico and Caribbean. *D. repens* is commonly cultivated as ornamental plant in both tropical areas as well as subtropical regions throughout the world, and has become accepted in many places. The leaves and the berries are toxic, the plant was confirmed to have killed cats and children. However, there

Plate 2.3: ***Duranta repens*** **tree (Saunders road, University of Ibadan, 7° 0.2 N/3° 28.8 E)**

were no toxic effects on songbirds when they eat the fruits (Thompson, 2007). The fruits are used traditionally as antimalaria and antihelmintic agent (Whistler, 2000). The leaves are utilised in the treatment of abscesses. The whole plant is used to treat intestinal worms, infertility, pneumonia, itches, neuralgic disorder and are insect repellent. The fruit and stem are natural larvicidals. The fruits are used in China as malaria remedy (Puri, 2018).

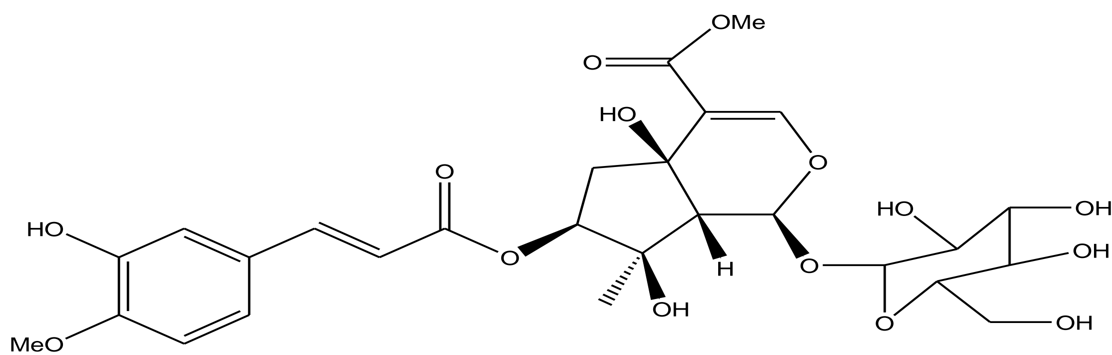
D. repens plants are active against wide range of bacteria, especially *shigella* species and also effective against pathogenic fungi (Nikkon *et al*., 2008). Extract from different parts of the plant have been documented to possess antioxidant, antibacterial, antifungi, antiviral, antifeedant, antidiabetic, cytotoxic and insecticidal properties (Iqbal *et al*., 2004; Ijaz *et al*., 2010; Bangou *et al*., 2012). Aqueous extract and ethyl acetate extracts of the fruit showed considerable antimalarial property in mice thereby supporting the traditional use of the plant (Castro *et al*., 1996).

Important phytochemicals namely flavonoids, phenols, saponins, sterols, tannins, alkaloids, triterpenoids and minerals such as Fe, Zn, and Cu were reported in the fruits and leaves. Wide variety of chemical constituents have been isolated from the plant. These include durantosides I **[2.8]**, II, III, duranterectoside A **[2.9]**, caryoptoside, (+)-5-‘methoxyisolariciresinol **[2.10]**, (-)-5-‘methoxyisolariciresinol **[2.11]**, lamiide and lamiidoside (Shankaraiah *et al*., 2013; Donkor *et al*., 2019). Triterpenes, which exhibited mosquitocidal activity, β-amyrin and 12-Oleanene 3β, 21β-diol were compounds from the stem (Nikkon *et al*., 2010). Repenins A-D isolated from chloroform soluble portion of whole plant exhibited anti-oxidative activity (Ahmad *et al.*, 2009 A). Also, flavonoid glycosides from chlorofom soluble fraction of whole plant showed anti-plasmodial property in both chloroquine-resistant and sensitive strains of *Plasmodium falciparum* (Ijaz *et al*., 2010). The leaf oil contains high quantity of vitamin A, vitamin D and iron while the seed oil has high magnessium, sodium, potassium and vitamin K. Phytochemicals in the oils were saponin, rutin, linamarin and catechin, kaempherol, anthocyanins, spartein, phytates and fatty acids.



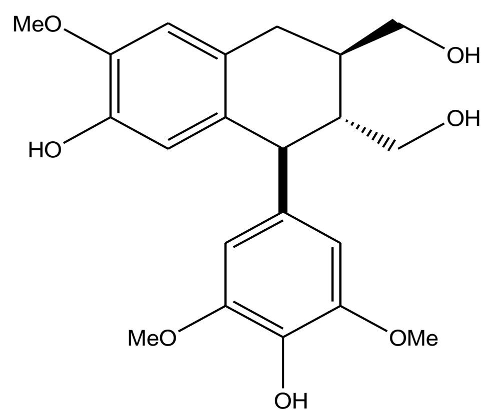
Durantosides I

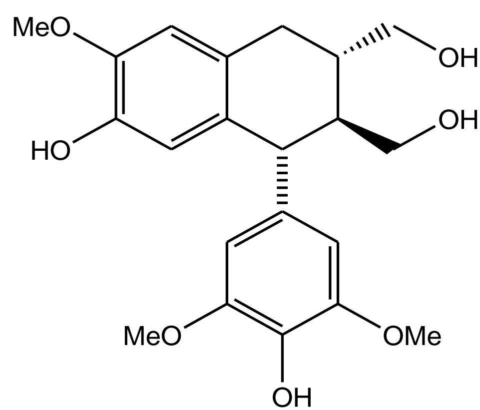
2.8



Duranterectoside A

2.9





(+)-5-'Methoxyisolariciresinol **2.10**

(-)-5-'Methoxyisolariciresinol **2.11**

Figure 2.3: Compounds in *D. repens*

2.1.4 Zanthoxylum zanthoxyloides (Rutaceae)

Zanthoxylum zanthozyloides Lam. belongs to the family Rutaceae and genus *Zanthoxylum*. *Zanthoxylum* contains about 250 species of evergreen decidous shrubs and trees among the Rutaceae. There are seven species of genus *Zanthoxylum* Linn. in Nigeria, all having close similarities and relationships. The major characteristic of these species is that the trunk, leaf stalk, branchlets, branches and inflorescence axes are covered by spines (also called prickles). They are principally plants of the forest vegetation. *Z. zanthoxyloides* is commonly found in dry forest vegetations and savannah areas, also found in the drier parts of Southwestern Nigeria as far as Niger State (Adesina, 2005).

Zanthoxylum zanthozyloides Lam, also known as *Fagara zanthoxyloides* is a small tree or shrub covered with spines and more or less scandent growing up to 6-8 m high (Plate 2.4). The leaves are alternate and glabrous up to 12 cm long. The petiole length is 2-5 cm, glabrous, spiny beneath with curved prickles while petiolules are 2-5 mm long. The leaflets which are 5-10 cm long and 2-4 cm wide are obovate to elliptical. They have round base and obtuse or round apex, sometimes notched or apiculate, having numerous glandular dots, giving the smell of lemon and pepper when crushed. The leaf is rigidly peppery when eaten, pinnately veined with 10-14 pairs of lateral veins, fusing near the margin. The pinacle with short branches has a length ranging from 5-25 cm while the flowers are unisexual with whitish or greenish colour and barely open corolla. The male flowers have stamens while the female flowers have superior ovary, one-celled, short style and lateral. The fruit is an ovoid follicle of about 5-6 mm wide, one-seeded, brown with glandular dots. The seeds are black to bluish in colour (Arbonier, 2004). The local name of *Z. zanthxyloides* is “igi ata” (Yoruba).

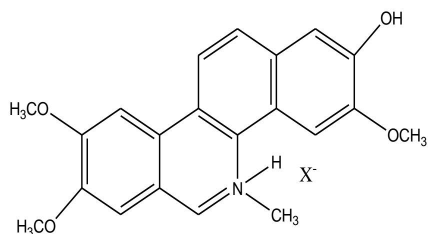
Ethnopharmacological uses of the plant include analgesic, anticancer and treatment of malaria, tuberculosis, oedema, intestinal worms, dysentery, gonorrhea, urethritis, eye infection, whooping cough and paralysis. It helps to reduce pain of migraine, neuralgia and labour. The root extracts are applied externally to haemorrhoids, swellings, snake bites, wound, leprosy, abscesses, syphilitic sores, arthritic and rheumatic pain (Guendéhou *et al*., 2018). Root extracts are also used in the treatment of elephantiasis, toothache, gonorrhea,

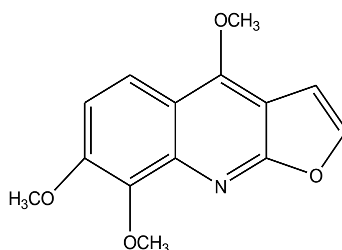
Plate 2.5*: Zanthoxylum zanthoxyloide* tree (Saunders road, University of Ibadan, 7° 0.2 N/3° 28.8 E)

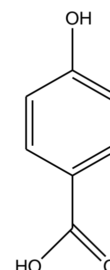
malaria, abdominal pain and sexual impotence (Odebiyi and Sofowora 1979; Awuah 1989; Okagu *et al*., 2021). When the root and stem bark are chewed, they produce an effect on the palate and thus help to cure toothache, sore gums as well as dental caries. The roots decoction is also used as remedy for sore throat and as mouthwash. Other economic importance includes construction of building, provision of firewood, poles and post, spices, necklaces from the seeds and also religious purpose (Nacoulma, 1996; Arbonier, 2004).

Previous studies on *Z. zanthozyloides* extracts have shown that the root and stem bark possess anti-malaria activity (Kassim *et al*., 2005; Gansane *et al*., 2010). Likewise, the root, stem bark and leaf extracts have pesticidal property (Udo, 2011), antimicrobial potential (Rojas *et al*., 1992), anti-inflammatory and anticancer properties (Folashade *et al*., 2006; Patel *et al*., 2010). The fruit essential oil exhibited antimirobial, antioxidant, antisickling and ant-inflammatory activities (Jazet *et al*., 2008). Anti-sickling activity and broad spectrum antimicrobial activity of the plant extracts were also reported (Oyedapo and Famurewa, 1995**;** Folashade *et al*., 2006; Ynalvez *et al*., 2012). Moderate to significant antibacterial activity and several chemovariants of fruits essential oils from different localities were also reported (Misra *et al*., 2013).

From the root bark, benzophenanthridines fagaronine **[2.12]** (antimalarial agent), dihydroavicine, chelerythrine, oxychelerythrine, furoquinoline skimmianine **[2.13]**, 8-methoxydictamine, berberine **[2.14]**, *N*-methylcorydine, tembetarine and aporphines magnoflorine were isolated while fagaronine and chelerythrine were found in the stem bark (Udo, 2011). Other compounds isolated from roots, fruits and stem bark are pungent N-isobutylocta-2,4-dienamide and N-isobutyldeca-2,4-dienamide. A series of acids also isolated from the roots are vanillic acid, parahydroxybenzoic acid **[2.18]**, hydroxybenzoic acid, 2-hydroxymethyl benzoic acid (anti-inflammatory property), parafluorobenzoic acid, divianilloylquinic acids in addition to burkinabin A, B and C. Aromatic amides arnottianamide, rubemamin, piperlonguminine, N-isopentyl-cinnamamide and fagaramide were found in the root bark. Other compounds reported in the root and the aerial parts were diosmin, zathoxylol, hesperidin, fagarol, lupeol, β-amyrin, β-sitosterol, stigmasterol and

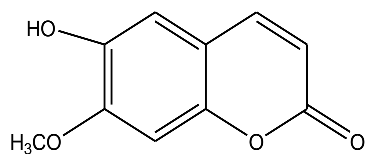


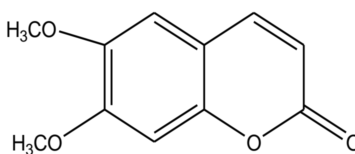


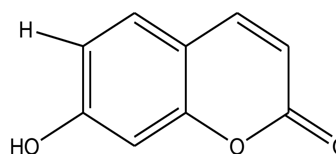


benzophenanthridines fagaronine **furoquinoline skimmianine** **berberine**

2.12 **2.13** **2.14**

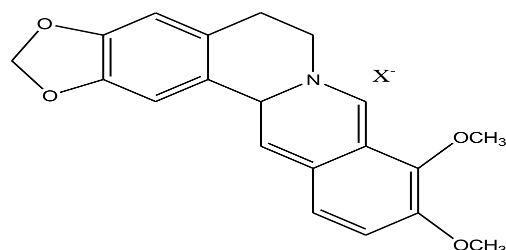


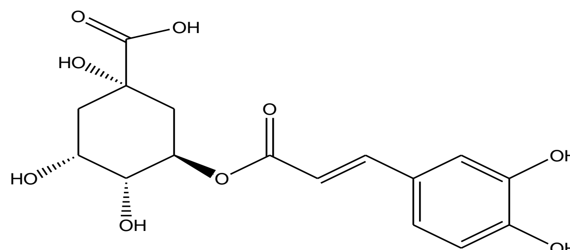




scoparone **scopoletin** **Umbelliferone**

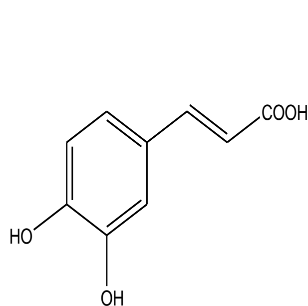
2.15 **2.16** **2.17**

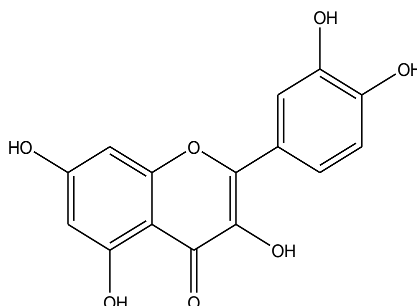


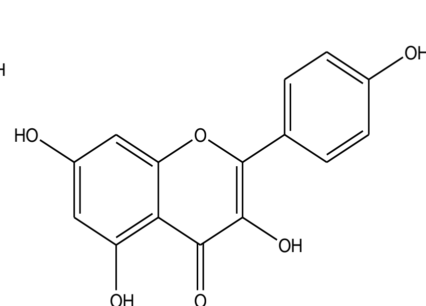


parahydroxybenzoic acid **chlorogenic**

2.18 **2.19**







caffeic **quercetin** **kaempferol**

2.20 **2,21** **2.22**

Figure 2.4: Structure of Compounds in *Z. zanthoxyloides*

campesterol. Coumarins isolated from the stem barks were: xanthotoxin, scoparone **[2.15]**, imperatorin, scopoletin **[2.16]**, marmesin, umbelliferone **[2.17]**, bergapten and pimpinellin. Adekunle et *al*., (2012) reported the presence of chlorogenic **[2.19]**, caffeic **[2.20]** (phenolic acids) and flavonoid compounds; rutin, quercetin **[2.21]** and kaempferol **[2.22]**. Monoterpene hydrocarbons (98.2%) are the only components of the leaf essential oil.

2.1.5 *Ceiba pentandra* (Bombacacea)

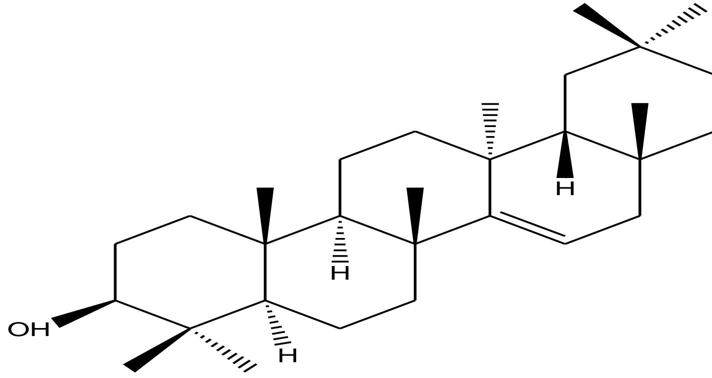
Ceiba pentandra (L.) Gaertn. (Syn *Bombax pentandrum* L.) of Bombacaceae family is a deciduous tall tree upto 60 m high, having short, sharp prickle all over the branches and the trunk, with a prominent buttress at the stem (Plate 2.5). The generic name is derived from a South American word while 'pentandra' is from Latin which means 5 stemmed or 'penta' (five) and 'andron' (male) in Greek. The common name includes white silk cotton tree, kapok tree, java kapok and cotton silk tree but locally called "Araba" in Nigeria by Yoruba tribes. The leaves are glabrous and digitate, each having 5-9 leaflets, alternate with slender green petioles 3-5 cm long, thickened at the base, apex expanded into almost circular disk. Flowers are bisexual, regular with strong but unpleasant scent. They cluster almost at the end of the twigs, colour ranging from white to red. There are 5 petals, calyx is cup-shaped with 5-10 shallow teeth, silky, with the outward densely hairy, also having 5 stemens longer than petals which joined together into a column at the bottom. Ovary is semi-inferior, 3-6 mm broad, 5-celled and style 2-4 cm long. Fruit is pendulous, oblong-ellipsoid capsule of length 10-30 cm. Pale yellow, white or grey floss originates from the internal wall of the fruit. Capsules of the seed split open along 5 lines, containing 120-175 dark brown seeds (Elumalai *et al*., 2012). The tree sheds its leaves during the dry season, blossom and bears fruits which is likely an adaptation that facilitates both mammal pollination and wind dispersal. This likely explains how the genus reached Africa from South America where they are believed to have originated.

Among the reported pharmacological activities of *Ceiba pentandra* are antioxidant, antifungal, antibacterial, antiinflammatory, antihelmintic, antidiarrheal, antiulcerogenic, hepatoprotective, hypoglyceamic, hypolipidaemic, angiogenesis, antihypertensive and

Plate 2.6*: Ceiba pentandra* tree (Chemistry Department, University of Ibadan, 7° 0.2 N/3° 28.8 E)

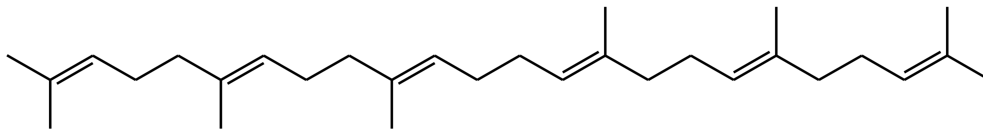
cytotoxicity (Nwachukwu *et al*., 1999; Elumalai *et al*., 2012; Aderogba *et al*., 2013; Oladimeji *et al*., 2015; Abouelela *et al*., 2018). Decoction of the boiled root is taken as medication for the cure of oedema. Tender shoot decoction is used as contraceptive. Cough and hoarse throat are treated with leaf infusion while compressed fresh leaves are used against dizziness. Other diseases reportedly treated with various part of the plant are constipation, peptic ulcer, fever, hypertension, diabetes, diarrhea, dysentery, arthritis, insect bites, bronchitis, headache and leprosy (Abouelela *et al*., 2018). The bark is used in Nigeria for curing microbial infections. Besides the plant's medicinal impotance, it is also grown as ornamental tree. The tree is also an important source of honey and foddder (containing 26% protein) for sheep, goats and cattle. The wood is used for making canoes and raft, furniture and matches, farm implements, pulp and paper products as well as constuction purposes. The seed oil which is similar to cottonseed oil is used in cooking, production of soap and as lubricant.

Iroka *et al.,* (2014) reported the presence of tannin, alkaloids, saponin, sterols, cyanogenic glycoside, flavonoid and phenols in the leaf, root and stem of *C. pentandra*. Minerals like calcium, sodium, phosphorus, potassium and magnesium were also reported (Osuntokun *et al*., 2017). Compounds that have been isolated from the plant include β-sitosterol-3-o-β-D-glocopyranoside, β-sitosterol, β-amyrin, 3β-taraxerol **[2.23]**, 3β-taraxerol acetate, oleic acid, 1-hexacosanol and all-trans-squalene **[2.24]**. Gas liquid chromatography analysis identified n-pentacosane (4.31%), n-docosane (4.71%), n-nonacosane (4.79%) and 3-ethyltetracosane (4.82%) as the major hydrocarbons in the unsaponifiable fraction of the parts above ground (leaves, petioles and young stems) of *C. pentandra*. Eight pure compounds: β-amyrin, β-sitosterol, β-sitosterol-3-o-β-D-glocopyranoside, 3β-taraxerol, 3β-taraxerol acetate, oleic acid, 1-hexacosanol and all-trans-squalene were also isolated from methylene chloride fraction of the extract (Abouelela *et al*., 2018).



3β-taraxerol

2.23



All-trans-squalene

2.24

Figure 2.5: Phytochemicals in *C. pentandra*

2.1.6: *Gliricidia sepium* (Fabaceae)

Fabaceae contains about 550 genera and 13000 species found all over the world. They are plant of great economic importance, being a source of food as well as timber used in furniture and construction purposes. They also provide dyes, gums, fodders, resin and more than 140 genera planted as ornamental plants. The family is divided into three sub-families which are Mimosaceae, Caesalpiniaceae and Papilonaceae (Mondal and Parul, 2011). The genus *Gliricidia,* comprises of a small number of taxa, native to tropical America, having *Gliricidia sepium* as one of its species, which is majorly cultivated as a shade and an ornamental plant.

Gliricidia sepium (Jacq.) Kunth also known as quickstick of Fabaceae (Leguminosae) and sub-family Papilionaceae is a small to medium-sized tree that grows up to 10-12 meters in height (Plate 2.6). The local name is "agunmaniye" (Yoruba). It has a smooth bark with colour varying from whitish grey to deep reddish brown. Leaves are odd pinnate, about 30 cm in length and contain 5-20 leaflets, ovate or elliptic, 1-3 cm wide and 2-7 cm long. The stem and branches are usually flecked with small white lenticels. Flowers are either bright pink or lilac, tinged with white and pale yellow spot at the base of the petals; calyx is glabrous, green and often tinged red. Mature fruit is yellowish-brown, while unripe fruit is green or reddish purple. Fruits are narrow, 10-18 cm long and 2 cm wide; seeds nearly round, with colour varying from yellowish-brown to brown (Hughes, 1987).

Gliricidia sepium is regarded as the second most important all-around legume tree, surpassed only by *Leucaena leucocephaia* (Daizy, 2007). It is propagated by seeds or cuttings. The plant is used as green manure, animal feed, shade, plant support and the wood as fuel. The bark and the leaves are applied to control rodents and insects when cooked with grains (Nazli *et al*., 2011).

The branches help to reduce fever and in the treatment of infections caused by *Trychophyton mentagrophytes*, *Neisseria gonorrohea* and *Microsporum canis* (Gupta 1995**;** Jasmine *et al*., 2017). In Costa Rica and Guatemala, the bark decoction is used as antibacterial and antiprotozoal agent. The leaves are used as mosquito repellants in Kerala,

Plate 2.7: ***Gliricidia sepium*** **plant (Saunders road, University of Ibadan, 7° 0.2 N/3° 28.8 E)**

India. Flowers are source of vegetables in Philippines and Central America. The plant is also used as antipyretic, antihistaminic, diuretic and expectorant. Phytochemicals from various part of *Gliricidia sepium* include melilotic acid, flavonoids, coumarins, coumaric acid, triterpenoids, saponins, stigmastanol glucoside and rhamnogalactoside of kaempferol (Griffiths, 1962; Rangaswami and Iyer, 1966; Manners and Jurd, 1979). Isolated and characterised compouds include gliricidin **[2.25]**, gliricidol **[2.26]** and 2-methoxygliricidol **[2.27]**, which exhibited activity against *Artemia salina* larvae, also oleanene glycosides from the root, vestitol and 2-*O*-methylvestitol from the bark as well as sepinol, (-)-isomucronulatol. Gentisic acid, gallic acid, ferrulic acid, syringic acid, vanillic acid, *p*-hydroxybenzoic acid, myricetin, β-resorcyclic acid, cis- and trans-sinapinic acid, protocatechuic acid, *m*-coumaric acid, *p*-coumaric acid, *o*-coumaric acid and coumarin, allelochemicals in the leaves extracts (Aulanni'am *et al*., 2021).

Essential oils from the leaves and flowers demonstrated significant activity against many bacteria. The bark oil also exhibited pronounced antimicrobial activity (Reddy and Jose, 2010). Ethanol extract likewise showed very good activity against Gram +ve organisms and fungi (Jasmine *et al*., 2017). The plant was reported to be active against bacteria and fungi causing dermatitis. Mineral constituents and nutritional factors of *G. sepium* leaf have also been accounted for. The leaf could serve as alternative material for supplementary feeding for livestock because it contains sufficient amounts of crude protein and minerals (Ab dulrazak *et al*., 1997). There was a report of weight gain and increase in milk production in small as well as large ruminants when fed with Gliricidia forage (Nochebueno and O' Donovan, 1986). Nitrogen, magnesium, calcium, potassium and phosphorus were present in the leaves, making them admirable green manure and fodder (Jones, 1996). Antimicrobial property of the plant has been exploited in many areas like preservation of both processed and raw food, alternative drug and conventional therapy (Lis-Balchin and Deans, 1997).

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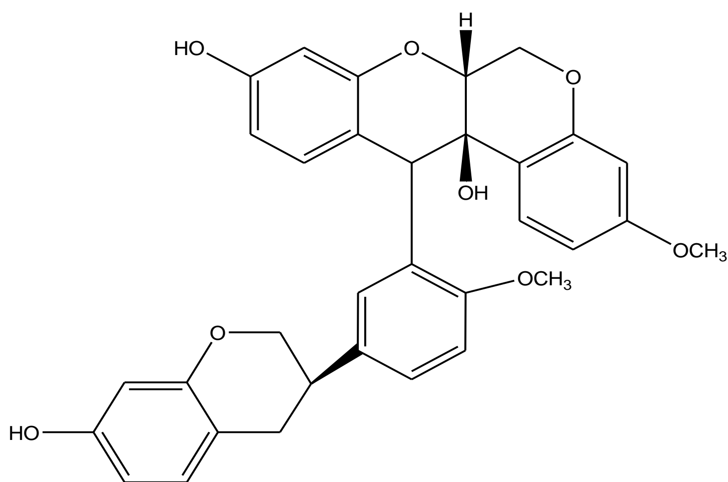
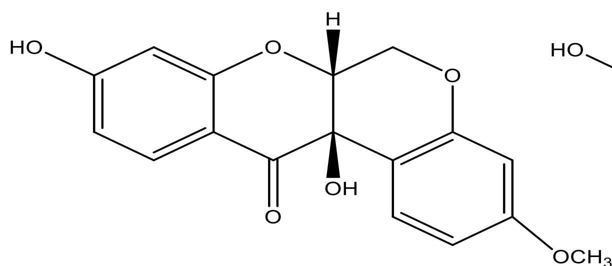
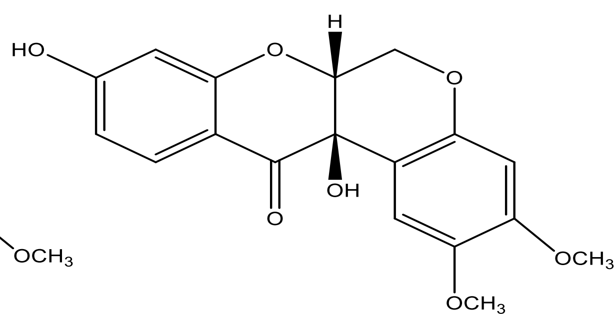


gliricidin

2.25





gliricidol **2.26**

2-methoxygliricidol **2.27**

Figure 2.6: Compounds from *G. sepium* that were active against *Artemia salina* larvae

2.1.7 *Delonix regia* (Fabaceae)

Delonix regia (Bojer ex Hook) Raffin (syn. *Poinciana regia*) is an umbrella-shaped tree reaching up to 40 feet high with large red-orange flowers, large trunk, buttressed and angled towards the base, greyish-brown smooth bark, sometimes slightly cracked (Plate 2.7). The plant belongs to the Fabaceae family and Caesalpiniaceae subfamily. It is usually called flame of the forest and the local name “sekeseke”. The leaves are alternate, bipinnate, light green, feathery, 20-60 cm long and having 10-20 pairs of major leaflets. The several leaflets are stalkless, round at the base and apex, slightly hairy and thin on both sides. The fruits are green and flaccid when young, but when matured the pod is hard, woody and turned dark brown. The pod length is between 30-70 cm, breadth is 5-7 cm and thickness of 3.8 cm with many horizontally partitioned seed chambers inside, which can be split into two parts. There are 30-45 seeds inside each pod, the seed is about 2 cm long, hard, grayish, glossy, oblong and transversely mottled with a bony testa which are arranged at right angles in the pod. The flowers are 5-13 cm wide with five equal petals, on a slim stalk of 5-7 cm long (Fatmawaty and Astuti 2013).

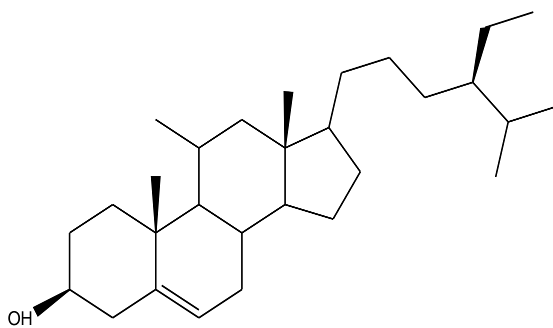
Phytochemicals screening of *D. regia* extracts showed the presence of flavonoids, saponins, alkaloids, carotene, hydrocarbons, phytotoxins, anthocyannin and carotenoids (Parekh *et al*., 2005; Fatmawaty and Astuti 2013). Isolated compounds from the stem bark include epilupeol, β -sitosterol **[2.28]**, lupeol, stigmasterol **[2.29]** and *p*-methoxybenzaldehyde with the extract showing good antimicrobial activities (Jahan *et al*., 2010). The plant also contains kaempferal, a flavonoid, having antiulcer property (Goel *et al.*, 1998) and quercetin **[2.30]**, which has the ability to elongate transit time in the small intestine of gastrointestinal system (Meli *et al*., 1990). Quercetin-3-rhamnoside, quercetin 3-glucoside (isoquercetrin), kaempferol-3-glucoside, kaempferol-3-rhamnoside (afzelin), kaempferol-3-rutinoside, quercetin-3-rutinoside and kaempferol-3-neohesperidoside were compounds reported from the leaf extracts (Azab *et al*., 2013). Stem bark, leaves, root bark, seeds and flowers of the plant are used in traditional medicine. Pharmacological activities include diuretic, anthelmintics, astringent and leucorrhoea (Maniruzzaman, 1993; Lawal *et al*., 2010).

Plate 2.8: ***Delonix regia*** **plant (Navic, 2016)**

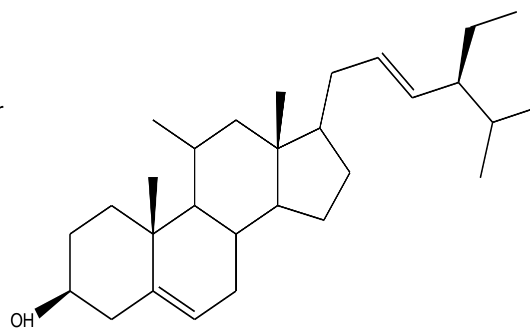
The flowers and bark extracts possess broad-spectrum antimicrobial and anti-inflammatory activity (Salem, 2013). Ahmad (2003) also reported a significant analgesic property of the bark and flower extracts. The plant also inhibited malaria parasite that infects humans. High concentration of polyphenol compounds including anthocyanines, phenolic acids and flavonols present in *D. regia* wood and bark are responsible for their antibacterial and antioxidant potentials (Einbond *et al*., 2003; Fatmawaty and Astuti 2013).

The flower ethanol extract showed good antioxidant activity and also inhibited β-lactamase producing both methicillin-sensitive and resistant *Staphylococcus aureus* (Aqil, 2005 *et al*., 2006). The flower ethylacetate extract exhibited molluscididal activity (El-Sayed *et al*., 2011). Chemical compounds reported in the flowers are anthocyanins (peonidin-3-*O*-glucoside, putunidin-3-*O*-acetyl-glucoside, cyanidin-3-*O*-rutinoside and cyaniding-3-*O*-glucoside), carotenoids (rubixanthin, lutein, β-carotene, zeaxanthin and β-cryptoxanthin) which are accountable for the vibrant colours of the flower petals (Adje *et al*., 2008; Veigas *et al*., 2012). The flower extract is a useful natural colour as well as acid-base indicator (Banerjee and De. 2001; Soltan and Sirry 2002). Other compounds isolated from flower ethanolic extract were β-sitosterol, β-stigmasterol, ursolic acid **[2.31]**, quercetin **[2.30]**, β-sitosterol-3-O-β-D-glucopyranoside, rutin, quercitrin, and isoquercitrin. The ethanol and non-polar extracts with the flavonoid rich portion and some isolated compounds exhibited cytotoxic activity against human liver cancer cell line (El-Sayed *et al*., 2011).

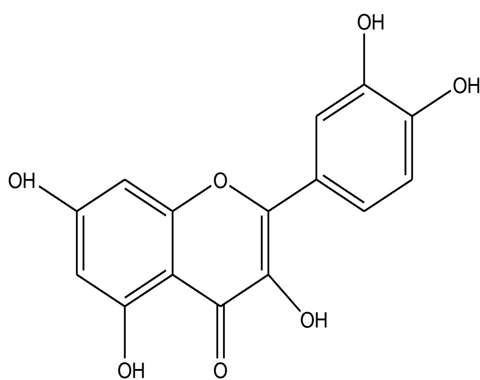
The flavonoid rich fraction also showed significant hepaprotective activity against tetrachloromethane induced hepatic cell damage in rats at 50 and 100 mg/kg dosage levels (El-Sayed *et al*., 2011). The leaves essential oil is fungitoxic, bark aqueous extract showed emetic effects in cats and monkeys while aqueous flower extracts are active against roundworm *Haemondus contorius* (Ragasa and Hofilena, 2011). The seed extract was reported to be a rich source of protein and also contains linoleic acid, palmitic acid and oleic acid. The seed extract is useful in food and nutraceutical applications (Barnaby *et al*., 2016).



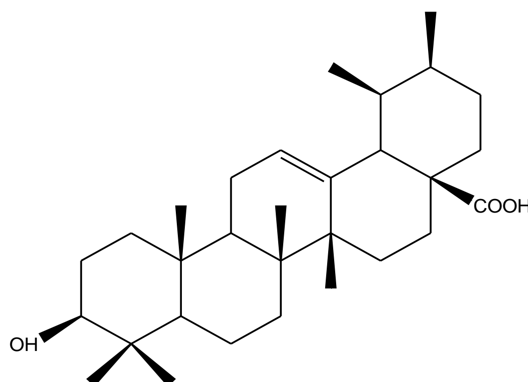
β-sitosterol
2.28



stigmasterol
2.29



quercetin
2.30



ursolic acid
2.31

Figure 2.7: Phytochemicals Isolated in *D. regia*

2.1.8 *Annona muricata* Linn (Annonaceae)

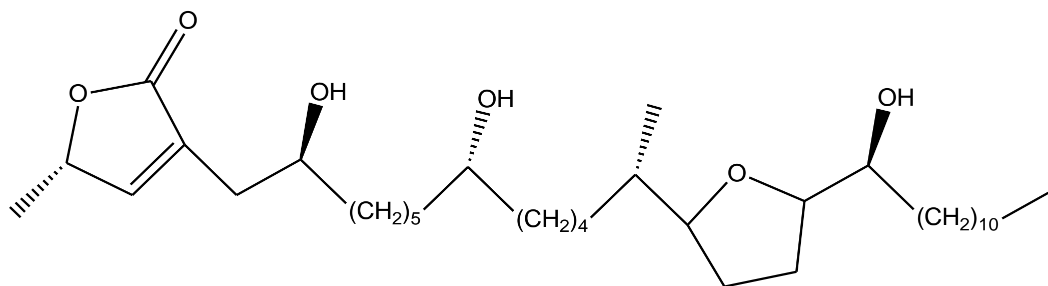
Annona muricata Linn, commonly called soursop or graviola and "ebo" in Yoruba belongs to the family of Annonaceae (Plate 2.7). It is a tropical semi-deciduous tree having the largest fruits among Annona genus. It is native to both tropical and subtropical areas in North and South America but at present distributed all through subtropical and tropical parts of the globe including Malaysia, India, Australia and African countries (Patel and Jayvadan, 2016). Graviola is a small, slender and cold-intolerant tree that normally reaches the heights of 4-8 m. It has flowers and can bear fruit after 3-5 years of planting. The flowers are borne singly and can appear anywhere on the twigs, branches or trunk. They are small stalked of about 4-5 cm long, pump and triangular-conical consisting 3 fleshy outer petals, yellowish-green in colour. The three close-set inner petals are light yellow. Leaves are glossy, dark green with a distinctive odour. The fruits are green, having curved spines and white edible juicy pulp. It has a sour or musky flavor and an aroma of pineapple, eaten fresh or processed as juices, custards, beverages as well as some dishes by the Caribbean, in South America and also Indonesia (Morton, 1987).

Annona muricata is a plant with long history of use in traditional medicine against a range of human diseases, particularly parasitic infections and cancer (Patel and Jayvadan, 2016). The fruit is used as remedy for fever, parasites, arthritis, neuralgia, diarrhea, malaria, dysentery, rheumatism, worms and skin rashes. After childbirth, the fruit is eaten by the mother to promote mother's milk. The leaves are utilised in the cure of insomnia, diabetes, headaches and cystitis. The leaf decoction gives anti-rheumatism and neuralgic effects when administered internally while the boiled leaves are used as remedy for rheumatism and abscesses (Leboeuf *et al*., 2006; Gajalakshmi *et al*., 2012). The crushed seeds exhibited antihelmintic activity against intestinal worms, external worms and parasites. In tropical Africa, *A. muricata* is used to treat coughs, skin diseases and also as pain killer, astringent, insecticide as well as pesticide. The plant is also used as remedy for asthma, diarrhea, hypertension, parasites and worms in West India (Patel and Jayvadan, 2016). The root bark and leaves extracts demonstrated antiphlogistic and antihelmintic activities (Morton, 1987).

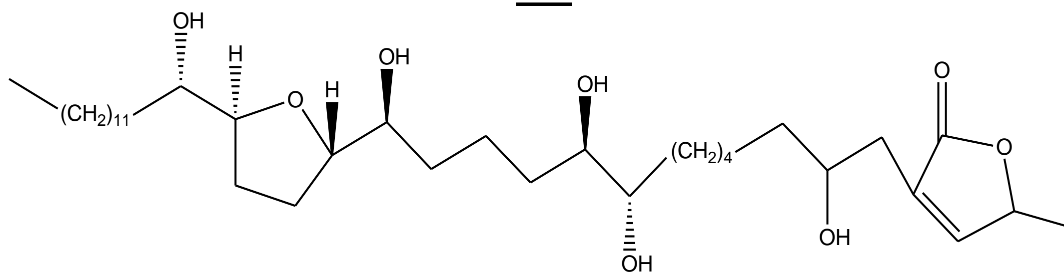
Plate 2.8*: Annona muricata* tree (Saunders road, University of Ibadan, 7° 0.2 N/3° 28.8 E)

Furthermore, smooth muscle relaxant, antispasmodic, sedative, hypoglycemic, hypotensive, antiplasmodial, antitumour, antiviral and anti-inflammatory effects are also ascribed to the leaves, barks and roots of the plant (Gajalakshmi *et al*., 2012; Yamthe *et al*., 2015; Roduan *et al*., 2019). The presence of copper, potassium, iron, magnesium, calcium and sodium as the major minerals in the fruit support regular consumption to provide essential nutrients to the human body.

Extensive phytochemical analysis of various parts of *A. muricata* confirmed the presence of diverse phytoconstituents which include alkaloids, phenolics, cyclopeptides, flavonol, triglycosides, megastigmanes and essential oils (Patel and Jayvadan, 2016). Some isolated compounds from the fruit and leaves of *A. muricata* includes annonaine **[2.32]**, nornuciferine, asimilobine (alkaloids with anti-depressive activity), epomusenin-A and B, epomurinin-A and B, muricin J, muricin K, muricin L, annomuricine A, B, C, E **[2.33]**, annohexocin, muricatocin A and B (annonaceous acetogenin with anticancer activity). Many compounds which showed anticancer properties were also obtained from the seeds, root and stembark. Previous investigations on *A. muricata* revealed antiviral, antioxidant, antiparasitic, antirheumatic, antihypertensive, anti-inflammatory, antinociceptive, antihyperglycemic and antiproliferative effect of different extracts from various parts of the plant (Rieser *et al*., 1996; Gleye *et al*., 1997; Baskar *et al*., 2007; Roslida *et al*., 2010; Hamid *et al*., 2012; Nwokocha *et al*., 2012). The leaves extract induced apoptosis in lung and colon cancer cells. The extract also inhibited the migration and invasion of colon cancer cells. It was also established in the earlier study that the plant is effective against cancer cell lines which were multi-drug resistant (Torres *et al*., 2011). Phytochemical investigations of *Annona muricata* showed that annonaceous acetogenins are the main chemical constituents of the plant and over 100 of this class have been reported from the fruits, leaves, seeds, barks and roots (Moghadamtousi *et al*., 2015). Acetogenins are the alkaloid compounds believed to be responsible for the excellent anticancer potentials displayed by the plant extracts, however, other compounds might have contributed to this property. Besides the health benefit of *A. muricata*, the plant has neurotoxic alkaloids linked to neurological effects when consumed regularly or in large amount (Torres *et al*., 2011).



Annonaine
2.32



Annomuricine E
2.33

Figure 2.8: Alkaloids in *A. muricata*

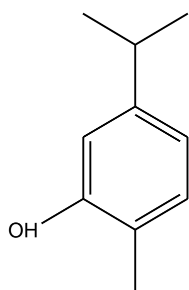
2.2 Essential Oils

Essential oils according to European Pharmacopoeia, are odorous products, commonly of complex composition, extracted from plant matter either by steam distillation or any suitable mechanical process which does not require heat. They are complex mixture of naturally occurring compounds or secondary metabolites (Ekundayo, 1986). Essential oils are hydrophobic liquids which are aromatic and highly volatile. They are also known as ethereal or volatile oils. Essential oils are produced by aromatic plants as secondary metabolites (Russo *et al*., 2015). In addition, they are obtained from animal sources e.g musk and civet oils. Other lower plants like liverworts, mosses, seaweeds and some fungi also produce essential oils. They are produced in several plant organs like buds, leaves, seeds, stem, flowers, twigs, root, bark as well as fruits and stored in secondary cells, epidermic cells, cavities, canals or glandular trichomes. They are mostly obtained through distillation process (Sharma *et al*., 2021).

Essential oils are water-insoluble liquids but soluble in alcohol, ether, hexane and fixed oils. They are commonly colorless at room temperature. They have very high vapor pressure at room temperature and atmospheric pressure, therefore, they exist to a certain extent in the vapor state. Volatile oil rich in one compound could serve as a commercial source of such compound. Example is citrus oil that contains 90 % of limonene (Guillen *et al*., 1996). However, some volatile oils possess complex mixtures of different kinds of compounds e.g *Passiflora mollissima* fruit pulp essential oil contains 140 volatile compounds (Frohlich *et al*., 1989).

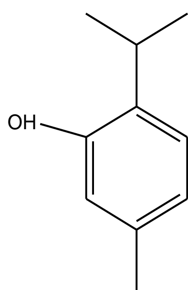
2.3 Factors Affecting Major Properties of Essential Oils

Plants producing essential oils are widely spread throughout the world. The early history of essential oils showed that there could be noticeable differences in the oils obtained from plants of the same species. These differences can affect major properties of the oil in the plant like composition, proportion and bioactivity. Some factors responsible for these differences include harvest period, extraction techniques, climatic and geographical conditions (Dhifi *et al*., 2016).



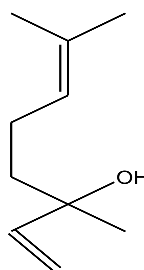
Carvacrol

2.34



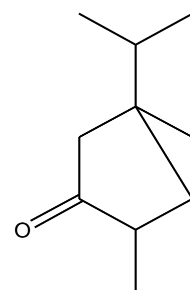
Thymol

2.35



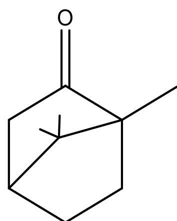
Linalool

2.36



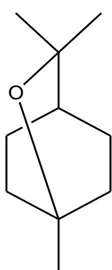
Thujone

2.37



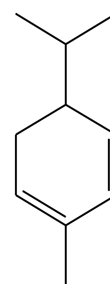
Camphor

2.38



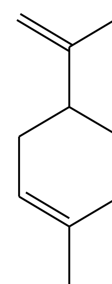
1,8-cineole

2.39



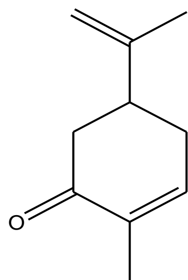
α-phellandrene

2.40



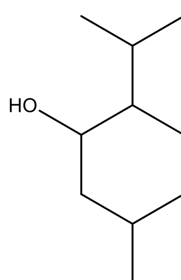
Limonene

2.41



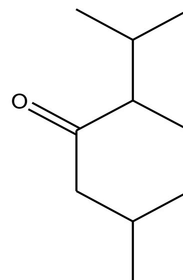
Carvone

2.42



Menthol

2.43



Menthone

2.44

Figure 2.9: Essential oil components

Plant organ where the oil is extracted, plant maturity and vegetative cycle stage, also can affect the quantity as well as the chemical composition of plant's volatile oil. Combination of these factors can offer a wide choice of yield and character change as the plant grows (Wilson, 1995).

2.4 Chemical Constituents of Essential Oils

Essential oils as earlier stated are mixture of compounds, which could be more than 100 components. The compounds may include monoterpenes, sesquiterpenes, diterpenes, low molecular weight branched, linear, unsaturated and saturated hydrocarbons, alcohols, acids, aldehydes, lactones or acyclic esters and occationally, nitrogen compounds, sulphur compounds and homologues of phenylpropanes (Frohlich *et al*., 1989). Essential oil components are broadly classified into two fractions:

Terpene fractions: The terpene fraction of essential oils are majorly monoterpenes and sesquiterpenes and sometimes diterpenes (Figure 2.9). Their oxygenated derivatives i.e aliphatic alcohols, esters and aldehydes may also be present.

Non-terpene fractions: These are hydrocarbons, fatty acids, apocarotenoids, sterols, waxes, lactones, sulphur and nitrogen containing compounds.

2.4.1 Terpenes

The word terpene originated from the English word "Turpentine" (Guenther, 1985). Turpentine is a resin of pine trees that contains acids and hydrocarbons known as terpenes built up from isoprene subunits. Thus, terpenes are unsaturated compounds of carbon and hydrogen with a distinct chemical and architectural structure relative to isoprene [**2.45]** molecule. Terpenoids (interchangeably used with terpenes) are modified terpenes with extra functional groups like oxygen (Sharma *et al*., 2022). Examples of terpenoids are carvacrol **[2.34]**, thymol **[2.35]**, linalool **[2.36]**, thujone **[2.37]**, 1,8-cineole **[2.39]**, carvone **[2.42]** and menthol **[2.43]**. Terpenes and terpenoids are the key constituents present in essential oils, which can either be acyclic (no ring), monocyclic (one ring), bicyclic (two rings) and tricyclic (three rings) giving rise to structural variations of the same empirical formula. Many of these components are unstable and are easily converted to another

compound by intramolecular rearrangement. As a result, terpenes are known to be the largest group of plant natural products. Generally, terpenes have names ending with "ene." For examples: α-phellandrene **[2.40]**, limonene **[2.41]**, α-bulnesene, α-pinene **[2.50]**, β-selinene [**2.51]** etc. Terpenes occur principally as hydrocarbons, aldehydes, ketones, ethers, carboxylic acids, esters, alcohols and their glycosides. Terpenes are classified based on the number of isoprene units in their carbon-carbon chain (Connolly and Hill, 2005) and they act as the principal odour carriers in essential oils (Pinder, 1996). Many plants like eucalyptus, lemon grass, roses, sage, thyme and balm tree are known for their spicy taste, sweet smell and pharmacological properties. They are able to show these potentials because of terpenes present in them. Plants also produce terpenes to expel certain animals that want to take them as food or attract some insects for pollination purpose. They as well serve as growth regulators in plants.

2.4.1.1 Classification of terpenes

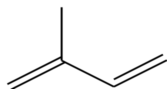
Hemiterpenes (C_5H_8)

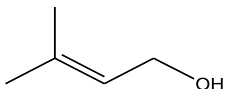
Hemiterpenes consist of one isoprene **[2.45]** unit. The only known hemiterpene is isoprene itself while hemiterpenoids i.e oxygenated derivatives of hemiterpene are prenol **[2.46]** and isovaleric acid **[2.47]**.

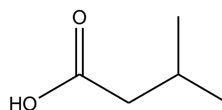
Monoterpenes ($(C_5H_8)_2$)

Monoterpenes are unsaturated hydrocarbons which are made up of two isoprene unit (C_{10}). They are compounds present in many plants' volatile oils including herbs, vegetables and spices. Their oxygenated derivatives are known as monoterpenoids. They can be linear e.g geraniol [**2.48**] and citronellol [**2.49**] or cyclic molecules: monocyclic- menthone **[2.44]**, limonene **[2.41]** or bicyclic-camphor **[2.38]** and α-pinene **[2.50]**. Geranyl pyrophosphate is the precursor for the monoterpenes. Aliphatic monoterpenes are converted to cyclic monoterpenes by a mechanism with many reaction steps which involve a universal intermediate, a terpinyl cation, which can undergo a lot of transformation into several compounds. From experimental reports, monoterpenes are regarded as effective, non-toxic dietary antitumorigenic agents (Loza-Tavera, 1999). These compounds also contribute to the aroma and flavor of plants from where they are isolated, therefore, they are used as

Hemiterpenes





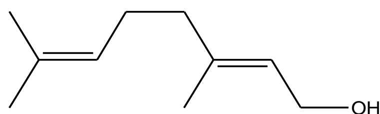


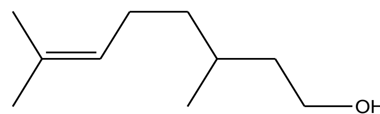
Isoprene **2.45**

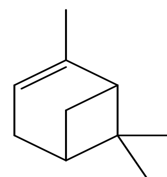
Prenol **2.46**

Isovaleric acid **2.47**

Monoterpenes





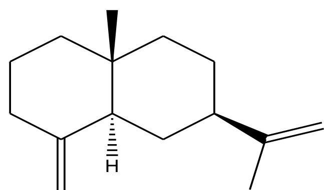


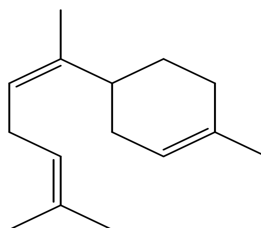
Geraniol **2.48**

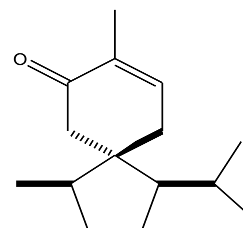
Citronellol **2.49**

α-pinene **2.50**

Sesquiterpenes





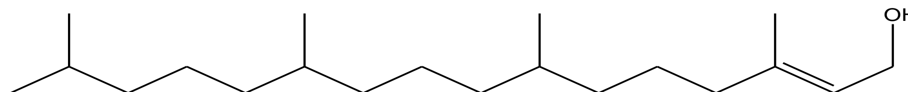


β-selinene **2.51**

α-bisabolene **2. 52**

Acorenone **2.53**

Diterpene



Phytol **2.54**

Figure 2.10: Examples of Terpenes

fragrance. Examples are geraniol **[2.48]**, nerol and citronellol **[2.49]** in rose oil as well as citra in lemon oil. They enhance the therapeutic values of other components like thymol, which possesss antimicrobial property making thyme and basil oils useful in herbal medicines.

Sesquiterpenes $(C_5H_8)_3$

Sesquiterpenes are biogenetically derived from farnesyl pyrophosphate. These compounds contain three isoprene units (C_{15}) which may be linear, monocyclic or bicyclic (Fraga, 2006). They have similar properties to monoterpenes but are less volatile (Dewick, 2009). They are made up of a very large group of secondary metabolites and some have shown to be stress compounds formed because of disease or injury. They are often used as fixatives in the perfume industry (Ansari and Curtis 1974). Medicinal activities of sesquiterpenes include anti-inflammatory, antiplasmodial, anticancer, painkiller, and antibacterial (Cox-Georgian *et al*., 2019) Examples include β-selinene **[2.51]**, bisabolene **[2.52]** and acorenone **[2.53]**.

Diterpenes $(C_5H_8)_4$

Diterpenes constitute four units of isoprene molecules (Hanson, 2000) with molecular formula $C_{20}H_{32}$. They are biosynthesised by plants, fungi and animals through HMG-CoA reductase pathway involving a primary intermediate known as geranylgeranyl pyrophosphate. They are generally found in resins like abietic acid. Diterpenes are heavy molecules hence they are difficult to extract from plants using steam distillation process for they require more energy to go into vapour state. Thus, longer distillation times are necessary to recover them from plants. Examples are phytol **[2.54]** and gibberellins (plant hormones) which occur as a side chain on chlorophyll are diterpene derivatives.

Diterpenes have many medicinal benefits which include anti-inflammatory, antitumor, cytotoxic, antispasmodics, antoxiolytics and in certain sedatives. (Cox-Georgian *et al*., 2019). They also possess antimicrobial and anti-inflammatory properties (Breitmaier, 2006; Tirapelli *et al*., 2008). Like sesquiterpenes, diterpenes can be linear (geranylcitronellol and phytol in essential oils), monocyclic (camphorene present in camphor oil), bicyclic (manool and manoyl oxide found in pine oils) or tricyclic (kaur-15-ene and phyllocladene also encountered in essential oils) (MacMillam and Beale, 1999; Hanson, 2005).

Sesterterpenes $(C_5H_8)_5$

Sesterterpenes consist of 25 carbons and five isoprene units. They are majorly found in marine organisms, fungi and plants. Many of them possess promising biological properties e.g anticancer, nematicidal, antifungal and antibacterial (Evidente *et al*., 2015).

Triterpenes $(C_5H_8)_6$

Triterpenes consist of 30 carbon atoms or six isoprene units with tail-to-tail arrangement in the center and with the general formula $C_{30}H_{48}$. Majority of triterpenes are derived from squalane, two farnesyl pyrophosphate units linked in the tail-to-tail manner. They include steroids and sterols (Cox-Georgian *et al*., 2019).

Sesquarterterpenes $(C_5H_8)_7$

They composed of seven isoprene units with the general formula $C_{35}H_{56}$. They are usually microbial in their origin e.g tetraprenyl curcumene.

Tetraterpenes $(C_5H_8)_8$

Tetraterpenes are also known as carotenoids. There are about 200 naturally abundant tetraterpenes. They are made up of 40-C atoms or eight isoprene residues with the molecular formula $C_{40}H_{56}$. Most of them represent structural variants or degradation derivatives of β-carotene. Examples of biologically active tetraterpenes are monocyclic γ-carotene, α-carotene and β-carotene. Carotenoids (oxygen derivatives) occur in the leaves, shoots and roots of all higher plants. They serve as color filters for photosynthesis in the leaves of plants (Singh and Sharma, 2015).

Polyterpenes $(C_5H_8)_n$

Polyterpenes are $(C_5)n$ where n is more than 8. Polyterpenes are polymers containing large number of isopentenyl unit (i.e polyisoprene). Polyisoprene that have *cis* double bond are natural rubber. There is also polyisoprene with *trans* double bonds e.g gutta-percha.

2.5 Biosynthesis of Terpenes

2.5.1 Mevalonic Pathway

Terpenes are derived biosynthetically from isoprene **[2.45]** units with molecular formula C_5H_8. Isoprene units are synthesised from acetyl-CoA through mevalonic acid pathway.

Three molecules of acetyl-CoA are joined together in stepwise manner to give a six-carbon intermediate known as mevalonic acid **[2.56]**. The mevalonic acid is then pyrophosphorylated using 2 ATP molecules to produce mevalonic acid pyrophosphate (MVA-PP). Dehydration and decarboxylation of MVA-PP yields activated 5-C unit called isopentenyl pyrophosphate (IPP) **[2.57]** or isopentenyl diphosphate which isomerises to another activated 5-C units dimethylallyl pyrophosphate (DMAPP) **[2.58]** or dimethylallyl diphosphate. Both IPP and DMAPP are building blocks of terpenes. They are linked together "head to tail" to form linear chains or rings. In the presence of metal ions, the enzyme isopentenyl pyrophosphate isomerase catalyses reaction between IPP and DMAPP to produce geranylpyrophosphate (GPP, 10-C compound), farnesyl pyrophosphate (FPP, 15-C compound) and geranylgeranyl pyrophosphate (GGPP, 20-C compound); precursors for mono-, sesqui- and di-terpenes respectively. FPP dimerises to form 30-C compound that produce squalene (a precursor of triterpenes and steroids) by elimination of two pyrophosphate groups. GGPP also dimerise to form 40-C compound, which produce phytene (a precursor to tetraterpenes) after elimination of two pyrophosphate groups (Singh and Sharma, 2015).

2.5.2 Deoxyxylulose phosphate (DOXP) pathway/ Methylerythritol phosphate (MEP) pathway

Methylerythritol phosphate (MEP) pathway or Deoxyxylulose phosphate (DOXP) pathway is also recognized as mevalonic acid-independent or non-mevalonate pathway. It takes place in the plastids of apicomplexan protozoa, plants and also in many bacteria. In this pathway, DOXP synthase and DOXP reductase converts pyruvate glyceraldehyde 3-phosphate to 1-deoxy-D-xylulose 5-phosphate **[2.55]** and 2-C-methyl-D-erythritol 4-phosphate respectively. It is also converted to 2-C-methyl-D-erythritol 2,4-cyclopyrophosphate (MEcPP) with the help of a catalytic enzyme. MEcPP later changes to (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) by action of HMB-PP synthase. HMB-PP is transformed to dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) by HMB-PP reductase. Isotope incorporation studies using bacteria, algae, plant cell cultures and tissues have confirmed the formation of IPP and DMAPP (the two terpenoids precursors) from exogenous 1-deoxyxylulose.

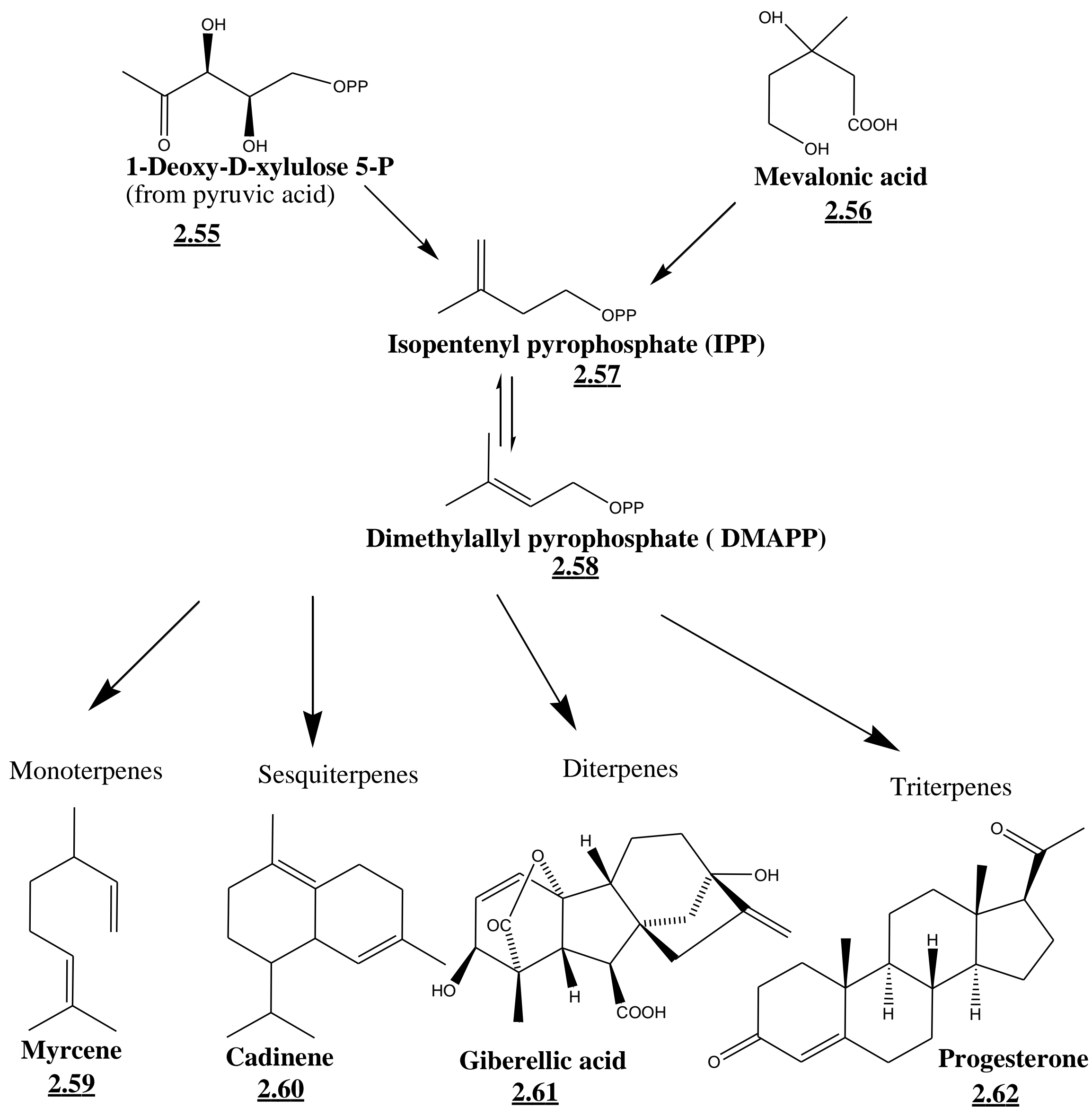


Figure 2.11: Terpenes biosynthesis

Many bacteria including human pathogens, green algae and possibly *Plasmodium falciparum* rely mainly on this pathway (Eisenreich *et al*., 2001).

The end product in both pathways are IPP and DMAPP (Figure 2.11), which are precursors to isoprene, monoterpenes, sesquiterpenes, diterpenes, carotenoids, plastoquinone and chlorophylls.

2. 6 Intramolecular Rearrangement of Terpenes

Volatility and thermolabile nature of terpenoids makes them to easily undergo oxidation or hydrolysis depending on their structure (Scott, 2005). Exposure of essential oils to heat, light or air can lead to isomerisation, oxidation, dehydrogenation, polymerisation or thermal rearrangements and thus converting its components from one form to another. This intramolecular rearrangement of terpenes is known as essential oil degradation. This is because conversion of one or more constituent in an essential oil to another compound by oxidation or any other reaction above is likely to have a strong effect on the odour, colour as well as the biologial activities of the oil.

Different cyclisation of terpinyl cation give rise to bornane-, camphene- and fenchane-type monoterpenes. Thujane-type and monoterpenes with a cyclopropane ring in a bicyclo [3.1.0] skeleton like sabinene, sabinol, isothujanol, α- and β-thujone are obtained from the terpinen-4-yl cation directly or via the sabinyl cation. All these are regular constituents found in many essential oils. Hydrolysis of carbon-oxygen bond of geranyl pyrophosphate (monoterpenoids' precursor) gives geranyl carbocation **[2.63]** that undergo many reactions to produce different monoterpenoids (Figure 2.12). Reaction of geranyl carbocation and water gives geraniol, which later converted to citral by oxidation. Isomerisation of geranyl carbocation gives linalyl carbocation **[2.64]** which loses proton to generate myrcene **[2.65]**. It also isomerises to acyclic hydrocarbons, α-terpinyl carbocation **[2.66]** which is later converted to terpinen-4-yl cation **[2.67]** by hydride shift and terpinen-4-yl cation to thujyl cation **[2.68]** by C2-C6 ring closure and afterward converted to sabinene **[2.69]** by loss of one molecule of hydrogen. Linalyl cation can also undergo intramolecular electrophilic addition giving rise to a monocyclic carbocation that can lose one hydrogen ion to give limonene or react with water to produce α-terpineol **[2.70]**. Another intramolecular addition

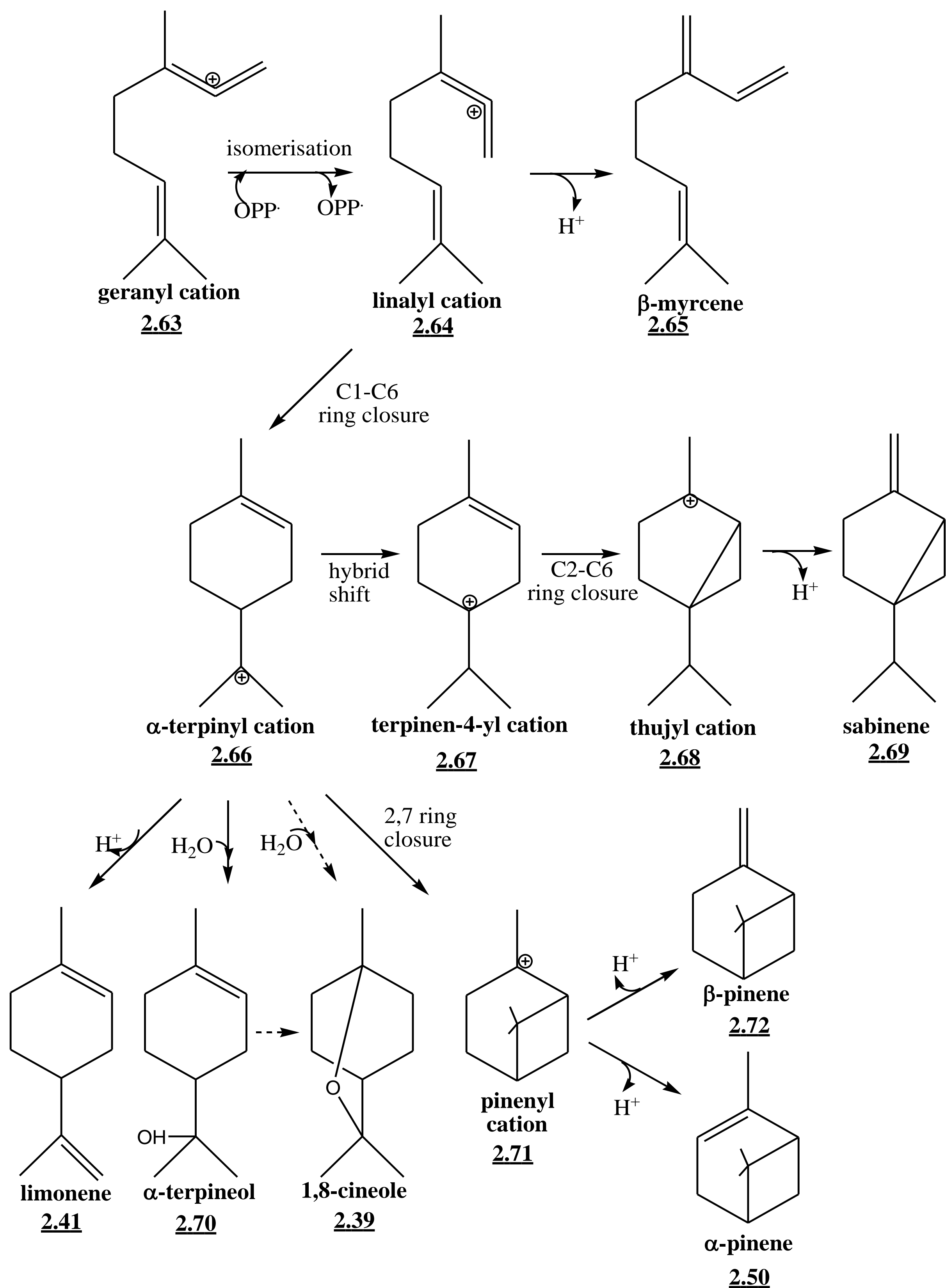


Figure 2.12: Intramolcular reaction of Terpenes

yields pinenyl carbocation **[2.71]** that can eliminate a proton to form either α- **[2.50]** or β-pinene **[2.72]**. Borneol and fenchone are also derived from pinyl carbocation. Oxidation of borneol gives camphor. Commonly encountered monoterpene hydrocarbons are formed by dehydration of alcohols, thus their occurrence in essential oils could be as artifacts from method of extraction. Alpha pinene is readily converted to menthol and rose oxide (Breitmaier 2006).

2.7 Non-terpene Essential Oils Components

The non-terpene constituents of essential oils include straight chain or cyclic hydrocarbons, heterocyclic compounds, aromatic compounds, waxes and apocarotenoids (Dhifi *et al*., 2016). The most commonly reported are the fatty acids (linoleic acid and palmitic acid), esters (ethyl palmitate and methyl salicylate), alkanes (eicosane and pentacosane), aldehydes (hexanal and nonanal), alcohols (1-octen-3-ol and octanol) as well as benzene derivatives (benzaldehyde and ethylbenzene),

Esters

Important esters found in essential oils include methyl salicylate, benzyl benzoate and benzyl isobutyrate. Most of them are perfumery ingredients (Dhifi *et al*., 2016).

Lactones

Lactones are cyclic esters found in many essential oils. They are derived from lactic acid ($C_3H_6O_3$). Lactones contain heterocyclic oxygen next to a carbonyl functional group in a three or more membered ring that is either saturated or unsaturated. Some of the lactones reported in essential oils are jasmolactone, δ-2-decenolactone and γ-decalactone. Coumarins are also types of lactones, which are naturally occurring lactone found in hay and tonka beans and also in essential oils from plant family like Rutaceae, Lamiaceae, Asteraceae and Apiaceae (Lončar *et al*., 2020).

Phthalides are lactones of 2-hydroxymethyl benzoic acid and are also refer to as benzofuran derivatives. They are found in essential oils of celery, lovage and angelica of the family Apiaceae (Perineau *et al*., 1992; Sajjadi *et al*., 2013). Examples are 3-butylphthalide and 3-butylhexahydrophthalide.

Nitrogen Containing Constituents

Nitrogen containing constituents found in essential oils include indole, pyridines, pyrazines, quinoline and methyl quinolines. Pyridines and pyrazines were detected in black oil and vetiver oils (Clery *et al*., 2005) while quinoline and methyl quinoline are present in fig leaf.

Phenylpropanoids

Phenylpropanoids are biosynthesized by the shikimic acid pathway through the amino acid *l*-phenylalanine to generate trans-cinnamic acid which is converted into a variety of phenylpropanoids by the action of various enzymes (Croteau and Karp, 1991). Phenylpropanoids consist atleast one fragment of C_6-C_3, in which the C_6 unit is a benzene ring. Examples of phenylpropernoids found in essential oil are safrole, vanillin, isoeugenol, eugenol and cinnamic aldehyde.

Sterols

Examples of sterol reported in the literature include 3-β-9,19-cyclolanost-24-en-3-ol and fucosterol in *Vigna unguiculata* (L) Walp oil (Abdel-Karim *et al*., 2016).

Apocarotenoids

Apocarotenoids are organic compounds resulting from the oxidative cleavage of double bonds in the carotenoid molecule. They are found widely in living organisms. They are also formed by chemical reactions in foods that contain carotenoids, catalyzed by carotenoid oxygenases. Carotenoid oxygenases cleave many carotenoids into a variety of biologically significant products. Examples are apocarotenoids (found in plants) and retinoids (present in animals). Apocarotenoids function as flavours, pigments, floral scents, hormones and defense compounds in plants, while retinoids are vitamins, visual pigments and signaling molecules in animals (Wyss, 2004).

Carotenoids are a family of tetraterpenoids characterised by tail-to-tail combination between two fragments of diterpenoid. The two main families of apocarotenoids are the ionones and damascones. The ionones are found in fruits, flowers and leaves of aromatic plants. They are important materials in perfumery. The damascones also are found naturally in many aromatic plants. Even when present at low percentage, they contribute largely to the odours of the oils containing them.

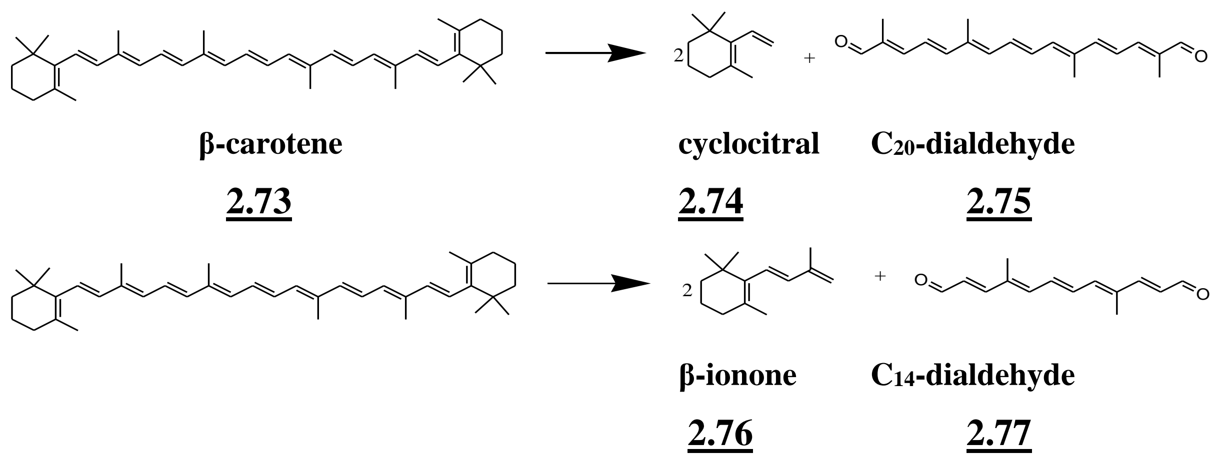


Figure 2.13: Oxidative cleavage of Carotenoid

Example of carotenoid degradation is the degradation of the central part of β-carotene [2.73] chain which, led to a number of fragments (Figure 2.13), like cyclocitral [2.74] and β-ionone [2.76] found in plant essential oils alongside with other products C_{20}- [2.75] and C_{14}-aldehyde [2.77] respectively.

2.8 Extraction of Essential Oils

Plants' essential oils are extracted using a variety of methods which include expression, solvent extraction, carbon dioxide extraction, enfleurage, hydrodistillation, steam distillation, microwave distillation, solid phase micro-extraction, supercritical fluid extraction, simultaneous distillation extraction, headspace sampling and microwave irradiation process.

2.8.1 Expression

Expression or cold pressing is an extraction method which is used to obtain essential oils from only citrus. The term expression refers to any non-mechanical process in which the essential oil glands in the peel are broken or crushed to release the oil (Pavia *et al.*, 1976). In the procedure, the citrus fruit is cut into two, and the pulp removed with the aid of sharpened spoon-knife (known as a *rastrello*). The oil is then separated from the peel either by pressing the peel against a hard object of baked clay (*concolina*) which is positioned under a large natural sponge or by bending the peel into the sponge. The oil emulsion absorbed by the sponge is removed from the sponge by squeezing it out into the *concolina* or some other container. Essential oil derived by this method contains more of the fruit odour character than using any other method of extraction.

Another method that resemble cold pressing is called equaling (or scodella method). It uses a shallow bowl of brass or copper with a hollow pipe at the center; the equaling tool is similar in shape to a shallow funnel. The bowl is equipped with brass points having blunt ends across in which the whole citrus fruit is rolled by hand with pressure until all of the oil glands have ruptured. The oil and aqueous cell contents are allowed to drip down the hollow cental tube into a container from which the oil is separated by decantation. Hand pressing is a very slow process e.g. on average only 2-4 lbs oil per day can be produced by

a single person using one of these hand methods, therefore, a number of machines have been designed over the years to extract volatile oils from citrus either by crushing the peels of the citrus fruit or crushng the whole fruit before separating the oil from the juice.

2.8.2 Solvent extraction

Solvent extraction is preferred when the odorous properties of plant materials like flower will be damaged or altered by steam distillation. The plant material is soaked in a hydrocarbon solvent to help dissolve the essential oil. The solvent also dissolves other plant constituents including fatty acid and waxes. The solution is filtered and concentrated by distillation to give a resin (resinoid), or a mixture of essential oil and wax also called concrete. Pure alcohol is used to extract the oil from the concentrate as the fatty acids and the waxes are insoluble in alcohol. Alcohol is separated from the essential oil by distillation at low temperature to avoid degradation of the latter. This method is not the best method of extracting essential oils because small amount of residue is retained in the oil leading to allergies and affecting immune system when used (Tooley, 1971).

2.8.3 Carbon dioxide extraction

Supercritical fluid extraction is a type of carbondioxide extraction which involves separation of one component from another using supercritical fluid as the extracting solvent. The most commonly used supercritical fluid is carbon dioxide and sometimes modified by adding solvents like ethanol and methanol.

Essential oil can be readily extracted with liquid CO_2 under pressure from all dry plant materials. There are similarities between carbon dioxide and other solvents used for extraction except that CO_2 can be used as subcritical liquid as well as a supercritical liquid. At some elevated pressure, CO_2 changes from gas to liquid, but when the pressure is raised to a critical point, it remains unchanged from a gaseous fluid to a liquid regardless of the exerted pressure. This is called a supercritical phase, whereas other liquid solvents are used only as subcritical phase.

The extraction of volatile concentrates of dried aromatic plants under subcritical condition is a commercially viable substitute of the conventional steam distillation because the

operating condition of 50-80 bar pressure and 0-10 ℃ are quite readily maintained in moderate pressure equipment (Moyler and Stephens, 1992). Many essential oils obtained by carbon dioxide extractions have their constituents unaltered than those extracted by steam-distillation since the procedure is carried out at lower temperature which preserves the original oil composition and properties (Irena *et al*., 2001). Hence, the former have fresher, cleaner and crisper aromas. As a result of this, many essential oils which composition and properties would be affected by steam distillation are obtained from plants by means of carbon dioxide extraction. The oil obtained from this method also possesses an aroma more similar to the starting materials from which it was derived (Aziz *et al*., 2018).

2.8.4 Enfleurage

An enfleurage is a rigorous and local method of extracting essential oil from flowers. The process involves covering the petals of flowers with a layer of fat. The fat absorbes essential oils from the flowers and alcohol is added to isolate the oil from the fat. The alcohol is then separated from the essential oil by distillation (Ibrahim *et al*., 2021).

2.8.5 Hydrodistillation

The plant material is boiled inside water and both steam and oil vapour from the boiling plant sample flow into a cooling tank. As they condense, the essential oil being lighter than water, stays above the condensed water. The water is referred to as plant-water essence or hydrosol. The temperature of distillation should be kept constant at 100ºC so as to prevent the plant materials from being damaged by overheating. The pressure in the still should be atmospheric. The duration for distillation depends on the nature of plant materials. The oil is then extracted from the hydrosol by organic solvent such as ether or hexane, giving a two phase system of water and the organic layer. The oil will dissolve in the organic layer. After separating the organic layer from the water layer, anhydrous sodium sulphate or magnesium sulphate is added to the organic layer to remove water molecules that may still be present in it (Sandra and Bicchi 1987). The advantage of this method includes the use of less steam and shorter extraction time compared to the steam distillation and higher yield (Ahmad *et al*., 2015).

2.8.6 Steam distillation

This method is different from hydrodistillation because the steam alone is allowed to come in contact with the plant material and not the water. The plant sample is placed inside a flask where pressurised steams are allowed to pass through the plant material. The steam is prepared in a separate chamber (steam generator) and directed into the flask. The heat burst open the globules of oil in the plant and both steam with the vapour of the essential oil then passed out through the top of the flask into a water cooled pipe which helps to condense both the oil vapour and the steam. At this point, the volatile oil separates from the water similar to hydrodistillation, using organic solvent like ether. Anhydrous magnesium sulphate is added to remove the unwanted water molecule. The essential oil is then filtered and kept in a vial. This method is more expensive than the other methods but good for plants that contain high boiling point essential oils. Other distillation methods used are vacuum distillation and vacuum degassing (Božović *et al*., 2017)

2.8.7 Solid phase micro-extraction

Solid phase micro-extraction is a technique that involves the use of a fiber covered with an extracting phase which can be either liquid (polymer) or solid (sorbent) (Spietelun *et al*., 2010). This phase extracts both volatile and non-volatile components from the plant materials (Mitra, 2003). This method is easy, rapid and can be done without solvents.

2.8.8 Simultaneous distillation extraction

The combination of the steam distillation with a simultaneous continuous extraction consists of a glass apparatus with two outlets in which one is connected to the aqueous medium and the other to low boiling solvent. The two separate flasks: one for the plant material and the other for solvent are connected through a condenser. The advantages of this combined method include the high concentration of the volatiles from a dilute solution, small amount of solvent used and reduction of thermal degradation using reduced pressure (Essien, 2007).

2.8.9 Microwave distillation

Microwave distillation is also known as microwave accelerated or microwave dry distillation. This method is good for the extraction of edible essential oils which are usually used in the fragrance, flavour and pharmaceutical industries as well as in aromatherapy. It combines microwave heating and dry distillation under atmospheric pressure without addition of any organic solvent or water. Isolation and concentration of the volatile constituents is performed in a single stage.

2.8.10 Headspace sampling

Extraction of essential oils by fats is gradually being replaced by headspace technique. Headspace sampling involves the extraction of volatile oils from flower in a distillation unit, coupled directly to a Gas chromatography or Gas chromatography/Mass spectroscopy. Studies of volatiles obtained by headspace sampling identify only the components responsible for the odour as perceived by human (Essien, 2007).

2.8.11 Microwave irradiation process

The microwave irradiation (or microwave assisted process) as reported by many authors is a technique for extracting volatile oils in order to get a very good yield in a short time (Collin *et al.*, 1991; Brosseau, 1997; Ghoulami *et al.*, 2001). The procedure includes using microwave to excite water molecules in the plant tissues. This causes the plant cells to rupture and then release the essential oils trapped in the plants' tissues (Belanger *et al.*, 1991).

2.9 Effects Extraction Methods on the Quality of Essential Oils

The essential oil quality is greatly influenced by the method of extraction. Therefore, the essential oils obtained from the same plant source but through different extraction methods may have completely different sensory properties even though they possess comparable odorous volatiles (Lemberkovics *et al.*, 2004). Furthermore, essential oils distillation at elevated temperature results in the transformation of the thermo-labile constituents to other compounds. Solvent extraction is generally used in the separation of heat-labile plant

materials, or if the volatile oil can be obtained only in low quantity. Thus, different extraction methods give rise to different number of chemical constituents and also different stereochemistry (Ibrahim *et al*., 2017).

2.10 Analysis of Essential Oils

The determination of exact composition of essential oils has been a serious challenge due to their complex nature. The rapid progresses in the techniques of spectroscopy and chromatography have made the study of essential oils chemistry simpler and more interesting. Chromatographic techniques are often used in the analyses of essential oil. Chromatography is a separation procedure that depends on the difference in the distribution of components of a mixture between a mobile phase and a stationary phase. The compound having greater affinity for the mobile phase or less affinity for the stationary phase will move faster than the compound with opposite properties. Those commonly used for essential oil analyses are gas chromatography and gas chromatography coupled with mass spectroscopy techniques (Wilkins and Madsen, 1991). The various type of chromatographic techniques employed in essential oils analyses are discussed as follows:

2.10.1 Thin layer chromatography (TLC)

The stationary phase is a finely divided absorbent material made up of silica gel, alumina or powdered cellulose. The absorbent is supported upon a glass plate. The mixture compoents is spotted at one end of the plate. The plate is developed in a closed container saturated with vapour of the Mobile phase. The plate is dried and then sprayed with a reagent for the determination of the separated constituents. This method is not often used for qualitative purpose due to the inability of the complex mixtures to separate very well. Thus, it is used for the control or monitoring of other chromatographic methods. An improved conventional preparative TLC is the Centrifugal Thin Layer Chromatography (CTLC), which makes use of centrifugal force to move solvent through the absorbent in contrast to the capillary action of the normal TLC (Forniss *et al*., 1980).

2.10.2 Liquid column chromatography (LCC)

In this chromatography technique, the stationary phase is a finely divided solid adsorbent packed in a glass column while the mobile phase is usually an organic solvent. The common adsorbents used as stationary phase are alumina, charcoal or silica gel. Column chromatography is useful for separating samples containing large compounds. The composition of the different components eluted from the column may be ascertained by gas chromatography or gas chromatography-mass spectrometry (Forniss *et al*., 1980).

2.10.3 High performance liquid chromatography (HPLC)

High performance liquid chromatography can be used to separate essential oil constituents into fractions, as preparation for analysis by another technique. It can also be used alone to analyse the non-volatile fractions present in the essential oil. The equipment consists of an injection port, a column, mobile phase reservoir, a pump, a detector, a chart recorder and an eluate collector. The sample to be separated or analysed is injected through the injection port into the column and the eluent which is the mobile phase is allowed to pass through the column under pressure. The components of the mixture are separated on the column by selective adsorption and carried by the mobile phase through the detector which sends the message to the chart recorder (Joseph, 2000). The eluates are then collected using suitable fraction collectors.

2.10.4 Gas chromatography (GC)

This method is known to be one among the best techniques used to identify the components of essential oil (Essien, 2009). When properly used, it can easily detect and identify the main constituents of essential oils, and also indicates the quality and authenticity of the oil. However, the technique has limitation of inability to detect minor components of essential oils (<0.01 %). GC usually carries out the separation of essential oil components with fused-silica capillary columns.

The chromatograph is made up of inlet where the samples are introduced into the column. At the inlet, the sample is heated to vapour at the heated injection port and mixed with the carrier gas before entering into the column. The oven temperature is controlled by a

thermostat and heating element. The vapourised sample is then swept into the column by the carrier gas. As the sample moves through the column, its components separate into individual bands in the carrier gas which then pass through the detector. The detector interprets these bands as electronic signals whose voltage is proportional to the quantity of each component in the gas stream mixture. The recorder plots this voltage as a function of time to give the gas chromatogram. The carrier gas is an inert gas such as hydrogen, helium or nitrogen (Dhifi *et al*., 2016).

2.10.5 Enantioselective Capillary Gas Chromatography (eGC)

Enantioselective GC is used to separate enantiomers with close retention times. Enantiomers are compounds that contain one or more chiral carbon atoms, thus exhibit optical activity (König, 1993).

2.10.6 Gas chromatography-Mass spectroscopy analysis (GC/MS)

The use of gas chromatography together with mass spectrometry allows fast and dependable identification of components of essential oils (Dhifi *et al*., 2016). The technique combines the features of gas-liquid chromatography and mass spectroscopy to identify different substances present in an unknown sample. The gas chromatography-mass spectroscopy instrument consists of two parts: The gas chromatography (GC) portion and the mass spectrometer (MS). The GC separates the constituents of the oil sample into pulses of pure chemicals while the MS identifies and quantifies the chemicals. The principle of GC-MS technique involves vapourisation of the samples and separation into different components (GC) followed by ionisation of the sample into different fragment ions and analyses of these individual components according to the molecular weight (mass/ charge ratio) (MS).

The GC-MS is the prime user of an automated data acquisition system. The vast number of spectra produced within a short time and the additional information contained in the consecutiveness of the data representing a dynamic record of the components of a mixture separated by a gas chromatograph cannot be exploited by any conventional recording system and manual evaluation of the resulting spectra. To ease the evaluation, GC-MS has

a computerised library from National Institute of Standards and Technology (NIST) and advanced search system. Mass spectrum of each compound detected can be taken immediately and compared with numerous mass spectra stored in the computer data (Essien, 2007).

2.11 Uses of Essential Oils

The various utilization and diverse biological properties of essential oils of plant origin are now gaining scientific interest. Chemical and biological activities of many herbal plants essential oils have been examined and utilized commercially (Maksimovic *et al*., 2008; Mohammadreza, 2008). Many industries make use of volatile oils in perfumery, cosmetics, ice creams, beverages, confectionary and food products as well as adding flavour and scent to other finished commodities of consumers (Guenther, 1985). About 10 percent of essential oils are commercially important. Many essential oils as well as their bioactive constituents like geranyl acetate, limonene or carvone are food preservatives and additives, hygienic products and also used in the treatment of diverse diseases in folk medicine systems. Few plant essential oils are now used in aromatherapy to cure dysfunction of organs.

Because essential oils are mixture of complex mixture of compounds which individually possess good biological activities, they have multibiological activities which is now of great interest to medicinal chemists. Some volatile oils showed better antimicrobial activity compared to synthetic antibiotics due to the presence of more than one active constituent which make them to exhibit broader spectrum of activity. A few have been found active against oral and dental microorganisms and thus can be incorporated into mouth washes to prevent bacterial aggregation (Yengopal, 2004a and b). Toxicity of *Croton cajucara* Benth essential oil to some bacteria and fungi associated with oral cavity diseases have been reported (Alviano *et al*., 2005). Besides, essential oils also possess interesting antiviral activities when compared to synthetic antiviral drugs. They also showed virucidal properties with low toxicity (Baqui *et al.,* 2001).

Some spices and herbs volatile oils like Satureja, thyme, sage and oregano have shown excellent antioxidant properties, therefore they can be used as alternative to synthetic antioxidants for the prevention of fats and oils oxidation and other correlated products.

Volatile oils are also used in the treatment of non-pathogenic diseases. For example, garlic essential oil helps to lower serum cholesterol and triglycerides while in patients with coronary heart diseases, the level of high-density lipoproteins is raised (Bordia, 1981). Some essential oils exhibited *in vivo* hypotensive activity and are therefore used in the treatment of hypertension. Essential oils and their individual fragrance components showed anticancer property against human cancer cells lines including leukaemia, glioma, breast cancer and others. The essential oil of lemon balm (*Mellisa oficinalis L*) was active against both mouse and human cell lines.

There was also report of essential oils insecticidal properties mainly ovicidal, larvicidal, growth inhibitor, repellence and antifeedant (Dale and Saradamma, 1981; Saxena and Koul, 1987; Isman *et al.,* 1990).

2.12 Antimicrobial Activities of Essential oils

Quite a large number of volatile oils are recognised to possess antimicrobial properties. This may be as a result of the presence of active constitutents mainly monoterpenes, sesquiterpenes as well as their related alcohols, phenols and other hydrocarbons (Wang *et al*.,2005)**.** Lipophilic nature of their hydrocarbon chain and hydrophilic character of their functional groups are very essential for essential oil constituents to display antimicrobial activity. Carvacrol and thymol, having phenolic structures, are very active against wide range of microorganisms (Khameneh *et al*., 2019). Conjugated double bonds with aldehyde functional group as well as presence of long hydrocarbon chain connected to the aromatic ring can enhance antimicrobial activity (Chang, 2011). The existence of cinnamaldehyde in leaf essential oil of *Cinnamomum osmophloeum* was directly related to its strong inhibitory effect against fungi (Lee *et al*., 2005; Wang *et al*., 2005). Aldehyde demonstrated moderate antimicrobial activities while ketones such as camphor and α-thujone were reported to display significant antimicrobial properties (Bezic *et al*., 2003). Many monoterpene hydrocarbons like limonene and terpinenes showed strong to moderate

inhibitory effects against gram-positive bacteria and pathogenic fungi (Guimarães *et al*., 2019). Strong to moderate activities were also observed when oxygenated monoterpenes like linalool was tested against many bacteria (Tzakou *et al*., 2001; Senatore *et al*., 2005). Likewise, essential oils with high percentage of sesquiterpenes, for example spathulenol, γ-muurolene, (Z)-β-farnesene, α-selinene and 8-cadinene, possess antifungal and antibacterial activity (Lago *et al.*, 2004; Cakir *et al*., 2005; Cheng *et al*., 2005; Yayli *et al*., 2005). There are diterpenes that were reported to exhibit antimicrobial activity e.g phytol, terpenone and geranylgeraniol (Inoue *et al*., 2004). Concentrations of individual sulfide constituents in essential oils also determine their sensitivity to microorganism. Sulfides with single sulfur atom are inactive whereas those with more than two sulfur atoms were highly active against *Staphylococcus aureus* and *Candida utilis* (Kim *et al*., 2004 a).

In most cases, the essential oils constituents that are present in high percentage are responsible for their antibacterial activity. Nevertheless, previous studies showed that it is possible for whole essential oil to have higher activity compared with the activity of the main compounds isolated from it. This indicates that minor constituents also contribute to the biological properties of the essential oil, which may be by creating a synergistic effect. For instance, the two main components of *Osmitopsis asteriscoides*, (-)-camphor and 1,8-cineole, showed greater antimicrobial effect on *Candida albicans* when combined than when tested separately. Presence of enantiomers also contributes to the oils' activities. There are cases when opposition may be observed among the oil constituents where activities of combined constituents will be less than that of individual constituents. For example, monoterpene hydrocarbons like *p*-cymene and γ–terpinene seem to offer an antagonistic effect with most active compounds by lowering its solubility, thereby making them inactive (Aggarwal *et al*., 2002).

2.13 Antioxidant Activity of Essential Oils

Oxidative deterioration of unsaturated fatty acids and DNA oxidative damage in human cells caused by reactive oxygen species (ROS) result in degradative diseases. Studies have shown that natural antioxidants like vitamins, phenolic compounds and essential oils, possess some degree of preventive and therapeutic effects on such disorder (Madsen and Bertelsen, 1995). This gives room for commercial improvement of plants as new sources

of antioxidants to improve health condition of human and preservation of food. Most essential oils possess antioxidant activity like that of phenolic groups. Generally, phenols can act as antioxidants of lipid peroxidation since they can trap the chain-carrying lipid peroxyl radicals. Plant phenols can act as reducing agents, hydrogen-donating antioxidant and single-oxygen quenchers, making consumption of dietary antioxidant important to reduce the cumulative effects of oxidative damage (Pietta, 2000). Essential oils have demonstrated superior antioxidant properties because of ability to penetrate cell membrane better and faster than water and salt as a result of their unique properties such as lipophilic nature, volatility and chemical compositions.

Many reports in the literature have proven some essential oil components to be good antioxidant agents. Terpinene and terpinolene showed high activity whereas α-pinene and limonene have weak antioxidant activity on DPPH (Kim *et al*., 2004). Among the oxygenated terpenes, geraniol showed high hydrogen-donating capacity towards the DDPH radicals (Choi *et al*., 2000). Other active terpenes are eugenol, 1,8-cineole, thymol, carvacrol (Wei *et al*., 2001). The high potential of phenolic constituents to scavenge radicals could be as a result of their ability to give out the hydrogen atom of their phenolic hydroxyl groups. Essential oils' antioxidant activity in most cases may not be attributed to the most prominent components, at times minor components might play significant role in the activity. Like antimicrobial activity of essential oils, there are reports of synergistic effects (Wei *et al*., 2001). Presence of electron-rich centers may contribute to the antioxidant activity of some terpenes as observed in germacrene D, α or β-pinene, menthadiene and caryophyllene (Hyldgaag *et al*., 2012).

2.14 Biological Analysis

2.14.1 Antioxidant assay

Antioxidants, from a biological point of view, have been described as materials that, when present in concentrations that are lower than the oxidised substrates, are capable of slowing down or hindering oxidative processes. Interactions of antioxidants with free radicals which play a major role in different health conditions, prevent the risk of so many diseases and

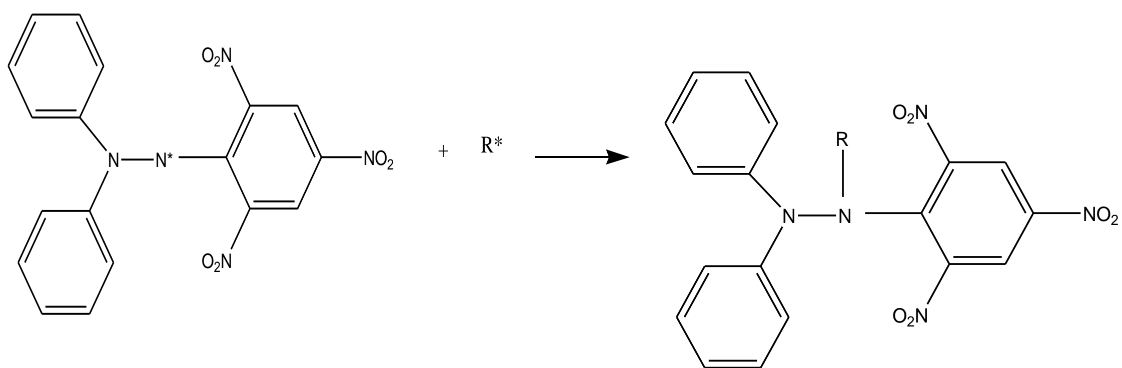
process like aging, cancer, atherosclerosis, ulcerative colitis, diabetes mellitus, rheumatoid arthritis, renal diseases, respiratory diseases and eye diseases (Aruoma, 1998).

The mechanism of interaction of natural antioxidant compounds with the free radicals includes: breaking the chain reactions of oxidation through donation of electrons or hydrogen atoms that will convert free radicals into more stable compounds; decomposition of lipid peroxides into more stable compounds and hindering the harmful action of peroxidant enzymes; chelating metal ions which help to generate reactive oxygen species. Natural antioxidants demonstrate a broad range of biological activities including antiallergic, antifungal, antiviral, antibacterial, antiinflammatory, vasodilatory and antithrombotic activities (Cook and Samman, 1996). Ascorbic acid, α-tocopherol, carotenoids, peptides, amino acids, proteins, flavonoids and other phenolic compounds which are food antioxidants, also play a significant role as dietary and physiological antioxidants (Shahidi, 2000).

Due to the complexity of essential oil components, isolation and studying of each antioxidant compound will be time consuming and tedious. The procedure usually used for the evaluation of plant essential oils' antioxidant activity are: bleaching of β- carotene in linoleic acid system; 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and inhibition of linoleic acid peroxidation assays.

2.14.1.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) **[2.78]** is a cell-permeable, free radical that is stable and often utilized to estimate free radical scavenging or hydrogen donor ability of a compound as well as evaluation of antioxidant potential of extracts obtained from plant materials (Kedare and Singh, 2011). The reaction of DPPH with an antioxidant or reducing compound produces 1,1-diphenyl-2-(2,4,6-trinitophenyl) hydrazine **[2.79]** (Figure 2.14), which can be monitored visually by colour change from purple to yellow (Molyneux, 2004; Sharma and Bhat, 2009; Pyrzynska and Pekal, 2013). There is also possibility of calculating the initial radicals from the change in the optical absorption at 515-528 nm or in the electron paramagnetic resonance signal of the DPPH (Mark, 1997).



2,2-diphenyl-1-picrylhydrazyl

<u>2.78</u>

1,1-diphenyl-2-(2,4,6-trinitrophenyl

<u>2.79</u>

Figure 2.14: Inhibition of DPPH

2.14.2 Antimicrobial assay

2.14.2.1 Antimicrobial Agent

Many *in vitro* evidences have revealed that essential oils possess antibacterial activity against a large number of pathogenic bacteria strains (Hulin *et al*., 1998: Dadalioglu and Evrendile, 2004). Antimicrobial agents are generally either bacteriostatic or bacteriocidal: fungistatic or fungicidal. The bacteriocidal antimicrobial agents show irreversible lethal action on their target organisms while bacteriostatic ones inhibit microbes reversibly and rely on the host defense mechanism for the final eradication of infected organism. Some of these chemotherapeutic agents have a narrow group of organisms against which they are active. Some may be active against Gram-positive bacteria while others are confined to Gram-negative, although, gram positive bacteria are more prone to chemical attacks which is likely due to the thinness of their cell walls and this has even been observed among most antibiotics. Gram-negative bacteria have thicker cell walls because of their additional outer membrane. The protein components of their outer membrane are also known to be involved in their pathogenicity (Essien, 2007). Chemotherapeutic (antimicrobial) agents are chemicals or drugs containing active components that are capable of waging war against invading microbial diseases as a result of inhibiting the growth of the microbes responsible for it without pronounced hindrance on the metabolic activities of the host (Brooks *et al*., 1995).

The potency of synthesised organic compounds in drug formulation is determination from their antimicrobial assay. Antimicrobial assays are also very useful in the study of the biological significance of isolated compounds from plants. The antimicrobial activity is determined by testing the sample of interest against gram-negative and gram-positive bacteria, and fungi of standard and clinical strains. Some of the common organisms used in the antimicrobial assay are pathogenic and are associated with various diseases. Examples are *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Bacillus cereus*, *Aspergillus niger* and *Candida albicans*. The common method used to determine the *in vitro* antimicrobial activity of test samples are agar-diffusion method. In this method, wells of the same diameter are created in a seeded agar and the wells are filled with the test sample or placing a paper disk impregnated with the sample (in case the sample is volatile plant

extract like essential oils) on the seeded agar plates. The extract is allowed to difuse into the seeded agar and diameter of zones of inhibition is recorded after 24 hours at 37 degree celcius and 48 hours at 25-32 degree celcius of incubation respectively, for bacteria and fungi. In agar dilution the extract is incorporated into the molten agar and the set agar is then streaked with the test organisms.

2.14.2.2 Characteristics of Microorganisms

Bacteria

Bacteria is a collective name for large number of single-celled, microorganisms that live in the soil, water, or animals including human. They vary in forms, sizes, shapes and aggregation. They are common in nature and live in almost every environment on Earth. They reproduce on their own by binary fission. Bacteria can be harmless or harmful. The harmful ones are responsible for so many health challenges of humans like cholera, tuberculosis, diarrheal, vaginal infection, syphilis, tooth decay and pneumonia. Bacterial cells are surrounded by an outer cell wall as well as an inner cell membrane. Their outer surfaces are covered with whip-like extentions (called flagella when long and pili when short) which help them to move around and attach to a host. Bacteria can be classified based on their shapes; comma-shaped (vibrios), spirals (spirilla), corkscrew (spirochaetes), cylindrical (bacilli) and spherical or round (cocci), and may organize themselves into chains or clusters. They are also classified based on the composition of their cell walls as Gram-positive and Gram-negative bacteria. Examples of Gram-negative bacteria are *Escherichia*, *Salmonella*, *Citrobacter, Morganella Leclericia* and *Klebsiella* while *Staphylococcus*, *Bacillus* and *streptococcus* are Gram-positive (Dahal, 2022)

Fungi

Fungi are non-photosynthetic organisms with rigid cell wall. The cells of most fungi are usually colourless, although some may be brown, red or yellow. Fungi may be saprophytic or parasitic. They take in soluble nutrients by diffusion through their cell surface. They include microorganisms like yeast (*Candida albicans*), moulds (*Fusarium solani, Aspergillus niger*) and mushrooms They are mainly found in soil rich in organic matter. Few are found in drier areas or habitat with little or no organic matter. Some of them are

parasites on plants or animals. Fungi are responsible for diseases like thrush, vaginal candidiasis, ringworm, histoplasmosis and aspergillosis (Mazheika *et al*., 2022).

2.14.3 Brine shrimp lethality bioassay

Brine shrimp lethality bioassay is a simple procedure used in the evaluation of the cytotoxicity of bioactive substances based on the ability of test sample to kill the simple zoological organism called brine shrimp (*Artemia salina*). It is a primary toxicity screening which preceeds more complex experiments involving mammalian animal models (Wu, 2014). Besides, this assay is capable of detecting a broad spectrum of biological activity exhibited by plant crude extracts (Pisutthanan *et al*., 2004) or essential oils including antitumor and pesticidal activity (Meyer *et al*., 1982). It was also reported to be a guide for the isolation of bioactive constituents from plant extract (Sam, 1993). This assay also provides a preliminary screening data which can be backed up by other bioassays that are more specific once the active compounds were isolated (Hamidi *et al*., 2014).
Artemia salina is an aquatic crustacean, specie of brine shrimps, having three eyes and eleven pairs of legs and can grow upto to a size of about 15 mm. They have similar blood pigment i.e haemoglobin like that of vertebrate animals. Males are different from female by having their second antennae magnified and modified into clasping organs used in mating. The females produce two types of eggs either through pathenogenesis or mating: eggs with thin-shells that hatch immediately and those with thick-shell, which can remain in a dormant state. The latter can last for many years and will only hatch when placed inside saltwater.

The advantages of this assay include low cost, simplicity, commercial availability in that the brine shrimp eggs are not expensive, high degree of repeatability and rapidity, making the assay to be a very helpful bench top technique (McLaughlin *et al*., 1991). In addition, several studies have reported a good correlation between the lethal concentration (LC_{50}) i.e concentration that kills 50% population of the shrimps (*Artemia salina*) exposed to the tested samples in Brine shrimps lethality assay and that of the Acute Oral Toxicity Assay in mice (Arlsanyolu and Erdemgil, 2006).

This *in vivo* test has been used as an alternative technique in the assessment of toxicity of dental materials, metal ions, heavy metals, algae, cyanobacteria, plant extracts, nano particles and also screening marine natural products (Hamidi *et al*., 2014).

One important aspect of brine shrimp lethality assay is the solvent used in the assay. Previous studies showed the effect of solvent used in the reconstitution of volatile plant extract. Some solvents interact with the activity of the tested samples due to their toxicity and thereby give inaccurate results. Dimethylsulfoxide (DMSO) is majorly used as solvent for the dissolution of plant extract because there is no evidence that brine shrimp nauplii are sensitive to this solvent (Wu, 2014) even up to 11% concentration. Preparation of stock solution and several dilutions make available different dose levels, which provides opportunity to determine the linear increase of toxicity with respect to increase in the concentrations of the plant extract (Hamidi *et al*., 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and Identification of Plant Materials

All the plants of study were collected within the University of Ibadan, Oyo state, Nigeria within the period February, 2014- October, 2014. The plants: *Tecoma stans*, *Plumeria acuminata*, *Plumeria rubra*, *Delonix regia,* *Gliricidia sepium*, *Duranta repens*, *Zanthoxylum zanthoxyloides*, *Ceiba pentandra* and *Annona muricata* were first identified at the herbarium in the Botany Department, University of Ibadan and later authenticated by Mr A. J. Egunjobi at the Forest Research Institute of Nigeria (Ibadan) where Voucher specimen was deposited in the Herbarium.

3.2 Extraction of Essential Oils

The fresh plant parts which include leaf, stem, twig, fruit, flower, root, stem bark, branches, heartwood and seed were separated and air-dried for three weeks and later pulverized. The flowers and leaves of *Plumeria* species were not air-dried due to their perishable character, but were chopped to pieces fresh. The pulverised plant materials (500 g dry samples and 200 g wet samples) were hydrodistilled for 3 h in an all-glass Clevenger-type apparatus, in accordance to the British Pharmacopeia (1980) specification using heating mantle as a source of heat. The volatile oils from the plant materials were collected in the receiver arm of the apparatus which contain analytical grade *n*-hexane and water. The hexane helps to trap the essential oils. The mixture of hexane and the oils were collected into glass vial after the distillation. Moisture was removed from the mix by passing it over sodium sulphate (Na_2SO_4). The volatile oils were stored in sealed glass vials inside the refrigerator at 4 °C prior to analysis and bioassay.

Table 3.1: List of Plants Used

Plant	Family	Common Name	Local (Yoruba) Name	Part Used	Voucher specimen
Tecoma stans	Bignoniaceae	Yellow trumpet tree	Awun	Leaf, seed, stem, flower	FHI 112524
Plumeria acuminata	Apocynaceae	White frangipani	-	Leaf, root, flower	FHI 112495
Plumeria rubra	Apocynaceae	Frangipani	-	Leaf, root, flower, stem	FHI 112567
Delonix regia	Fabaceae	Flame of the forest	Sekeseke	Twig, flower, root	FHI 112523
Duranta repens	Verbenaceae	Pigeon berry	Peregun	Leaf, fruit, root, stem	FHI 112525
Zanthoxylum zanthoxyloide	Rutaceae	Fagara	Igi ata	Leaf, stem bark	FHI 112982
Ceiba pentandra	Bombacaceae	Silk cotton tree	Araba	Stem bark, heartwood	FHI 112962
Gliricidia sepium	Fabaceae	Quickstick	Agunmaniye	Leaf, stem	FHI 112496
Annona muricata	Annonaceae	Soursop	Ebo	Leaf, stem bark, branches	FHI 112526

3.3 Gas Chromatography/Mass Spectrometry Analysis

The chemical constituents of the essential oils of *Tecoma stans* were identified using GC-MS technique carried out on a Varian CP-3800 gas chromatograph equipped with a HP-5 capillary column (30 m x 0.25 mm; 0.25 µm coating thickness) interfaced to a Varian Saturn 2000 ion trap Mass Detector. The MS was operated in electron impact mode with ionization voltage 70 eV. The transfer line and injector temperatures were 240 ºC and 220 ºC, respectively. The initial oven temperature of the GC was 60 ºC and increased to 240 ºC at 3 ºC/min. Helium was used as a carrier gas at 1 mL/min flow rate. Identification of the chemical constituents was based on comparison of their retention indices with those of n-hydrocarbon series and their mass spectra with the literature (Davies, 1990; Adams, 2017) and reference compounds in NIST 05.

Plumeria acuminata, *Plumeria rubra*, *Delonix regia, Gliricidia sepium*, *Duranta repens*, *Zanthoxylum zanthoxyloides*, *Ceiba pentandra* and *Annona muricata* essential oils were investigated using a Shimadzu GC-MS-QP2010 which is ultra operated in the electron impact mode (electron energy=70 eV). The scan range were 40-400 a.m.u. at the rate of 3.0 scan/s and GC-MS solution software v. 4.20 (Shimadzu Scientific Instruments, Columbia, MD, USA). The column of the gas chromatography was a ZB-5 fused silica capillary column (Phenomenex, Torrance, CA, USA) with stationary phase (5 % phenyl)-polymethylsiloxane. Helium was used as carrier gas with a column head pressure and flow rate of 552 kPa and 1.37 mL/min respectively. The initial temperature of injector was 50 ºC, and increased to 260 ºC at 2 ºC/min. The sample with concentration of 0.1 µL prepared by dissolving 5 percent weight per volume of the sample solution in dichloromethane was injected through a splitting mode of 30:1.

The constituents of the essential oils were identified based on comparison of their retention indices relative to the homolgous series of n-alkanes and comparison of their mass spectra fragmentation patterns with the ones in the literature (Adams, 2017) and also with reference compounds stored in home database library (Satyal, 2015). The relative percentage of each constituent was calculated by integration of the GC peak areas.

3.4 Antimicrobial Assay

In order to assess the antimicrobial potency of the volatile oils under study, a pilot study was carried out on all the oils using one representative Gram-positive bacterium (*Staphylococcus aureus*), Gram-negative (*E. coli*) bacterium and one fungus (*Candida albicans*). The volatile oil with good inhibitory zones were further tested against more microbes (*Salmonella typhi, Leclericia adecarboxylata, Morganella morganii, Citrobacter freundii, Klebsiella pneumoniae, Aspergillus niger, Fusarium solani*) at three different concentrations (1 µL/mL, 10 µL/mL, 100µL/mL). The activities of the essential oils were evaluated and compared with that of a synthetic antibiotic (positive control), using ketoconazole (200 µg) as antifungal and gentamycin (10 µL/mL) as antibacterial. The effect of the solvent, dimethyl sulphoxide (DMSO) was also checked against the microbes and recorded as negative control. The experiment was performed in duplicates and diameters of inhibitory zones were reported in millimeter as mean approximately to 1 decimal place without the diameter of the well.

3.4.1 Test organisms

All the tested organisms were clinical isolates besides *E. coli* (ATCC 25925) and *S. aureus* (ATCC 6571). The microbes were obtained from the Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria where the bioassay was carried out.

3.4.2 Sample preparation

The solution of each essential oil was prepared by dissolving 0.1 mL of the oil in 1.0 mL of Dimethylsulfoxide (DMSO) to give 100 mg/mL stock solution. Further dilution was done to give 10 mg/mL and 1 mg/mL.

3.4.3 Preparation of the bacteria medium

The medium used for antibacterial assay was Mueller Hinton Agar (MHA). It was prepared by suspending 38 g of the powdered agar in 1 L of distilled water. The agar was homogenized on water bath, later dispensed in 20 mL portion into universal bottles and sterilized by autoclaving for 15 mins at a temperature of 121 ºC.

3.4.4 Antibacterial assay procedure

The antibacterial assay was carried out using the method described by Hoods *et al*., (2003) with some modifications. Overnight cultured microorganism was prepared by taking two loopful of the organism from the stock and inoculating each into 5 mL of sterile nutrient broth (Lab M) for 18-24 h at 37 ºC. The mixture of 0.1 mL organism from the overnight culture and 9.9 mL of sterile distilled water to achieve 1:100 (10^{-2}) dilution was done for each organism. At 45 ºC, the sterilized agar was aseptically poured in to the sterile plates and allowed to set before the diluted overnight cultured organisms were seeded on the surface of the agar. Using a sterile cork borer of 8 mm, 7 wells were introduced and well labeled based on the serial dilution of each essential oil with the control and organisms used. The same amount (3 drops) of the solutions of the essential oils as well as positive and negative controls were introduced into the 8 mm wells accordingly and the plates were left for 30 mins to allow diffusion of the samples into the medium before been incubated for 18-24 h at 37 ºC. The assay was performed in duplicate and the activity of the essential oils against the microbes were evaluated by measuring the diameter of zones of inhibition of the test organisms in millimeters and comparing them with that of the controls.

3.4.5 Preparation of the fungi medium

The medium used for antifungal assay was Potato Dextrose Agar (PDA). It was prepared by dissolving 62 grams of the agar in 1 liter of distilled water and later homogenized on water bath after which is dispensed in 20mL portion into universal bottles. These were sterilized by autoclaving for 15 mins at a temperature of 121 ºC.

3.4.6 Antifungal assay procedure

The antifungal assay has the same procedure with antibacterial assay. The sterile PDA prepared was aseptically poured into the sterile plates and allowed to set. The 0.2 mL of 10^{-2} of each fungus was spread on the surface of the set agar using a sterile spreader to cover all the surface of the agar. Seven wells of uniform diameter using 8 mm cork borer were made in all the plates and labelled. After putting the oil samples, negative control (DMSO) and the standard drug (ketoconazole (200 µg) in the wells according to the labels on the

plates, the plates were left for 30 mins to allow the diffusion of the solutions into the seeded agar, then incubated at 28 ºC for 48 h after which the inhibitory zones were measured. The activity of the oils was compared with the standard drug.

3.5 Antioxidant Assay (2,2-diphenyl-1-picrylhydrazyl radical-scavenging assay)

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to evaluate the antioxidant ability of the essential oils because the method is simple and fast (Kumawat *et al*., 2012). The assay was carried out based on the procedure highlighted by Saleh *et al.,* (2010) with some modifications. In the method, 1.5 mL of three different concentrations (5 mg/mL, 25 mg/mL and 100 mg/mL) of the essential oil samples (dissolved in methanol) were mixed separately with 1.5 mL of 0.2 mM DPPH. The mixtures were incubated in the dark at room temperature for 20 minutes. The absorbance at 517 nanometers was recorded as $A_{(sample)}$ via CE 2021, 2000 series double beam ultraviolet-visible spectrophotometer. The same procedure was repeated for blank experiment but without essential oils and the absorbance was recorded as $A_{(blank)}$. Each experiment was performed in triplicates and the percentage inhibition which is the measure of the free-radical scavenging activity of the oils was calculated according to the formula below:

$$\% \text{ inhibition} = A_{(blank)} - A_{(sample)} / A_{(blank)} \times 100$$

The concentration of essential oil that gives 50% inhibition (IC_{50}) was determined by plotting the values of percentage inhibition against concentration using Microsoft EXCEL as described by Kumawat *et al*., 2012. The activity of α-tocopherol and BHA were likewise evaluated as positive control.

3.6 Brine Shrimp Lethality Assay

In this assay, all the hydrodistilled oils were subjected to the brine shrimp lethality assay at the concentration level of 10, 100 and1000 ppm to determine their toxicity. The number of mortality was subjected to Probit Regression analysis to obtain the lethal concentration of the essential oils, which is the concentration that killed 50 % of the larvae (LC_{50}). Negative control was likewise subjected to the same procedure to validate the test method and ensure the activity of the tested oil samples was only due to their chemical costituents and not from the solvent used.

The procedure includes soaking *Artemia salina* eggs inside seawater, in a plastic plate. The plate has two unequal chambers with several holes on the divider in between which enable the hatched *Artemia* nauplii to move from the hatching chamber to the illuminated compartment. Both sides were filled with seawater. The eggs were introduced in the wider part of the chamber and protected from light. Then the hatched nauplii were attracted by the light source and swimmed into the smaller chamber. The experiment was conducted after 48 hrs of soaking the eggs, by this time the eggs were hatched and mature as nauplii (larva) (Hamidi, 2014).

3.6.1 Dilution procedure

Different concentrations of the essential oils dissolved in Dimethyl sulphoxide (DMSO) were tested against the brine shrimps. The mixture of 0.02 mL of essential oils in 0.2 mL of DMSO and 1.8 mL seawater gave 2 mL of 1000 ppm. From this, 0.5 mL was taken and introduced into three well-labeled test tubes for each oil sample. From the remaining 0.5 mL, 0.2 mL was taken and added to 1.8 mL of seawater to give 2 mL of 100 ppm and 0.5 mL of this was placed in three test tubes like 1000 ppm. The last concentration, 10 ppm was prepared from 100 ppm in the same manner and 0.5 mL was introduced into three different labeled test tubes. Ten (10) active shrimps were introduced into each of these test tubes and seawater was added to make it up to 5 mL mark making 30 shrimps per dilution. Blank experiment was also carried out by introducing 10 shrimps into 5 mL seawater with DMSO to serve as negative control in other to eliminate other factors contributing to the total number of dead nauplii. All the test tubes were left for 24 h after which the number of surviving larvae were counted and recorded.

The lethality concentration (LC_{50}), the concentration at which 50 % mortality was observed, was analysed at 95 % confidence intervals using a probit regression analysis (Finney, 1971). LC_{50} less than 100 ppm are considered as toxic. LC_{50} value between 100-500 ppm is moderately toxic, between 500-1000 ppm is less toxic while the one greater than 1000 ppm is non-toxic (Meyer *et al*., 1982).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1: Extraction Results

The colours, odours and percentage yields of all the essential oils investigated in this study are given in Table 4.1.

The yields ranged from 0.06 to 0.62 % (w/w). The leaf oil of *T. stans, P. acuminate, P. rubra, D. repens, G. sepium and A. muricata* have greater yield than other parts of the plant. The disparity in the yields of the essential oils for the different parts could be attributed to the amount of volatile oils stored in specialised structures such as glandular hairs on the epidermis, oil tubes in the pericarp or isolated oil cells in the plant tissue (Masotti *et al*., 2003; Essien, 2007).

The odour of essential oil is useful in the determination of organoleptic quality of the oil for consideration as perfume or flavours (Sarkic and Stappen, 2018). The observed odours of the oils in this work were similar to the plant part from which they were extracted. The odour of *D. regia*, *G. sepium*, *Z. zanthoxyloides* and *A. muricata* leaves essential oil were described as leafy. The volatile oil from the flowers and leaves of the two *Plumeria* species and the root of *P. rubra* possessed minty odour and this might be connected to the use of the plant in perfumery industry. Minty odour was also perceived in *T. stans* leaf oil. These oils contained many compounds used in perfumery which include methyl salicylate, limonene, benzyl benzoate and linalool. The essential oil isolated from the stem of *T. stans*, *P. rubra*, *D. repens* and *G. sepium*; root of *P. acuminata* and *D. regia*; branches and stem bark of *A. muricana* as well as heartwood and stem bark of *C. pentandra* were described as woody. The essential oil of *T. stans*, and *D. regia* flower were flowery in aroma; *D. repens* fruit oil was fruity while *T. stans* seed oil, *D. repens* root oil and *Z. zanthoxyloides* stembark

oil were spicy. Variation in chemical profiles of the volatile oils obtained from different organs of the same plant is responsible for different odour observed in the oils.

All the volatile oils were colourless beside *D. repens* leaf, *P. acuminata*, *P. rubra* and *T. stans* leaf and flower oils which were yellow. The difference in colour of the volatile oils extracted from different parts of the same plant may be ascribed to the nature and concentration of the chemical components in each of the oils and how they are readily oxidized during extraction. Some essential oils possess inherent colours while some acquire colours on exposure to air (Essien, 2007).

Table 4.1: Physical Properties of the Essential Oils

Plant/Part used	Percentage yield (w/w)	Colour	Odour
Tecoma stans			
Leaf	0.62	Pale yellow	Minty
Seed	0.36	Colourless	Spicy
Stem	0.32	Colourless	Woody
Flowers	0.49	Pale yellow	flowery
***Plumeria acuminata* (White flower)**		Golden yellow	Minty
Leaf	0.48	Golden yellow	Minty
Flowers	0.32	Golden yellow	Woody
Root	0.25		
***Plumeria rubra* (Pink flower)**			
Leaf	0.32	Golden yellow	Minty
Stem	0.11	Golden yellow	Woody
Flower	0.18	Golden yellow	Minty
Root	0.25	Golden yellow	Minty
Delonix regia			
Twig	0.06	Colourless	Woody
Flowers	0.29	Colourless	Flowery
Root	0.22	Colourless	Woody
Duranta repens			
Leaf	0.26	Pale yellow	Leafy
Stem	0.10	Colourless	Woody
Fruits	0.20	Colourless	Fruity
Root	0.15	Colourless	Spicy
Zanthizylum zanthozyloids			
Leaf	0.36	Colourless	Leafy
Stem back	0.18	Colourless	Spicy
Ceiba pentandra			
Heartwood	0.09	Colourless	Woody
Stem back	0.10	Colourless	Woody
Gliricidia sepium			
Leaf	0.20	Colourless	Leafy
Stem back	0.16	Colourless	Woody
Annona muricata			
Leaf	0.32	Colourless	Leafy
Branches	0.12	Colourless	Woody
Stem bark	0.08	Colourless	Woody

4.2 *Tecoma stans* Essential Oil Chemical Constituents

The volatile compounds identified in T. stans essential oils are listed in Table 4.2 in order of their elution from a HP-5 capillary column. A total of 31, 22, 31 and 35 compounds were identified amounting to 91.5 %, 81.4 %, 88.5 % and 95.8 % of the whole essential oil from the leaf, stem, seed and flower respectively. The *T. stans* essential oils were composed of a mixture of terpenes and non terpene derivatives mainly, aliphatic alcohols, aldehydes, ketones, aromatic and aliphatic hydrocarbons. The constituents of the leaf, stem, seed and flower essential oils of *T. stans* have not been investigated before. However, the compositions of the oils were compared with some of the volatile constituents of plants belonging to the Bignoniaceae family as reported in the literatures.

The leaf oil was dominated by 1-octen-3-ol [**4.3**] (24.8 %), 2,6,10-trimethylpentadecane [**4.1**] (10.4 %), pentadecanal [**4.2**] (8.9 %), linalool [**2.36**] (7.7 %), 3-octanol [**4.6**] (6.0 %), citronellol [**2.49**] (5.1 %) and patchouli alcohol **[4.11]** (4.3 %). The previous works on the leaves essential oils from Bignoniaceae family have shown linalool and 1-octen -3-ol as their characteristics constituents (Bintu and Lajubutu, 1994; Guilhon *et al*., 2012). *Jacaranda acutifolia* essential oil and hexane extract contain methyl linolenate, 1- octen-3-ol, methyl pnonyl acetate, beta-linalool and palmitic acid as major constituents. GC (FID) and GC/MS analysis of *Memora nodosa* Miers leaf volatile oil gave benzaldehyde, 1- octen-3-ol, linalool and mandelonitrile as the most prominent components. Essential oil from the leaves of *Mansoa alliacea* of Brazil origin showed the presence of sulphur compounds and 1-octen-3-ol (Zoghbi *et al.*, 2009). Other notable representative compounds found in the *T. stans* leaf oil are patchouli alcohol (4.3 %), acorenone [**2.52**] (3.8 %) and (*E*)-β-ionone [**2.76**] (2.7 %). Two uncommon essential oil constituents were also identified only in the leaf oil, they are (*Z*) and (*E*)- rose oxide (0.7 % and 0.8 % respectively). *Z*-rose oxide **[4.15]** (isobutenyl-4-methyl tetrahydropyran) is a perfumery ingredient (Connoly and Hill, 2003) which is likely to have contributed to the light minty odour of the leaf oil. Other chemical compounds present only in the leaf oil are (*Z*)-3-octen-1-ol (0.5%), (*E*)-4-dodecene (0.9%), tetradecanal (0.5%), isocedranol (1.1%), 1-tetradecanol (0.7%) and patchouli alcohol. Monoterpene and sesquiterpene hydrocarbons were absent in the essential oil. Fourteen compounds made up the bulk of the non-terpenes (58.1%) in the leaf oil while seven and

five constituents equivalent to 16.1 % and 11.8 % of oxygenated monoterpenes and sesquiterpenes respectively were detected. The remaining components are apocarotenoids (5.5 %) which are product of carotenoid double bonds oxidative cleavage and are known to be good scent impacting compounds.

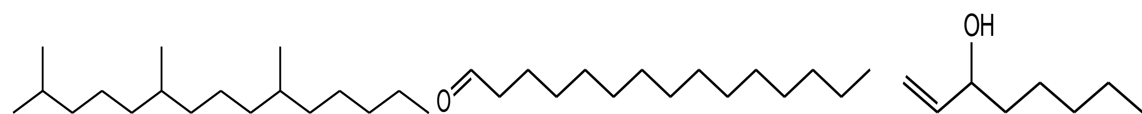
Of the twenty-two components identified in the stem volatile oil, linalool **[2.36]** (11.4 %) was the most abundant constituents. Two other major compounds were 2,6,10-trimethylpentadecane [**4.1]** (10.7 %) and 3- octanol [**4.6]** (7.0 %). Some important terpenes were also present in noteworthy quantity like β-eudesmol [**4.8]** (5.9 %), β-selinene [**2.51]** (5.4 %) and α-terpineol [**2.70]** (5.0 %), (*E*)-nerolidol (3.7 %) **[4.10]**, epi-α-bisabolol (2.4 %), acorenone (2.4 %) [**2.53]** and spathulenol [**4.13]** (2.0 %). Among the non-terpene derivatives (40.1 %), there were two aldehydes (3.8 %), five aliphatic hydrocarbons (18.9 %), two aliphatic alcohols (10.7 %) and one aromatic hydrocarbon making a total of ten compounds. The only aromatic compound was methyl salicylate [**4.7]** (5.7 %) while limonene **[2.41]** (0.7 %) was the only monoterpene hydrocarbon and both are odour impacting constituents used in perfumery industry. Spathulenol and β-eudesmol are present only in the stem oil.

Monoterpenoid compounds (30.6 %) were the dominating constituents in the seed essential oil, largely linalool [**2.36]** (7.4 %) and α-terpineol [**2.70]** (11.7 %). 1-Octen-3-ol [**4.3]** (5.5 %) and 3-octanol [**4.6]** (6.0 %) were present in good quantity and the only non-terpene aliphatic alcohols present in the seed oil while β- selinene [**2.51]** (9.3 %) was the major component in the sesquiterpenoid class. Five aromatic compounds (10.1 %): ethylbenzene (2.0 %), *p*-xylene (3.2 %), *o*-xylene (0.9 %), 1-ethyl-4-methylbenzene (1.8 %) and methylsalicylate **[4.7]** (3.4 %) known to be odour impacting constituents were also identified in the essential oil. Other classes of compounds identified were non-terpene aliphatic hydrocarbons (7.0 %), aldehydes (7.8 %) and one apocarotenoid i.e (*E*)-geranylacetone (1.4%). Constituents that were present only in the seed volatile oil include γ-terpinene (1.4 %), terpinolene (0.8 %), (*E*)-2-decenal (0.9 %), β-elemene (1.5 %), dodecanal (1.0 %) and dendrolasin (1.8 %).

On the other hand, the floral essential oil contained more chemical constituents than the oil samples extracted from the leaf, stem and seed of *T. stans*. The floral oil had higher

percentage of non-terpene derivatives (56.2 %), low sesquiterpenoids (7.4 %) and appreciable quantity of apocarotenoids (20.1%). There was no monoterpene hydrocarbon in the floral oil but two oxygenated monoterpenes amounting to 12.1 % of the oil were detected. The most prominent components were *(E)*-β-ionone [**2.76**] (11.2 %), linalool [**2.36**] (10.8 %), 2,6,10-trimethylpentadecane [**4.1**] (8.4 %), 1-octen-3-ol [**4.3**] (7.9 %), (*E*)-β-damascenone (4.1 %) and *p*-xylene (4.0 %). *(E)*-β-ionone **[2.76]** and (*E*)-β-damascenone [**4.5**] were apocarotenoids that possess intense floral aromas and are reported as character impact compounds in plant essential oils (Shi *et al*., 2020). These compounds would have contributed largely to the flowery odour observed in the floral oil. The terpenoids detected only in the flower oil are α-cardinol (1.4 %) **[4.9]**, garmacrene-D-4-ol (2.3 %) [**4.12]** and (*E*,*E)*-α-farnesene [**4.14**]. Higher percentage of apocarotenoids in the flower volatile oil agreed with the presence of β-carotene in the *T. stans* flower extract reported earlier (Marasco *et al*., 2006; Anand and Basavaraju, 2018). The chemical constituents identified in flowers from other Bignoniaceae plants agree not with the present work (Mostafa *et al*., 2015; Villarreal *et al*., 2015; Araoju *et al*., 2020).

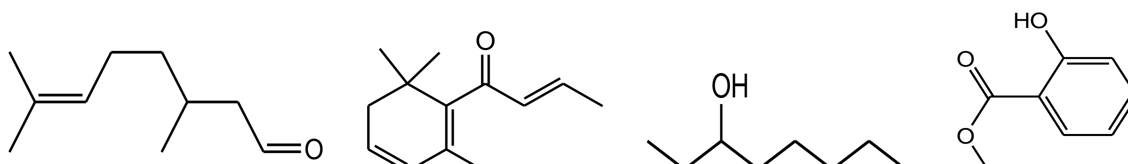
The classes of compounds identified in the *T. stans* essential oils are given in Table 4.3. The common constituents in the four oils were 1-octene-3-ol [**4.3**], linalool [**2.36**], nonanal, n-dodecane, α-terpeneol [**2.70]** and *n*-hexadecane. Oxygenated monoterpenes namely 6-camphene, citronellol [**2.49]** and citronellal [**4.4]** occur as the major constituents in both seed and leaf essential oils. (*E*)-nerolidol, an important aroma compound in perfumery and food industry (Essien, 2007) was found in the seed, leaf and stem oils. *E*-nerolidol [**4.10]** and α-terpineol [**4.11]** were minor components in leaf oil (0.6 % and 0.5 %) but present in high concentration in the seed oil (3.6 % and 11.9 %). β-cyclocitral [**2.73]**, β-cyclohomocitral, β-ionone **[2.76]**, (*E*)-β-demascenone and (*E*)-geranylacetate were the apocarotenoids identified in the essential oil samples.



2,6,10-trimethylpentadecane **4.1**

pentadecanal **4.2**

1-octen-3-ol **4.3**

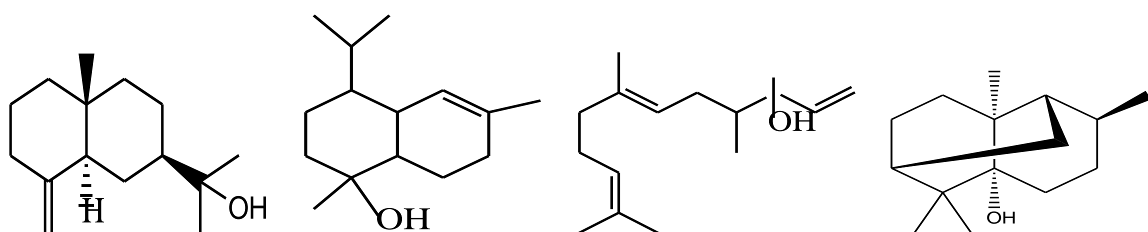


citronellal **4.4**

(*E*)-β-damascenone **4.5**

3-octanol **4.6**

Methylsalicylate **4.7**

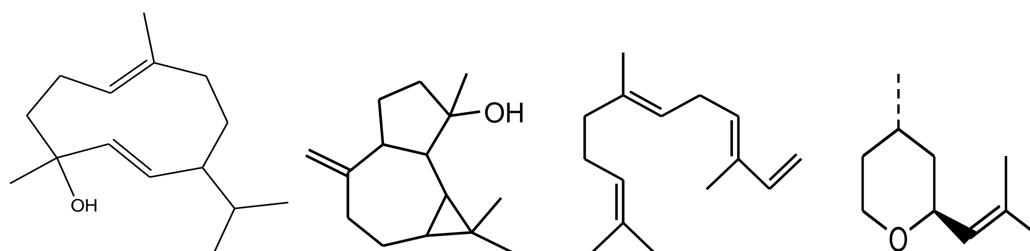


β-eudesmol **4.8**

α-cadinol **4.9**

***E*-nerolidol** **4.10**

patchouli alcohol **4.11**



Germacrene-D-4-ol **4.12**

Spathulenol **4.13**

(E,E)-α-farnesene **4.14**

Z-rose oxide **4.15**

Figure 4.1: Constituents of *T. stans* Essential oils

Table 4.2: Chemical Constituents of *Tecoma stans*

Constituents	l.r.i.	Percentage compositions (%)			
		Leaf	Stem	Seed	Flower
Ethylbenzene	864	-	-	2.0	3.4
p-xylene	867	-	-	3.2	4.0
o-xylene	895	-	-	0.9	1.8
1-ethyl-4-methylbenzene	965	-	-	1.8	2.2
1-ethyl-3-methylbenzene	967	-	-	-	0.8
1-octen-3-ol	981	24.8	3.7	5.5	7.9
3-octanol	994	6.0	7.0	6.0	-
Mesitylene	996	-	-	-	2.7
n-decane	1000	-	-	-	1.1
Limonene	1032	-	0.7	2.1	-
(Z)-3-octen-1-ol	1051	0.5	-	-	-
4-methyldecane	1059	-	-	-	1.5
γ-terpinene	1063	-	-	1.4	-
Terpinolene	1090	-	-	0.8	-
6-camphenone	1092	0.5	-	1.0	-
Linalool	1101	7.7	11.4	7.4	10.8
Nonanal	1104	0.4	1.6	2.1	1.4
(*Z*)-rose oxide	1111	0.8	-	-	-
(*E*)-rose oxide	1128	0.7	-	-	-
Citronellal	1155	0.8	-	3.0	-
2,4-dimethylbenzaldehyde	1179	-	-	-	1.9
4-terpineol	1179	-	0.9	1.1	-
α-terpineol	1191	0.5	5.0	11.7	1.3
methyl salicylate	1192	2.2	5.7	3.4	-
(E)-4-dodecene	1193	0.9	-	-	-
n-dodecane	1200	1.1	2.1	1.2	1.5
Decanal	1206	0.4	2.2	2.6	-
β-cyclocitral	1222	0.6	-	-	1.6

Citronellol	1230	5.1	-	2.1	-
β-cyclohomocitral	1256	0.8	-	-	1.7
(E)-2-decenal	1263	-	-	0.9	-
3-methyldodecane	1273	-	-	-	1.0
(E,E)-2,4-decadienal	1316	-	-	1.2	1.0
3-methylundecanol	1326	-	-	-	1.0
(E)-β-damascenone	1382	0.9	-	-	4.1
β-elemene	1392	-	-	1.5	-
n-tetradecane	1400	-	1.2	1.6	1.6
Dodecanal	1409	-	-	1.0	-
(*E*)-α-bergamotene	1437	-	0.8	2.7	-
(E)-geranylacetone	1457	0.5	-	1.4	1.5
2-methyltetradecane	1462	-	-	-	1.0
β-selinene	1487	-	5.4	9.3	-
(E)-β-ionone	1487	2.7	-	-	11.2
n-pentadecane	1500	0.5	-	1.1	0.9
2-tridecanol	1506	-	-	-	2.3
(E,E)-α-farnesene	1508	-	-	-	2.2
δ-cadinene	1524	-	0.7	-	1.5
5-methylpentadecane	1551	-	0.8	-	0.9
(E)-nerolidol	1564	0.6	3.7	3.6	-
germacrene D-4-ol	1575	-	-	-	2.3
Spathulenol	1577	-	2.0	-	-
Dendrolasin	1580	-	-	1.8	-
n-hexadecane	1600	0.8	5.1	3.1	2.2
Tetradecanal	1614	0.5	-	-	-
2,6,10-trimethylpentadecane	1642	10.4	10.7	-	8.4
β-eudesmol	1650	-	5.9	-	-
α-cadinol	1654	-	-	-	1.4
patchouli alcohol	1660	4.3	-	-	-
Isocedranol	1668	1.1	-	-	-

1-tetradecanol	1675	0.7	-	-	-
epi-α-bisabolol	1687	2.0	2.4	-	-
Acorenone	1688	3.8	2.4	-	-
2-pentadecanone	1699	-	-	-	2.6
Pentadecanal	1716	8.9	-	-	3.1
Number Identified		31	26	31	35
Percentage identified		91.5	81.4	88.5	95.8

lri- Linear retention indices from the analyses value.

Table 4.3: Classes of components identified in *T. stans* essential oils

Classes	Leaf		Stem		Seed		Flower	
	PC (%)	**NI**	**PC (%)**	**NI**	**PC(%)**	**NI**	**PC(%)**	**NI**
Monoterpene hydrocarbons	-	-	0.7	1	4.3	3	-	-
Oxygenated monoterpenes	16.1	7	17.3	3	26.3	6	12.1	2
Sesquiterpene hydrocarbons	-	-	6.9	3	13.5	3	3.7	2
Oxygenated sesquiterpenes	11.8	5	16.4	5	5.4	2	3.7	2
Apocarotenoids	5.5	5	-	-	1.4	1	20.1	5
Non-terpene derivatives	58.1	14	40.1	10	37.6	16	56.2	24

PC- Percentage composition NI- Number identified

4.3. *Plumeria acuminata* (white flower) Essential Oil Chemical Constituents

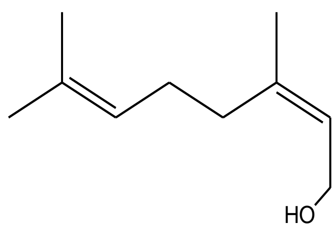
Table 4.4 shows the chemical constituents of *P. acuminata* leaf, flower and root essential oils as they are eluted from a HP-5MS column. Twenty-six components were identified in the essential oil of the leaf amounting to 69 0 % of the whole volatile oil out of which 9 are non terpenes (22.5 %) and 17 terpenes (46.5 %). The major compounds were linalool **[2.36]** (16.1 %), (*E*)-β-ionone (6.1%), phytol (6.8%) and (*E*)-hexadecatrienal (5.0 %). Other notable components are 1-(1-methyl-cyclopentyl)-ethanone (4.8 %), 4-methyl-3-hexanol (3.0 %), viridiflorene (2.5 %), o-xylene (2.2 %), 2-pentyl furan (2.1 %), ethylbenzene (2.0 %), limonene (2.0 %), α-pinene (1.8 %), β-elemene (1.8 %) and α-copane (1.7 %) which are scent impacting components. In addition, (*E*)-β-ionone (0.1 %), (*E*)-β-farnesene (0.6 %), germacrene D (0.6 %), (*E*)-α-bergamotene (0.7 %), (*E*)-p-menth-2-en-1-ol (0.7 %), α-terpineol (0.8 %) **[2.70]**, nonanal (0.9 %), β-caryophyllene (1.0 %), (Z)-p-menth-2-en-1-ol (1.1 %), β-cyclocitral (1.1 %) and mustakone (1.1 %) were also odour impacting constituents identified in the leaf volatile oil.

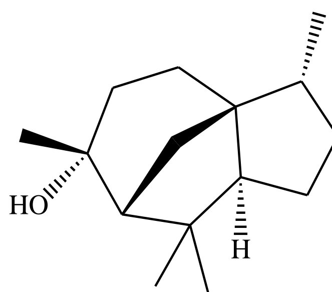
Eighty-five compounds corresponding to 99.8 % of the entire oil were identified in the flower essential oil. The volatile oil comprised of 33 terpenes (26.5 %) and 52 non terpene (73.3 %) derivatives. The classes of compounds identified include monoterpenes, sesquiterpenes, diterpenes and apocarotenoids. In addition, alkanes, aldehydes, alcohol, ketones, amide, ester, fatty acid and aromatic compounds of non-terpene derivatives were present. The most prominent constituents were nonanal (20.5 %), linalool (12.6 %), eicosanal (10.6 %), nonadecane (8.6 %), octane (4.4 %) and (*E*)-nerolidol (4.3 %) **[4.10]**. The flower essential oil was mainly odour impacting compounds some of which are utilised as flavor and fragrant ingredients in food and cosmetic industries. Examples were linalool (12.6 %), nerol (0.2 %), citronellol (0.2 %) and geraniol (0.9 %) known as rose alcohols because they are the key constituents responsible for rose odour character in rose essential oil (Ahmad *et al*., 2009). Majority of the non terpenes found in this oil are also good scent company like nonanal (20.5 %), benzaldehyde (0.8 %), heptanal (0.5 %), 1-octen-3-ol (0.5 %), (2*E*)-decenal (0.1 %), (2*E*,4*E*)-decadienal (0.1 %), (2*E*)-undecenal (trace), 4,6,8,10-tetramethyltridecane (0.1 %) and many more which could have added to the good scent of the flower volatile oil.

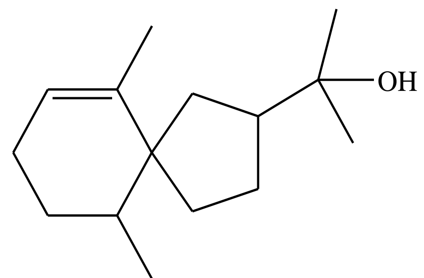
On the other hand, the root essential oil contained 12 compounds equivalent to 97.4 % of the whole oil and mainly oxygenated sesquiterpenes with non-terpenes. The major constituents were β-eudesmol (43.0 %), a sesquiternoid, and palmitic acid (27.6 %), 11-hydroxy-(8*E*)-dodecenoic acid lactone (14.9 %) and oleic acid (3.7 %), which are non-terpenes. Palmitic acid is a useful ingredient in food, soaps, cosmetic and industrial mold release agents' production (Mank and Polonska, 2016). It is an important constituent of most vegetable fats such as soybean and sunflower oils. Other isomers of eudesmol, α- (1.2 %) and γ- (1.7 %) were also present, and none of these isomers were found in the other parts (leaf and flower oils). Palmitic acid was the only constituent present in all the *P. acuminata* essential oils. The common constituents in the flower and root oils were carryophyllene oxide (0.2 %, 0.7 %), pentadecenal (1.8 %, 0.3 %), (9Z)-octadecenamide (0.3 %, 1.3 %), 1-docosanol (0.3 %, 0.6 %) and pentacosane (0.5 %, 1.4) respectively.

Seven components were found in both the leaf and flower essential oils, which are likely to have contributed largely to the minty odour of the two oils. They were nonanal (0.9 %, 20.5 %), α-terpineol (0.8 %, 1.2 %), (*E*)-α-bergamotene (0.7 %, 0.1 %), (*E*)-β-farnesene (0.6 %, 0.1 %), α-pinene (1.8 %. 0.2 %), limonene (2.0 %, 0.3 %) and linalool (16.2 %, 12.6 %).

Little variation was observed by Tohar *et al***.** (2006) in the flower essential oil compared with the present study. They have few common dominant constituents such as linalool (8.9%, 12.6 %), (*E*)-nerolidol (10.6 %, 4.3 %) and benzyl benzoate (17.2 %, 0.2 %) while other prominent constituents reported like benzyl salicylate (39.0 %) and neryl phenylacetate (10.5 %) were not found. The major compounds in the flower oil reported by Lawal *et al*., (2014) compared with the present study were limonene (9.1 %, 0.3 %), linalool (7.9 %, 12.6 %), α-cedrene (8.0 %, absent), caryophyllene oxide (7.9 %, 0.2 %) and (*E,E*)-α-farnesene (6.6 %, 0.3 %). Likewise, there was variation in the leaf oil from the two sources. Phenyl acetaldehyde (8.5 %) and neryl acetone (5.3 %) reported as prominent constituents were not identified in this study while n-decanal (5.1%) was only detected in low percentage (0.9 %). The variations could be as a result of different geographical locations, plant age, time and season of harvest, handling of sample and condition of extraction (Teles *et al*., 2013).







Nerol **cedrol** **hinesol**

4.16 **4.17** **4.18**

Figure 4.2: Sesquiterpenes in *P. acuminata* essential oils

Table 4.4: Chemical Constituents of *Plumeria acuminata* Essential Oils

		Percentage compositions (%)		
Constituents	RI	Leaf	Flower	Root
Octane	800	-	4.4	-
2*E*-Hexanal	851	1.0	-	-
Ethylbenzene	874	2.0	-	-
o-Xylene	879	2.2	-	-
Heptanal	901	-	0.5	-
4-Methyl-3-hexanol	905	3.0	-	-
α- pinene	928	1.8	0.2	-
Benzaldehyde	959	-	0.8	-
1-(1-Methyl-cyclopentyl)-ethanone	961	4.8	-	-
1-Octen-3-ol	976	-	0.5	-
2-Pentylfuran	986	2.1	-	-
Octanal	1001	-	0.7	-
Limonene	1026	2.0	0.3	-
(*Z*)-β-Ocimene	1033	-	0.1	-
Benzeneacetaldehyde	1041	-	0.9	-
(*E*)-β-Ocimene	1043	-	2.3	-
1-Octanol	1067	-	0.6	-
E-Linalool oxide	1084	-	0.7	-
2-Nonanone	1088	-	0.2	-
Methyl benzoate	1092	-	1.0	-
Linalool	1098	16.1	12.6	-
n-Nonanal	1107	0.9	20.5	-
Z-*p*-Menth-2-en-1-ol	1119	1.1	-	-
Non-3-en-2-one	1135	-	2.7	-
E-*p*-Menth-2-en-1-ol	1138	0.7	-	-
(2*E*)-Nonenal	1157	-	0.3	-
α-Terpineol	1183	0.8	1.2	-
Methyl salicylate	1189	-	0.6	-

Decanal	1204	-	0.1	-
β-Cyclocitral	1218	1.1	-	-
Nerol	1221	-	0.2	-
Citronellol	1223	-	0,2	-
Geraniol	1247	-	0.9	-
(2*E*)-Decenal	1260	-	0.1	-
Nonanoic acid	1262	-	0.2	-
Geranial	1265	-	0.1	-
Hydroxycitronellal	1285	-	0.2	-
2-Undecanone	1289	-	0.1	-
1-Nitro-2-phenyl ethane	1295	-	0.3	-
(2*E*,4*E*)-Decadienal	1316	-	0.1	-
α-Terpinyl acetate	1343	-	tr	-
(2*E*)-Undecenal	1361	-	tr	-
α-Copaene	1372	1.7	0.1	-
(*E*)-β-Demascenone	1375	-	0.2	-
β-Elemene	1389	1.8	-	-
β-Caryophyllene	1414	1.0	0.1	-
(*E*)-α-Ionone	1418	-	tr	-
(*E*)-α- Bergamotene	1433	0.7	0.1	-
11-Hydroxy-(8*E*)-dodecenoic acid lactone	1443	-	-	14.9
Geranyl acetone	1444	-	0.1	-
(*E*)-β-Farnesene	1456	0.6	0.1	-
α-Humulene	1452	-	0.1	-
4,6,8,10-Tetramethyltridecane	1457	-	0.1	-
γ-Decalactone	1461	-	tr	-
Germacrene D	1479	0.6	-	-
(*E*)-β-Ionone	1483	6.1	0.1	-
Viridiflorene	1499	2.5	-	-
(*E*,*E*)-α-fernesene	1500	-	0.3	-
β-Bisabolene	1504	-	tr	-

Tridecanal	1508	-	0.1	-
δ-Cadinene	1514	-	0.1	-
(*E*)-Nerolidol	1557	-	4.3	-
Dendrolasin	1569	-	0.1	-
Caryophyllene oxide	1574	-	0.2	0.7
Cedrol	1605	-	0.5	-
Tetradecanal	1609	-	0.1	-
γ-Eudesmol	1628	-	-	1.7
Hinesol	1639	-	-	1.0
β-Eudesmol	1647	-	-	43.0
α-Eudesmol	1651	-	-	1.2
1-Tetradecanol	1673	-	0.2	-
Mustakone	1676	1.1	-	-
epi-α-Bisabolol	1681	-	0.2	-
α-Bisabolol	1683	-	0.2	-
Heptadecane	1700	-	0.4	-
Pentadecanal	1711	-	1.8	0.3
Myristic acid	1754	-	0.4	-
Benzyl benzoate	1762	-	0.2	-
Octadecane	1800	-	0.2	-
Neophytadiene	1832	-	0.1	-
Phytone	1837	-	0.7	-
2-Methylbenzyl benzoate	1850	-	0.2	-
(*Z*)-Hexadecatrienal	1881	-	0.6	-
(*E*)-Hexadecatrienal	1888	5.4	0.4	-
Heptadecenal	1915	-	0.3	-
Nonadecane	1900	-	8.6	-
Palmitic acid	1959	1.1	2.7	27.6
Eicosane	2000	-	0.2	-
Octadecanal	2016	-	0.5	-
1-Octadecanol	2079	-	0.4	-

Heneicosane	2100	-	2.4	-
Phytol	2102	6.8	0.5	-
Nonadecanal	2118	-	1.3	-
Linoleic acid	2123	-	0.2	-
Oleic acid	2129	-	-	3.7
Eicosanal	2221	-	10.6	-
1-Eicosanol	2283	-	1.0	-
Tricosane	2300	-	0.5	-
Heneicosanal	2322	-	0.2	-
(9Z)-Octadecenamide	2349	-	0.3	1.3
Docosanal	2424	-	0.8	-
1-Docosanol	2484	-	0.3	0.6
Pentacosane	2500	-	0.5	1.4
Heptacosane	2700	-	2.6	-
Percentage identified		69.0	99.8	97.4
Number identified		26	85	12

RI- Retention indices from the analyses value. tr-trace

4.4 *Plumeria rubra* (Pink flower) Essential Oil Chemical Constituents

The results of the chemical anlysis of essential oils from the leaf, stem, root and flower of *Plumeria rubra* are reported in Table 4.5. The compounds identified in the essential oils appeared in the table according to their elution from a HP-5MS column. The GC-MS analyses of the leaf oil resulted in the identification of 93 compounds representing 97.7 %. Aldehydes (65.3 %), alkanes (0.9 %), alcohols (1.5 %), ketones (0.8 %), esters (0.8 %), fatty acids (9.4 %), aromatic compounds (0.4 %), apocarotenoids (0.9 %), diterpene (7.4 %), monoterpenes (1.8 %) and sesquiterpenes (6.7 %) were identified in the leaf volatile oil. Most of the non-terpene derivatives are odour impacting constituent (Resconi *et al*., 2013), even though they were present in small quantity. Among these were hexanal (0.4 %), (4*Z*)-heptenal (0.8 %), heptanal (0.6 %), 1-octen-3-ol (0.1 %), 6-methyl-5-hepten-2-one (0.1 %), 2-penthyl furan (1.2 %), 2,2,6-trimethylcyclohexanone (0.1 %), nonanal (0.4 %), (2*E*,6*Z*)- nonadienal (1.9 %), (3*E*,6*Z*)-nonadienol (0.4 %), (2*E*)-nonenal (1.0 %), (2*E*,6*Z*)-nonadien-1-ol (0.1 %), (2*E*)-nonen-1-ol (0.1 %), (6*Z*)-nonen-1-ol (0.1 %), 1-nonanol (0.1 %) and 2,5-dimethylstyrene (0.4 %). The dominant constituents were pentadecanal (51.2 %), palmitic acid (9.0 %) and (*E*)-phytol (7.4 %) was the only diterpene alcohol present. Monoterpenes in the leaf essential oil were α-pinene (0.1 %), β-pinene (tr), p-cymene (0.1 %), limonene (0.2 %), linalool (0.7 %), α-terpineol (0.1 %) and carvacrol methyl ether (0.1 %). Eugenol (0.1 %), α-copaene (0.8 %), β-elemene (0.4 %), β-caryophyllene (0.3 %) and (*E*)-α-bergamotene (0.4 %) were among the sesquiterpenes identified in the oil. Although all the terpenes in the oil occured in small amount i.e less than 0.8 %, but in one way, they have contributed to perfumery property of the *Plumeria* leaf since they have pleasant aroma. The apocarotenoids seen in the oil were safranal (trace), which was known to have antioxidant properties and free radical-scavenging activities and anti-inflammatory activities (Alayunt *et al*., 2019); β-cyclocitral (0.2 %), β-cyclohomocitral (0.1 %) and (*E*)-β-ionone (0.6 %), are all important materials in perfumery.

The root volatile oil was composed largely of non-terpenes, 37 compounds making up the bulk of the oil (94.3 %). The non terpenes were mainly 5 fatty acids (59.0 %) with palmitic acid (30.0 %), oleic acid (18.8 %) and linoleic acid (8.0 %) as the most abundant constituents. Two amides (14.9 %) namely (9Z)-docosenamide (11.4 %) and (9Z)-

Octadecenamide (3.5 %) along with 5 aliphatic aldehydes (13.0%) dominated by nonanal (8.1 %). There were 1 unsaturated aldehyde, 12 aliphatic alkanes and 1 cycloalkane, 3 aromatic compounds and 1 ester as well as 2 alcohols and 2 ketones were detected. The remaining terpenes constituents in the root volatile oil were α-pinene (0.3 %), limonene (0.3 %), linalool (0.5 %), α-copaene (0.9 %), β-elemene (0.2 %), β-caryophyllene (0.3 %), geranyl acetone (0.6 %), allo-aromadendrene (0.2 %), spathulenol (0.2 %), thujopsan-2α-ol (0.2 %), (5*Z*,9*E*)-farnesyl acetone (0.4 %) and acorenone (0.2 %).

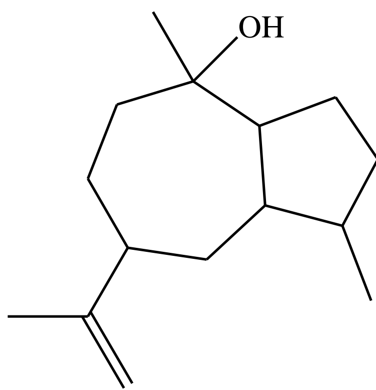
Forty-two compounds were identified in the stem essential oil representing 93.6 % of the whole oil. The oil was rich in oxygenated sesquiterpenes (61.7 %). Spathulenol (34.7 %) and caryophyllene oxide (17.8 %) were the most prominent compounds. Other terpenes present which are odour impacting constituents were of low concentration i.e monoterpenes like α-pinene (0.4 %), *p*-cymene (2.2 %) and limonene (0.7 %); and α-copaene (1.8 %), β-elemene (2.5 %), β-caryophyllene (0.6 %), (*E*)-α- bergamotene (1.2 %), (*E*)-β-farnesene (1.2 %), α and β-selinene (0.5 %, 0.8 %), bicyclogermacrene (0.4 %), β-bisabolene (0.3 %) and δ-cadinene (0.8 %) which are sesquiterpene hydrocarbons. The non terpene fraction of the oil include unsaturated aliphatic aldehyde (1.9 %), saturated aldehyde (10.7 %) with pentadecanal as the main component (7.0 %), aliphatic alcohols (1.7 %), amide (0.6 %) and heterocyclic compound (1.1 %). Other examples of fragrance impacting constituents that are non-terpene derivatives were hexanal (0.7 %), heptanal (0.5 %), 2-pentylfuran (1.1 %), nonanal (0.9 %), (3*E*)-nonen-1-ol (0.3 %), (2*E*)-nonenal (0.8 %), tridecanal (0.2 %), tetradecanal (0.9 %), pentadecanal (7.0 %), hexadecatrienal (0.6 %) and heptadecenal (0.5 %). All these compounds contributed to the good odour of the stem essential oil.

The flower essential oil is largely different from the stem, leaf and root oils since they were secreted in different trochomes, epidermal cells and internal secretory cells ot the plant (Dhifi *et al*., 2016). It contained fewer compounds which were all non-terpenes. Ten constituents corresponding to 100 % of the whole oil were identified in the flower oil. These are aliphatic alkanes (38.0 %), aliphatic alcohols (34.6 %), aliphatic aldehydes (21.2 %) and aromatic esters (6.2 %). The prominent components were 1-docosanol (34.6 %), nonadecane (16.3 %), heneicosane (12.5 %), pentadecanal (10.1 %) eicosanal (11.2 %) and pentacosane (4.9 %). In contrast, flower oils of Egypt origin consist of farnesol, geraniol,

phenylethyl benzoate, methyl pentadecane and α–terpinolieine.as the major constituents (Sengab *et al*., 2010). Goswami *et al*., (2016) reported benzyl salicylate (26.7 %), benzyl benzoate (22.3 %), (*E*,*E*)-geranyl linalool (9.4 %), tricosane (8.3 %), nonadecane (7.0 %) and (*E*)-nerolidol (7.0 %) as dominant compounds in the flower oil in India. Although, pentacosane and nonadecane were both found in appreciable quantity in the flower oil in the present study and the one grown in India, there are marked differences in the quality and quantity of the two volatile oils due to the difference in their geographical locations and some other facters explained by Dhifi *et al*., (2016). Benzyl benzoate, tricosane, heptacosane and heneicosane were found at varying concentrations in both oils. The terpenes identified in the latter was absent in this study even though most of them were found in traces. No aldehyde was reported in the formal whereas pentadecanal (10.1 %) and eicosanal (11.2 %) were in the present study. Essential oil of *P. rubra* flower from Hawaii had β-phenyl ethyl alcohol, phenyl acetaldehyde and methyl cinammate as the most abundant components (Omata *et al*., 1992) while the flower oil from Chinese source presented linalool (20.7 %), geraniol (16.2 %) and nerolidol (14.1 %) as major components (Xiao *et al*., 2011). Phenyl ethyl benzoate, lauric acid, palmitic acid, linalool, benzyl benzoate and benzyl salicylate were prominent components of reddish-orange flower of *P. rubra* as well as in the Malysian *P. rubra* red flower with the exception of linoleic acid, myristic acid, benzyl salicylate and benzyl benzoate (Tohar *et al*., 2006). Other reports from Malaysia gave account of high percentage of fatty acids like linoleic acid, palmitic acid, myristic acid and lauric acid in the pink and orange flowers (Shaida *et al*., 2008). Reddish-orange flower from Nigeria earlier characterised showed heneicosane (19.15 %), nonadecane (15.63 %), citronellol (14.63 %) and geraniol (9.17 %) as dominant constituents (Obuzor and Nweke 2011) while Lawal *et al*., (2015) reported (*E*)-non-2-en-1-ol (15.7 %), limonene (10.8 %), phenyl acetaldehyde (9.0 %) and *n*-tetradecanol (8.8 %) from different locations; east and west respectively. None of the principal compounds of Lawal *et al* (2014) were found in the present work. Analysis of flower essential oils of two *Plumeria rubra* Linn of China origin revealed high percentage of hydrocarbons (38.6 %) and carboxylic acids (59.7 %) respectively (Liu *et al*., 2012). The carboxylic acid was largely *n*-tetradecanoic acid (11.2 %) and *n*-hexadecanoic acid (35.8 %) while the main

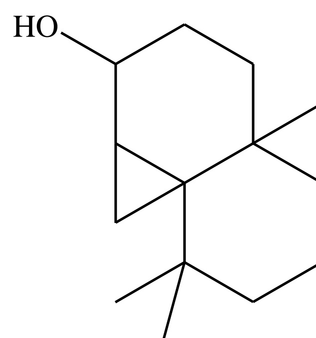
hydrocarbon constituents were 9-hexacosane (14.6 %), octadecanal (11.5 %) and *n*-octadecanol (8.4 %).

Comparing the oils extracted from *P. rubra* in this study, only pentadecanal which appeared in appreciable quantity in leaf, stem and flower oils was common to all out of 125 compounds identified altogether. 78 compounds were present in at least three oils, 32 appear in at least two oils while 20 were found only in one oil sample most of which were detected at low concentration as low as ≤ 0.1 %. As observed in the leaf oil, benzaldehyde, β-pinene, safranal and *Z*-cadina-1(6),4-diene were present in trace amount along with 0.1 % of 1-octanol, (2*E*,6*Z*)-nonadienal-1-ol, (2*E*)-nonen-1-ol, (6Z)-nonen-1-ol, 1-nonanol, methyl salicylate, β-cyclohomocitral, α-humulene, ishwarane, α- and γ-muurolene, eugenol, germacrene D, (*E*)-γ- and β-bisabolene, γ-cardinene, (*E*)-nerolidol, τ-cadinol, 1-pentadecanol and (9Z)-hexadecenal. Majority of the dominant components reported from other geographical zones in the previous work were absent in the *P. rubra* L. examined. The classes of compounds identified in the essential oils of *P. rubra* and *P. acuminata* are given in Table 4.6.



pogostol

4.19



thujospan-2α-ol

4.20

Figure 4.3: Sesquiterpenoids identified in *P. rubra* volatile oil

Table 4.5: Chemical Compositions of *Plumeria rubra* (Pink flower)

Constituents	RI	Percentage compositions (%)			
		Leaf	Stem	Flower	Root
Methylcyclohexane	718	0.1	-	-	0.1
Octane	798	0.1	-	-	0.5
Hexanal	800	0.4	0.7	-	0.8
Styrene	890	0.1	-	-	0.6
(4Z)-Heptenal	898	0.8	-	-	-
Heptanal	901	0.6	0.5	-	0.1
α- pinene	930	0.1	0.4	-	0.3
Benzaldehyde	960	tr	-	-	-
β-pinene	975	tr	-	-	-
1-Octen-3-ol	976	0.1	-	-	0.8
6-Methyl-5-hepten-2-one	981	0.1	-	-	-
2-Pentylfuran	987	1.2	1.1	-	-
Octanal	1002	-	-	-	0.3
p-Cymene	1022	0.1	2.2	-	-
Limonene	1027	0.2	0.7	-	0.3
2,2,6-trimethylcyclohexanone	1032	0.1	-	-	-`
1-Octanol	1068	0.1	-	-	-
Linalool	1097	0.7	-	-	0.5
Nonanal	1103	0.4	0.9	-	8.1
(2E,6Z)-Nonadienal	1150	1.9	-	-	-
p-Vinylanisole	1151	-	-	-	0.4
(3Z)-Nonen-1-ol	1152	-	0.3	-	-
(3E,6Z)-Nonadienol	1153	0.4	-	-	-
(2E)-Nonenal	1157	1.0	0.8	-	0.8
(2E,6Z)-Nonadien-1-ol	1162	0.1	-	-	-
(2E)-Nonen-1-ol	1165	0.1	-	-	-
(6E)-Nonen-1-ol	1167	0.1	-	-	-
1-Nonanol	1169	0.1	-	-	-

2,5-dimethylstyrene	1182	0.4	-	-	-
Methyl salicylate	1189	0.1	-	-	-
α-Terpineol	1193	0.1	0.4	-	-
Safranal	1196	tr	-	-	-
Decanal	1204	0.1	0.5	-	1.3
(2*E*,4*E*)-Nonadienal	1213	0.1	-	-	-
β-Cyclocitral	1216	0.2	-	-	-
Carvacrol methyl ether	1236	0.1	1.5	-	-
β-Cyclohomocitral	1253	0.1	-	-	-
(2*E*)-Decenal	1260	0.1	-	-	-
4,6-Dimethyldodecane	1271	-	-	-	0.2
(2*E*,4*E*)-Decadienal	1316	0.2	-	-	-
Eugenol	1348	0.1	-	-	-
α-Copaene	1373	0.8	1.8	-	0.9
β-Elemene	1386	0.4	2.5	-	0.2
Methyleugenol	1396	0.4	-	-	-
2,5-Dimethoxy-p-cymene	1409	-	0.3	-	-
β-Caryophyllene	1416	0.3	0.6	-	0.3
E-α- Bergamotene	1429	0.4	1.2	-	-
Geranyl acetone	1444	0.1	0.3	-	0.6
(*E*)-β-Farnesene	1449	0.4	1.2	-	-
α-Humulene	1452	0.1	-	-	-
allo-Aromadendrene	1457	0.1	-	-	0.2
Z-Cadina-1(6),4-diene	1459	tr	-	-	-
Ishwarane	1466	0.1	-	-	-
γ –Muurolene	1471	0.1	-	-	-
(*E*)-β-Ionone	1475	0.6	-	-	-
Germacrene D	1477	0.1	-	-	-
β-Selinene	1485	0.1	0.8	-	-
Bicyclogermacrene	1492	-	0.4	-	-
α-Selinene	1493	0.1	0.5	-	-

α-Muurolene	1495	0.1	-	-	-
β-Bisabolene	1504	0.1	0.3	-	-
Tridecanal	1507	0.4	0.2	-	-
γ-Cadinene	1509	0.1	-	-	-
Cubebol	1512	-	0.4	-	-
δ-Cadinene	1514	0.9	0.8	-	-
(*E*)-γ-Bisabolene	1523	0.1	-	-	-
10-epi-Cubenol	1526	0.2	-	-	-
Isocaryophyllene oxide	1548	-	0.9	-	-
(*E*)-Nerolidol	1557	0.1	-	-	-
Spathulenol	1573	0.1	34.7	-	0.2
Caryophyllene oxide	1578	0.2	17.8	-	-
Thujopsan-2α-ol	1589	-	-	-	0.2
Viridiflorol	1591	-	0.4	-	-
Hexadecane	1600	-	-	-	0.3
Humulene epoxide II	1606	0.1	1.7	-	-
Tetradecanal	1609	2.6	0.9	-	-
1,10- di-epi-Cubenol	1611	0.2	-	-	-
Caryophylla-4(12),8(13)-dien-5α-ol	1629	-	1.0	-	-
τ-Cadinol	1638	0.1	-	-	-
τ-Muurolol	1640	0.1	0.6	-	-
Cadalene	1669	0.1	-	-	-
α-Cadinol	1652	-	1.8	-	-
Pogostol	1655	-	1.1	-	-
14-Hydroxy-9-epi-(*E*)-caryophyllene	1667	-	1.3	-	-
1-Tetradecanol	1673	0.2	-	-	-
Acorenone	1686	-	-	-	0.2
(2Z,6Z)-Farnesal	1690	1.3	-	-	-
2-Pentadecanone	1694	0.3	-	-	-
Heptadecane	1700	0.1	-	1.6	0.2
Pentadecanal	1713	51.2	7.0	10.1	1.6

Myristic acid	1754	-	-	-	0.2
Benzyl benzoate	1762	-	-	2.3	-
(2E)-Pentadecenal	1771	0.3	-	-	-
1-Pentadecanol	1775	0.1	-	-	-
(9Z)-Hexadecenal	1789	0.1	-	-	-
Octadecane	1800	-	-	-	0.3
Hexadecanal	1813	0.2	-	-	-
Phytone	1837	0.3	-	-	0.4
2-Methylbenzyl benzoate	1850	-	-	3.9	-
1-Hexadecanol	1876	0.2	-	-	-
(*Z*)-Hexadecatrienal	1882	0.7	0.6	-	-
(*E*)-Hexadecatrienal	1886	1.9	-	-	-
Heptadecenal	1889	1.8	0.5	-	-
Nonadecane	1900	0.2	-	16.3	0.6
(5E,9E)-Fernesyl acetone	1904	0.2	-	-	0.4
Heptadecanal	1915	0.5	-	-	-
Methyl palmitate	1920	0.5	-	-	0.5
Palmitic acid	1959	9.0	-	-	30.0
Eicosane	2000	-	-	-	0.4
Heneicosane	2100	-	-	12.5	0.2
(E)-Phytol	2103	7.4	-	-	-
2-Nonadecanone	2104	-	-	-	0.3
Methyl stearate	2121	0.3	-	-	-
Linoleic acid	2123	-	-	-	8.0
Oleic acid	2130	0.4	-	-	18.8
Stearic acid	2155	-	-	-	2.0
Ethyl stearate	2188	-	-	-	0.3
Docosane	2200	-	-	-	0.4
Eicosanal	2221	-	-	11.2	-
Tricosane	2300	0.1	-	2.7	0.3
(9Z)-Octadecenamide	2349	-	0.6	-	3.5

Tetracosane	2400	0.1	-	-	0.3
1-Docosanol	2484	-	1.4	34.6	0.3
Pentacosane	2500	0.2	-	4.9	0.4
(9Z)-Docosenamide	2754	-	-	-	11.4
Percentage identified		97.9	93.6`	100.0	99.0
Number identified		94	42	10	47

RI- Retention indices from the analyses value.

Table 4.6: Comparison of Classes of Components Identified in *P. acuminata* and *P. rubra* Essential Oils

Classes	L1		L2		F1		F2		R1		R2	
	PC (%)	NI	PC (%)	NI	PC (%)	NI	PC (%)	NI	PC (%)	NI	PC (%)	NI
Monoterpene hydrocarbons	3.8	2	0.4	4	2.9	4	-	-	-	-	0.6	2
Oxygenated monoterpenes	18.7	4	1.4	6	16.2	10	-	-	-	-	0.5	1
Sesquiterpene hydrocarbons	8.9	7	4.3	19	1.1	10	-	-	-	-	1.6	4
Oxygenated sesquiterpenes	1.1	1	2.4	9	5.6	6	-	-	47.6	5	0.6	3
Apocarotenoids	7.2	2	0.9	4	0.1	2	-	-	-	-	1.0	2
Diterpene	6.8	1	7.4	1	0.5	1	-	-	-	-	-	-
Non-terpene derivatives	22.5	9	80.5	51	9.0	52	100.0	10	49.8	7	94.3	37
Total identified	69.0	26	97.7	94	99.8	85	100.0	10	97.4	12	98.6	49

1- *P. acuminata* PC- Percentage Composition NI- Number identified
2- *P. rubra*
L- Leaf
F- Flower
R- Root
(-)- Not detected

4.5 *Delonix regia* Essential Oil Chemical Constituents

The chemical profile of the essential oils of *Delonix regia* arranged in order of elution from a HP-5MS is presented in **Table 4.7**. Twenty-three (23) volatile components were identified in the twig oil, 13 in the flower oil and 39 in the root oil, representing a total of 99.9 %, 96.9 % and 94.5 % of the whole oils respectively. The twig oil was largely made up of non-terpene hydrocarbons (97.6 %) which were long chain aliphatic alcohols, alkanes, amide and aldehydes. The dominant constituents were (9Z)-docosenamide (51.6 %) **[4.23]**, pentacosane (6.1 %), (9Z)-octadecenamide (5.1 %) **[4.21]** and 1-docosanol (4.9 %) **[4.24].** Acorenone B is the only sesquiterpenoid while iso-phytol (0.4 %) and phytol (0.7 %) were the diterpenoids identified in the oil as the terpene fraction. Other compounds that were also present in noticeable quantity were heptacosane (4.0 %), tetracosane (3.9 %), tricosane (3.8 %), docosane (2.7 %), pentadecanal (2.5 %), hexacosane (2.3 %), 3-methylheptacosane (1.7 %), hexadecanal (1.6 %) and heneicosane (1.5 %). Docosanol (also known as behenyl alcohol), one of the prominent constituents is a saturated fatty alcohol used as nutritional supplement, emulsifier, emollient and thickener in cosmetics (Sacks *et al*., 2001), and also possesses antiviral activity (Treister and Woo, 2010).

The root essential oil comprises twenty-eight non-terpenes (74.0 %) and eleven terpenes (20.5 %). The most abundant compounds in the root oil were phytone (28.0 %) **[4.22]** and phytol (12.7 %). High percentage of non-terpenes mainly palmitic acid (6.2 %), (9*Z*)-tetradecen-1-ol (6.0 %) and (9*Z*)-octadecenamide (5.0 %) were also present. Other diterpenes found were neophytadiene (1.5 %), (5*E*, 9*E*)-fernesyl acetone (1.5 %), (*E*)-phytyl acetate (1.0 %) and isophytol (0.9 %). Monoterpenes and sesquiterpenes with their oxygenated derivatives were also identified as minor constituents in the root oil. Among these minor components were odour impacting compounds like α-pinene (0.7 %), limonene (0.7 %), linalool (0.8 %), geranyl acetone (0.6 %) as well as (*E*)-β-ionone (0.8 %), the only apocarotenoid found in the root oil.

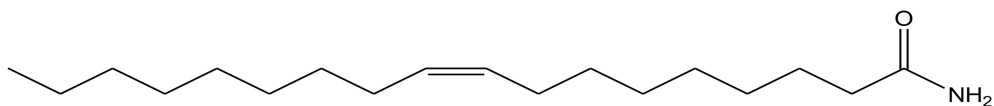
In the flower oil, 2 oxygenated sesquiterpenes namely τ and α cadinol and non-terpenes; 1 amide, 6 hydrocarbons, 1 alcohol, 1 acid and 2 ketones-all together thirteen compounds were identified. The two major components were (9Z)-docosecenamide (40.1 %) and heptacosane (40.2 %). Docosenamide recently recognized as oleamide is a monocarboxylic

acid amide derived from oleic acid. Heptacosane is a hydrocarbon lipid molecule that can be found in many food items like sweet cherry and wild carrot (Staniszewska *et al*., 2003; Peschel *et al*., 2007).

Table 4.8 showed comparison of the classes of compounds identified in the twig, flower and root essential oils of the plant. There were marked variations in the oil constituents of *D. regia* root and the other two parts studied (twig and flower). Monoterpene and sesquiterpene hydrocarbons along with oxygenated monoterpenes were found in small percentages in the root oil but absent in the twig and flower oils. Both twig and flower oils contained at different levels, (9Z)-docosecenamide, pentacosane and heptacosane as dominant constituents. These compounds account for approximately 61.7 % and 86.8 % of the chemical constituents of the twig and flower essential oils respectively. (9Z)-octadecenamide was present in almost the same proportion in the root and twig oils (5.0 % and 5.1 %). Other regular compounds in the twig and root were two oxygenated diterpenes (*iso*-phytol and phytol), two aliphatic aldehydes (nonanal and pentadecanal) and four alkanes (octadecane, eicosane, docosane and tetracosane). All the oil samples have large percentage of non-terpenes, with nonadecane, heneicosane, tricosane, pentacosane and 1-docosanol as the common constituents. Some compounds were common to both flower and twig oils; they are nonadecane, heneicosane, heptacosane, (9Z)-docosecenamide and 3-methylheptacosane, all of which are likely to contribute largely to their character note. Only 2 compounds were common to flower and root oils but absent in the twig oil. The essential oil constituents of twig, flower and root of *D. regia* are characterised for the first time in this study.

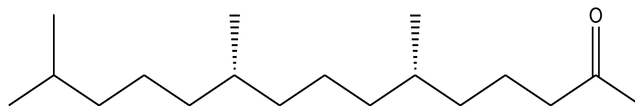
No data was found in the literature on the chemical profile of essential oils from the twig, flower and root of the plant but Salem *et al*., (2014), gave account of the wood essential oil comprising of naphthalene derivatives, fatty acids, alkanes as well as monoterpenoids. The only report from Nigeria is the determination of the phytochemical constituents of the seed fixed oil, examination of the nutritional value of the oil and its benefit in both nutrition and industrial sector (Abulude and Adejayan 2017; Oyedeji *et al*., 2017). However, a large number of compounds were identified in the *Cassia fistula* flower oil, a member of the Fabaceae family but a different genus (Tzakou *et al*., 2007) from *D. regia*. Compounds like

(*E*)-nerolidol (38.0 %), 2-hexadecanone (17.0 %), methyl eugenol (7.3 %) and nonacosane (6.5 %) were the most abundant constituents, but these were absent in the present work. Tricosane (2.1 %, 5.9 %), heptacosane (40.2 %, 12.8 %) and pentacosane (6.5 %, 6.1 %) were the common components in both *D. regia* and *C. fistula* flowers essential oils.



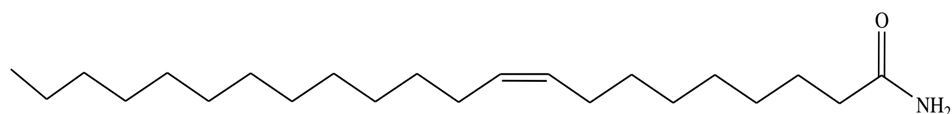
(9Z)-octadecenamide

4.21



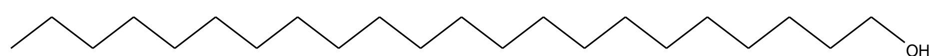
phytone

4.22



(9Z)-docosenamide

4.23



1-docosanol

4.24

Figure 4.4: Constituents of *D. regia* volatile oils

Table 4.7: Chemical Constituents of *Delonix regia*

		Percentage compositions (%)		
Constituents	RI	Flowers	Root	Twig
Octane	800	-	0.5	-
Hexanal	801	-	0.5	-
α-Pinene	931	-	0.7	-
1-Octen-3-ol	977	-	0.5	-
3-Octanone	983	-	0.6	-
Limonene	1027	-	0.7	-
Linalool	1098	-	0.8	-
Nonanal	1103	-	2.8	1.2
(2E)-Nonen-1-al	1159	-	0.8	-
Geranyl acetone	1444	-	0.6	-
Ishwarane	1466	-	0.6	-
(E)-β-Ionone	1475	-	0.8	-
Pentadecane	1500	-	0.6	-
Hexadecane	1600	-	0.6	-
τ-Cadinol	1638	1.0	-	-
α-Cadinol	1651	0.3	-	-
(9Z)-Tetradecen-1-ol	1665	-	6.0	-
1-Tetradecanol	1674	-	0.8	-
Acorenone B	1686	-	-	1.2
2-Pentadecanone	1693	1.3	-	-
Heptadecane	1700	-	0.9	-
Pentadecanal	1711	-	1.5	2.5
Octadecane	1800	-	0.8	0.3
Neophytadiene	1832	-	1.5	-
Phytone	1837	1.4	28.0	-
Phytadiene	1875	-	0.6	-
(*Z*)-Hexadecatrienal	1881	-	-	1.2
(*E*)-Hexadecatrienal	1886	-	-	1.0

Hexadecenal	1889	-	-	1.6
Nonadecane	1900	0.7	1.1	0.7
(5E,9E)-Fernesyl acetone	1904	-	1.5	-
Heptadecanal	1915	-	-	0.5
Methyl palmitate	1921	-	0.5	-
iso-Phytol	1941	-	0.9	0.4
Palmitic acid	1953	0.7	6.2	-
Eicosane	2000	-	1.1	1.0
Heneicosane	2100	0.9	1.2	1.5
Phytol	2103	-	12.7	0.7
Methyl stearate	2121	-	0.6	-
Oleic acid	2129	-	1.7	-
Docosane	2200	-	1.0	2.7
(*E*)-Phytyl acetate	2208	-	1.0	-
Tricosane	2300	2.1	1.3	3.8
(9Z)-Octadecenamide	2350	-	5.0	5.1
Tetracosane	2400	-	1.2	3.9
1-Docosanol	2484	1.6	3.6	4.9
Pentacosane	2500	6.5	2.7	6.1
Hexacosane	2600	-	-	2.3
Heptacosane	2700	40.2	-	4.0
(9Z)-Docosecenamide	2754	40.1	-	51.6
3-Methylheptacosane	2778	1.1	-	1.7
Percentage identified		96.9	94.5	99.9
Total identified		13	39	23

RI- Retention indices from the analyses value.

Table 4.8: Classes of Components Identified in *D. regia* Essential Oils

Classes	Twig		Flower		Root	
	PC (%)	NI	PC(%)	NI	PC(%)	NI
Monoterpene hydrocarbons	-	-	-	-	1.4	2
Oxygenated monoterpenes	-	-	-	-	1.4	2
Oxygenated Sesquiterpenes	1.2	1	1.3	2	1.5	1
Sesquiterpene hydrocarbons	-	-	-	-	0.6	1
Diterpenoids	1.1	2	-	-	13.4	2
Apocarotenoids	-	-	-	-	0.8	1
Non-terpene derivatives	97.6	20	96.6	11	75.0	27

PC- Percentage composition; NI- Number identified

4.6 *Duranta repens* Essential Oil Chemical Constituents

The results of GC-MS analysis of essential oils from *D. repens* leaf, fruit, stem and root are displayed in Table 4.9 and compunds were listed as eluted from HP-5MS column. The leaf oil contained 20 non terpenes, 3 oxygenated monoterpenes, 1 oxygenated sesquiterpene, 3 oxygenated diterpene and 3 apocarotenoids: altogether 30 compounds amounting to 92.5 % of the entire oil. The non-terpene derivatives were aliphatic alcohols mainly 1-octen-3-ol (12.9 %) and 3-octanol (4.0 %), and aromatic and aliphatic aldehydes (1.4 % and 4.2 %). In addition, heterocyclic compound (2-pentylfuran, 0.4 %), aromatic ketone (acetophenone, 0.5 %), aromatic ether (*p*-vinylanisole, 11.7 %) **[4.25]**, aliphatic ketone (4-methyl-2-pentanone, 0.4 %) were found in the leaf oil. Other compounds identified were cyclic alkane (methylcyclohexane, 1.3 %), saturated fatty acids dominated by palmitic acid (6.8 %) and amide ((9*Z*)-octadecenamide, 6.4 %) **[4.21]**. Furthermore, benzene derivatives present in the volatile oil were toluene (13.2 %) and styrene (0.6 %). In addition, geranyl acetone (4.3 %), phytone (3.7 %), phytol (3.3 %), (*E*)-β-ionone (2.7 %), (5*E*,9*E*)-farnesyl acetone (2.7 %), and (*E*)-α-ionone (2.6 %), were found in significant quantities. Quite the reverse was reported by Thomas *et al*. (2019).

The *D. repens* leaf volatile oil afforded 47 constituents equivalent to 95.8% of the whole oil and the oil contained appreciable quantity of sesquiterpenes (43.1%) and monoterpenes (26.3%). The dominant constituents were limonene 11.6%, β-caryophyllene 7.5%, pentadecanal 6.7% and humulene 5.0%. In the *Lippa stachyoides* var. martiana (Verbenaceae) leaf oil analysed by Silva *et al*. (2014), spathulenol, δ-cadinene, caryophyllene oxide, (*E*)-nerolidol and cubebol were reported as principal constituents, which were all absent in the *D. repens* leaf oil.

A total of twenty compounds were identified in the fruit oil, equivalent to 98.1% of the whole oil. The volatile oil was dominated by nonterpene derivatives (91.2 %). The prevailing constituents are styrene (52.5 %) **[4.26]** and vinylanisole (26.0 %) **[4.25]**. The monoterpenes that were present include α- pinene (0.4%), mesitylene (0.6%), γ-terpinene (0.3%), limonene (0.4%), terpinen-4-ol (0.3%) and linalool (0.6%). Cuparene (0.7%), and α-bisabolol (0.8%) were the sesquiterpenes detected in the fruit volatile oil and (*E*)-β-ionone is the only apocarotenoid present in the oil. Styrene is known to be carcinogenic

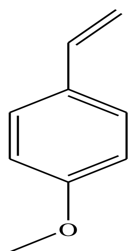
especially when high level is inhaled, ingested or come in contact with skin or eye (Arnedo-Pena *et al*., 2003). Although the *D. repens* friut was earlier reported to be poisonous but there was no report on the presence of styrene as the component of the fruit that is responsible for the toxicity. Styrene is a precursor to many copolymers manufacture which is its main commercial significance.

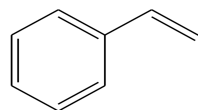
Thirty-five compounds were detected in the root essential oil corresponding to 93.2 % of the oil. Like the leaf and fruit oils, the root oil is mainly non-terpenes. The terpenes found in the volatile oil were limonene (0.3 %), acorenone (0.3 %) and bulnesol (0.2 %). On the other hand, ester (0.7 %), fatty acids (67.5 %), aliphatic aldehyde (2.1 %), ketone (0.3 %), alcohols (3.0 %), amide (4.4 %), alkene (0.1 %) and benzene derivatives (4.4 %) along with cyclic and acyclic alkanes (2.2 % and 6.7 %) were classess of compounds identified as the non-terpene components. The oil was dominated by palmitic acid (55.7 %) **[4.27]** followed by oleic acid (6.3 %) **[4.28]**. Other notable constituents were methylcyclohexane (5.0 %) an hydrocarbon, linoleic acid (4.3 %) **[4.29]** and (9Z)-octadecenamide (3.8 %) **[4.21]**. Palmitic acid, oleic acid and linoleic acid which made up 66.5 % of the root oil are important fatty acid with significant industrial applications. Palmitic acid is a significant material in food industry as well as soap and cosmetic ingredients (Hermans *et al*., 2016), Oleic acid, which was the second most abundant compound in the fruit oil is known as an emulsifying agent in aerosol products and soap as well as excipient in drug industries. It is an insect pheromone, and also an emollient. Oleic acid has many health benefits such as wound healing, improves immune system, antiiflammatory, anticancer, reduces cholesterol and blood pressure reduction (Sales-Campos *et al*., 2013). Linoleic acid possesses skin lightening, acne reductive, anti-inflammatory and moisture retentive properties when applied on the skin and this makes it popular in cosmetic world (Shigeta *et al.,* 2004; Zulet *et al.,* 2004). Besides, linoleic acid is a vital ingredient in oil paints and varnishes, an essential fatty acid in human diet for good health, a pheromone and its lipid radical can be utilized to demonstrate the antioxidant activity of natural phenols and polyphenols (Zheng and Wang, 2001).

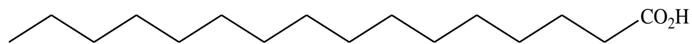
Analysis of the stem volatile oil revealed the presence of twelve compounds. The oil comprised mainly alkanes of high molecular weight: tetracosane, tricosane, pentacosane,

docosane and heneicosane which were present as the abundant constituents in the quantities 22.2 %, 19.3 %, 18.0 %, 14.2 % and 8.1 % respectively. Other constituents in appreciable quantity were (9Z)-octadecenamide (3.8 %) and 1-docosanol (5.0 %). A diterpene, phytol (0.8 %) was the only terpene found in the oil was which is not often present in hydrodistilled essential oils because of its high molecular weight. Higher alkanes (hexadecane upward) are essential constituents of lubricating oil because they act at the same time as anti-corrosive agents. They are solid at ambient temperature and many find applications in the manufacture of candles and electrical insulators. They also constitute major components of fuel oil. Heneicosane is a termites and mosquitoes' pheromone. Eicosane has little application in petrochemical industry but n-eicosane (straight chain structural isomer of eicosane) is an important component of paraffin waxes that is useful in candle production (Stachowiak and Batchelor, 2014).

There is marked difference among the oil samples from different plant parts. None of the compounds found in the stem oil were seen in the fruit oil. Furthermore, 1-octene-3-ol is present in the leaf, fruit and root oils at 12.9 %, 0.7 %, 0.5 % in that order, likewise n-nonanol 0.4 %, 0.4 % and 1.7 %. (9Z)-octadecenamide is common to the leaf, stem and root oils in the quantity 6.4 %, 3.8 % and 3.8 % respectively. There were many components present in the leaf and root oils but absent in both the stem and fruit oils. Generally, not many terpenes were discovered in the *D. repens* oils. Phytol was the only terpene in the stem oil. Terpene fraction of the leaf oil was also absent in other oils extracted from the plant except linalool that was found in the fruit volatile oil and phytol in the stem oil Table 4.9. The summary of classes of compounds identified in *D. repens* essential oils is presented in Table 4.10.



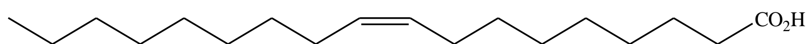




vinylanisole **4.25**

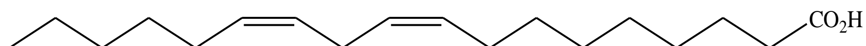
styrene **4.26**

palmitic acid **4.27**



oleic acid

4.28



linoleic acid

4.29

Figure 4.5: Most abundant constituents in *D. repens* volatile oils

Table 4.9: Chemical Compositions of *Duranta repens*

Constituents	RI	Percentage compositions (%)			
		Leaves	Fruit	Stem	Root
Z-1,3-Dimethylcyclopentane	716	-	-	-	0.8
Methylcyclohexane	718	1.3	-	-	5.0
2-Methyl-2-pentanol	723	2.4	-	-	0.7
Ethylcyclopentane	726	-	-	-	0.2
3-Methyl-3-pentanol	745	1.4	-	-	0.4
2-Methylheptane	754	-	-	-	0.2
Toluene	760	13.2	-	-	3.6
3-Methylheptane	763	-	-	-	0.3
Z-1,3-Dimethylcyclohexane	777	-	-	-	0.3
E -1,3-Dimethylcyclohexane	780	-	-	-	0.2
2-Hexanone	785	-	-	-	0.3
4-Methyl-2-pentanone	786	0.4	-	-	-
1-Methylcyclopentanol	792	1.4	-	-	1.0
Octane	800	-	-	-	0.4
Hexanal	801	1.9	-	-	0.4
(2*E*)-Octene	828	-	-	-	0.1
Ethylcyclohexane	832	-	-	-	0.2
(2*E*)-Hexanal	849	1.9	-	-	-
Ethylbenzene	856	-	-	-	0.5
p-Xylene	866	-	-	-	0.3
Ethylbenzene	891	-	1.2	-	-
o-Xylene	895	-	1.3	-	-
Styrene	890	0.6	**52.5**	-	-
α-Pinene	935	-	0.4	-	-
Mesitylene	957	-	0.6	-	-
Benzaldehyde	960	1.4	-	-	-
1-(1-Methyl-cyclopentyl)-ethanone	962	-	3.5	-	-
1-Octen-3-ol	977	12.9	0.7	-	0.5

2-Pentylfuran	987	0.4	-	-	-
3-Octanol	995	4.0	-	-	-
Limonene	1027	-	0.4	-	0.3
γ-Terpinene	1050	-	0.3	-	-
Acetophenone	1064	0.5	-	-	-
Linalool	1098	1.4	0.6	-	-
n-Nonanal	1103	0.4	0.4	-	1.7
p-Vinylanisole	1150	11.7	26.0	-	-
Terpinen-4-ol	1175	-	0.3	-	-
Naphthalene	1177	-	0.4	-	-
α-Terpineol	1193	0.4	-	-	-
β-Cyclocitral	1216	0.4	-	-	-
p-Ethylguaiacol	1285	-	0.9	-	-
4-Ethyl-1,2-dimethoxybenzene	1335	-	1.2	-	-
3,4-Dimethoxystyrene	1362	0.6	2.5	-	-
(*E*)-α-Ionone	1418	2.6	-	-	-
Geranyl acetone	1444	4.3	-	-	-
(*E*)-β-Ionone	1475	2.7	0.4	-	-
Cuparene	1500	-	0.7	-	-
Dodecanoic acid	1556	-	-	-	0.6
Bulnesol	1662	-	-	-	0.2
Acorenone	1686	-	-	-	0.3
α-Bisabolol	1682	-	0.8	-	-
Myristic acid	1754	-	-	-	0.6
Phytone	1837	3.7	-	-	-
Nonadecane	1900	-	-	1.8	-
(5*E*,9*E*)-Farnesyl acetone	1904	2.7	-	-	-
Palmitic acid	1957	6.8	-	-	55.7
Ethyl palmitate	1989	-	-	-	0.7
Eicosane	2000	-	-	4.2	-
Abietadiene	2080	0.5	-	-	-

Heneicosane	2100	-	-	8.1	-
Phytol	2102	3.3	-	0.8	-
Linoleic acid	2123	-	-	-	4.3
Oleic acid	2130	-	-	-	6.3
(9*E*)-Hexadecanamide	2170	0.9	-	-	0.6
Docosane	2200	-	-	14.2	-
2-Methyltetracosane	2259	-	-	0.9	-
Tricosane	2300	-	-	19.3	0.3
(9*Z*)-Octadecanamide	2350	6.4	-	3.8	3.8
3-Methyltricosane	2368	-	-	1.2	-
Tetracosane	2396	-	-	22.2	0.5
1-Docosanol	2484	-	-	5.0	0.4
Pentacosane	2495	-	-	18.0	0.5
Percentage identified		92.5	95.1	99.5	92.2
Total identified		30	20	12	35

RI- Retention indices from the analyses value.

Table 4.10: Classes of Components Identified in *D. repens* Essential Oils

Classes	Leaf		Fruit		Stem		Root	
	PC (%)	**NI**	**PC (%)**	**NI**	**PC (%)**	**NI**	**PC (%)**	**NI**
Monoterpene hydrocarbons	-	-	1.1	3	-	-	0.3	1
Oxygenated monoterpenes	6.1	3	0.9	2	-	-	-	-
Sesquiterpene hydrocarbons	-	-	0.7	1	-	-	-	-
Oxygenated sesquiterpenes	2.7	1	0.8	1	-	-	0.5	2
Apocarotenoids	5.7	3	0.4	1	-	-	-	-
Diterpenes	7.5	3	-	-	0.8	1	-	-
Non-terpene derivatives	70.5	21	91.2	12	98.7	11	91.4	32

PC- Percentage composition; NI- Number identified

4.7 *Zanthoxylum zanthoxyloides* Essential Oils Chemical Constituents

The chemical compounds identified in the volatile oils of the laef and stem bark of *Zanthoxylum zanthoxyloides* as eluted from HP-5MS column were reported in Table 4.1 while the classes of the identified constituents were given in Table 4.12. The leaf essential oil comprised of 11 components equivalent to 88.4 % of the total oil. Two oxygenated sesquiterpenes; carryophyllene oxide (27.0 %) and humulene epoxide II (8.0 %); two monoterpene hydrocarbons, α-pinene (16.0 %) and limonene (5.2 %); a ketone, 1-(1-methyl-cyclopentyl)-ethanone (11.2 %); and an alcohol, 4-methyl-3-hexanol (8.5 %) were the prominent constituents identified in the leaf oil. Other compounds present were ethylbenzene (3.7 %) and *o*-xylene (4.3 %), which were benzene derivatives, decanal (2.0 %) an aliphatic aldehyde, β-carryophyllene (1.6 %) and α-humulene (0.9 %) which are sesquiterpene hydrocarbons. In contrast, the leaf oil reported by Menut *et al* (2000) from Benin city contained only monoterpene hydrocarbons (98.2 %) dominated by myrcene (30 0 %) and (*E*)-β-ocimene (31.9 %) (that were absent in the present study) and α-pinene (26.5%) (which was detected in 16.0 % and 38.5 % concentration of the leaf and stembark oils in the current work). Other reported volatile compounds from previous work done in Nigeria were farnesol, farnesal, nerolidol, methyleugenol, α- and β- farnesene which are all absent in the present work (Oyedeji *et al*., 2008). *Z. zanthoxyloide*s leaf from Côte d'Ivoire was characterised largely by sesquiterpene and monoterpene hydrocarbons with the high percentage of germacrene D (10.2 %), myrcene (10.0 %) (which were not detected in this study), in addition to limonene (7.1 %) and β-caryophyllene (7.0 %) present at 5.2 % and 1.6 % respectively in this report (Affouet *et al*., 2012). The observed disparity in the chemical constituents of the *Z. zanthoxyloide*s leaf volatile oils from different regions of Nigeria and Côte d'Ivoire could be attributed to period of harvest, extraction procedure, geographical and genetic factors (Dhifi *et al*., 2016).

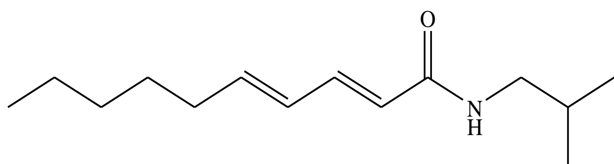
Forty-six compounds corresponding to 99.5 % of the whole oil were identified in the volatile oil of the stembark. There were 43.3 % monoterpenes, 6.8 % hydrogenated sesquiterpenes majorly α-humulene (2.5 %) and β-caryophyllene (2.4 %) and 2.8 % oxygenated sesquiterpenes as terpene fractions. The non-terpenes derivatives were aliphatic alcohols (1.0 %), cyclic alkanes (7.0 %), unsaturated amide (13.9 %), aromatic compounds

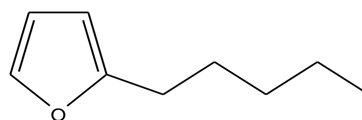
(3.0 %), fatty acids (7.2 %), ketones (0.9 %), aliphatic alkanes (0.4 %) and heterocyclic compound (0.9 %). The oil was composed largely of monoterpene hydrocarbons (42.0 %), mainly α-pinene (38.5 %), a good scent compound. Most of the components identified in the stem bark oil were good fragrant ingredients. They include 2-pentyl furan (0.9 %) **[4.31],** bornyl acetate (0.3 %) **[4.32],** ethyl-(*E*)-cinnamate (0.3 %) **[4.33]**, limonene (1.4 %), linalool (0.2 %) and cedrol (2.4 %). Other dominant constituents were pellitorine (13.9 %) **[4.30]**, hexanal (9.7 %), palmitic acid (6.2 %) and methylcyclohexane (5.2 %). Hexanal is a perfume ingredient while pellitorine is an unsaturated secondary amide, toxic and possessing insecticidal activity. It is likely one of the components responsible for the peppery taste of the stem bark because Bowden and Ross had earlier identified pellitorine as hot peppery component of the root extract of the plant (Guendéhou *et al*., 2018). It had also been isolated from other *Zanthoxylum* species (Mizutani *et al*., 1988; Gregor, 1984; Adesina *et al*., 1997). On the contrary, the stem bark essential from Côte d'Ivoire was dominated by linalool 49.5 % which was minor constituent in this study and *E*-nerolidol 11.3 % which was not detected at all (Affouet *et al*., 2012)

Comparing the chemical constituents of the leaf and stem bark oils, all the compounds in the leaf oil were also found in the stem bark oil beside 4-methyl-3-hexanol, 1-(1-methyl-cyclopentyl)-ethanone and humulene epoxide II which were among the main constituents of the leaf oil. The compounds present in both oils were, α-pinene which was prominent in the leaf and stem bark essential oils at 16.0 % and 38.5 % concentrations respectively. Others are ethylbenzene (3.7 %, 0.4 %), *o*-xylene (4.3 %, 0.3 %), limonene (5.2 %, 1.4 %), decanal (2.0 %, 0.4 %), β-carryophyllene (1.6 %, 2.4 %), α-humulene (0.9 %, 2.5 %) and carryophyllene oxide (27.0 %, 0.3 %) in the leaf and stembark volatile oils respectively.

The chemical composition reported in this study is quite different from earlier work on other *Zanthoxylum* species from other geological region (Table 4.13)**.** *Zanthoxylum leprieurii* Guill and Perr. leaf and stem bark oils from Cote d' Ivoire have undecan-2-one (10.0 % and 30.3 %) and tridecan-2-one (Tanoh *et al*., 2018) as prominent constituents. The Cameroonean fruit oil is composed of (*E*)-β-ocimene as the most abundant component (Misra *et al*., 2013, Lamaty *et al*., 1989). None of these dominant constituents were found in this study. The leaf volatile oil of *Z. alatum* from Himalaya and Northern Indian leaf oil

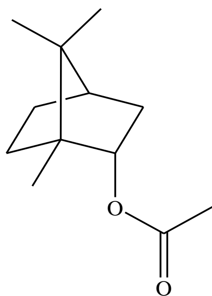
have high percentage of linalool (30.58 %, 71.0 %) and β-phellandrene (5.99 %, 5.7 %) (Guleria *et al*., 2013; Jain *et al*., 2001). Additional major constituents in the oil from Himalaya were 2-decanone (20.85 %), β-fenchol (9.43 %) and 2-tridecanone (8.86 %) while the other contained limonene (8.2 %). Shafi *et al*., (2000) gave account of the leaf essential oil from Kerala, India having caryophyllene oxide (12.7%) and β-caryophyllene (9.6 %) as the most abundant constituents. Limonene (11.94%) and undecan-2-one (11.74%) were the main components in *Z. armatum* leaf volatile from Nepal (Phuyal *et al*., 2019). Like the leaf oil presented by Affouet *et al* (2012), *Z. nitidum* leaf essential oil from Vietnam (Tuyen *et al*., 2021) was predominated by monoterpene (limonene 44.3 %, linalool 11.0 %, germacrene D 5.3 %) and sesquiterpene hydrocarbons (β-caryophyllene 12.5 %). Likewise, Negi *et al*., (2012) accounted for high monoterpenes and sesquiterpenes in *Z. armatum*, having bornyl acetate (16.61 - 22.66 %), *p*-cymene (8.25 - 12.50 %) and α-copaene (7.54 - 7.59 %) as the major constituents. Comparison of the main constituents of *Zanthoxylum* leaf and stembark oils from different geographical areas are summarised in Table 4.13.

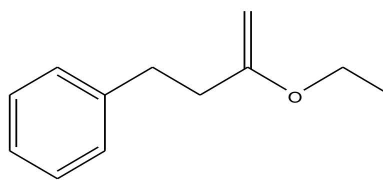




Pellitorine **2-pentyl furan**

4.30 **4.31**





bornyl acetate **ethyl-(*E*)-cinnamate**

4.32 **4.33**

Figure 4.6: Non-volatile constituents present in *Z. zanthoxyloides* essential oil

Table 4.11: Chemical Constituents of *Zanthoxylum zanthoxyloide*

		Percentage compositions (%)	
Constituents	RI	Leaf	Stem bark
(*Z*)-1,2-dimethylcyclopentane	716	-	0.8
Methylcyclohexane	718	-	5.2
Ethylcyclopentane	726	-	0.3
3-methyl-3-pentanol	744	-	0.2
2-methylheptane	754	-	0.2
Toluene	760	-	1.7
3-methylheptane	763	-	0.2
(*Z*)-1,3-Dimethylcyclohexane	777	-	0.3
(*E*)-1,3-Dimethylcyclohexane	780	-	0.2
Hexanal	799	-	9.7
(*Z*)-1,4-Dimethylcyclohexane	807	-	0.2
Ethylbenzene	856	3.7	0.4
o-Xylene	865	4.3	0.3
1-Hexanol	862	-	0.2
p-Xylene	867	-	0.2
Heptanal	901	-	0.3
4-Methyl-3-hexanol	905	8.5	-
α-Pinene	930	16.0	38.5
Camphene	947	-	0.2
1-(1-Methyl-cyclopentyl)-ethanone	961	11.2	-
Sabinene	970	-	0.4
β-pinene	975	-	1.3
6-Methyl-5-hepten-2-one	981	-	0.3
2-Pentylfuran	987	-	0.9
1-Octanal	1002	-	0.4

2-Ethylhexanol	1025	-	0.3
Limonene	1026	5.2	1.4
(2*E*)-Octenal	1055	-	0.2
Terpinolene	1083	-	0.2
Linalool	1097	-	0.2
Nonanal	1103	-	1.3
Naphthalene	1182	-	0.4
Decanal	1204	2.0	0.4
Bornyl acetate	1281	-	0.3
α-Terpinyl acetate	1343	-	0.8
β-Caryophyllene	1416	1.6	2.4
α-Humulene	1452	0.9	2.5
Ethyl-(*E*)-cinnamate	1464	-	0.3
Germacrene D	1477	-	0.8
δ-Cadinene	1514	-	0.8
Germacrene-D-4-ol	1573	-	0.1
Caryophyllene oxide	1575	27.0	0.3
Humulene epoxide II	1602	8.0	-
Cedrol	1605	-	2.4
Pellitorine	1929	-	13.9
Palmitic acid	1954	-	6.2
1-Octadecanol	2079	-	0.3
Oleic acid	2129	-	1.0
Oxacyclononadec-6-en-2-one	2133	-	0.6
Number Identified		11	46
Percentage identified		88.4	99.5

RI- Retention indices from the analyses value.

Table 4.12: Classes of Components Identified in *Z. zanthoxyloides* Essential Oils

Classes	Leaf		Stem bark	
	PC (%)	**NI**	**PC (%)**	**NI**
Monoterpene hydrocarbons	21.2	2	42.0	6
Oxygenated monoterpenes	-	-	1.3	3
Sesquiterpene hydrocarbons	2.5	2	6.8	5
Oxygenated sesquiterpenes	35.0	2	2.8	3
Non-terpene derivatives	29.7	5	46.6	29

PC- Percentage composition; NI- Number identified

Table 4. 13: Comparison of Dominant Constituents in Present and Previous Studies

Constituents	Percentage composition										
	ZZ1	ZZL	ZLL	ZAL1	ZAL2	ZAL3	ZRL	ZGL	ZNL	ZZ2	ZGSb
carryophyllene oxide	27.0	2.0	-	-	-	2.23	12.7	-	0.96	0.3	-
α-pinene	16.0	2.8	2.48	0.42	-	4.11	6.6	0.3	4.94	38.5	0.80
limonene	5.2	7.1	-	11.94	-	-	tr	0.4	44.3	1.4	-
germacrene D	-	10.2	1.96	-	-	-	0.8	-	5.3	0.8	0.9
1-(1-Methyl-cyclopentyl)-ethanone	11.2	-	-	-	-	-	-	-	-	-	-
β-caryophyllene	1.6	7.0	13.51	-	-	-	9.6	2.0	12.5	2.4	1.8
humulene epoxide II	8.0	1.2	-	-	-	-		-	0.18	-	-
tridecan-2-one	-	-	18.74	2.03	-	8.86		-	-	-	-
E-β-ionone	-	-		-	-	-	tr	-	-	-	-
Undecan-2-one	-	-	8.51	11.74	-	-	tr	-	0.42	-	-
myrcene	-	10.0	0.71	2.08	-	-	-	-	0.90	-	1.30
Bornyl acetate	-	-	-	-	19.03	-	-	-	-	0.3	-
linalool	-	0.7	6.44	62.77	3.38	30.58	0.1	47.0	11.0	0.2	49.5
decanal	2.0	6.4	-	-	-	-	-	-	-	0.4	-
β-fenchol	-	-	-	-	-	9.43	-	-	-	-	-
Pellitorine	-	-	-	-	-	-	0.1	-	-	13.9	-
E-nerolidol	-	-	-	-	-	-	0.4	1.0	-	-	11.3
cymene	-	-	1.48	-	9.70	-	tr	-	-	-	-
2-decanone	-	-	-	-	-	20.85		-	-	-	-
α-copaene	-	0.4	-	-	7.55	-	1.0	1.0	0.21	-	-
(E)-β-ocimene	-	4.2	23.57	-	3.32	-	-	32.3	0.83	-	10

ZZ1- *Z. zanthoxyloide* leaf (Present work)

ZZL-*Z. zanthoxyloide*s leaf from Côte d'Ivoire (Affouet et al 2012)

ZLL- *Z. leprieurii* from Côte d'Ivoire (Tanoh et al., 2020)

ZAL1-*Z. armatum* leaf from Nepal (Phuyal *et al*., 2019)

ZAL2-*Z. armatum* leaf from India (Negi *et al*., 2012)

ZAL3-*Z. alatum* leaf from N/W Himalaya (Guleria *et al*., 2013)

ZRL-*Z. rhetsa* leaf from Kerala india (Shafi *et al*., 2011)

ZNL-*Z. nitidum* leaf from Vietnam (Tuyen *et al*., 2021)

ZZ2-*Z. zanthoxyloide* stem bark (Present work)

ZGSb-*Z. gilletti* stem bark from Côte d'Ivoire (Affouet *et al* 2012)

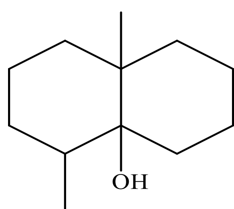
4.8 *Ceiba pentandra* Essential Oil Chemical Constituents

The results of GC-MS analysis of *Ceiba pentandra* essential oils are given in Table 4.14**.** Twenty-eight components were identified in the stem bark oil equivalent to 98.8 % of the whole oil. High proportion of sesquiterpenes (85.7 %) dominated by β- caryophyllene (28.7 %), β-elemene (18.5 %), δ-cadinene (8.0 %), α-muurolene (7.8 %), caryophyllene oxide (4.8 %) and α-humulene (4.2 %) were present in the oil. Other sesquiterpenes in noticeable quantity were bicyclogermacrene (2.3 %), germacrene-D-4-ol (2.4 %), γ-cardinene (1.6 %), γ-muurolene (1.5 %). The non-terpenes identified were hexanal (0.7 %), nonane (1.5 %), 3-octanone (0.8 %), nonanal (1.1 %), vanillin (1.7 %) **[4.35]**, 1-docosanol (1.0 %), pentacosane (0.8 %), methyl palmitate (1.4 %) and methyl stearate (1.4 %) and one monoterpene, geranyl acetone (0.8 %). Vanillin is an uncommon essential oil component with a molecular formula $C_8H_8O_3$. It is a phenolic aldehyde that has three functional groups; ether, hydroxyl and aldehyde. It is the major component of vanilla bean extract. Although, the bean extract is a natural flavouring agent, synthetic vanillin is now frequently used as a flavouring agent in pharmaceuticals, beverages and foods due to the high cost and scarcity of vanilla extract from plant. Vanillin is synthesised commercially from more redily available natural compound eugenol, guaiacol, or lignin (Buttery and Ling, 1995; Esposito *et al*., 1997; Hocking 1997).

Analysis of the volatile oil obtained from the heartwood led to the identification of 23 components corresponding to 76.8 % of the total oil. The non-terpene derivatives found in the oil include esters (12.2 %) dominated by 2-ethoxyethyl acetate (11.3 %), and aldehydes (12.1 %) majorly nonanal (7.3 %), decanal (2.5 %) and hexanal (2.5 %). Other non-terpenes were alcohols (11.8 %) with 1-docosanol (7.0 %) as the main component, alkanes (1.2 %), ketone (0.8 %), aromatic compounds (0.7 %) and heterocyclic compounds (0.7 %). The heartwood oil contained large percentage of oxygenated sesquiterpenes (24.9 %) precisely α-eudesmol (21.1 %), (5*E*,9*E*)-farnesyl acetone (3.0 %) and 10-epi-γ-eudesmol (0.8 %) Table 4.15. In addition, 3.7 % sesquiterpene hydrocarbons which are ar-curcumene (1.2 %) and α-bulnesene (2.5 %), then pinene (3.2 %) as monoterpene hydrocarbons and lastly geranyl acetone (4.5 %) as the only oxygenated monoterpene were also identified. A rare essential oil constituent, geosmin, also known as *E*-1,10-dimethyl-*E*-9-decalol) **[4.34]** was

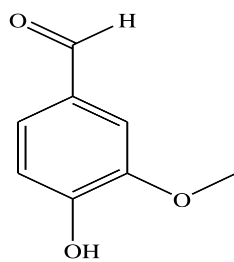
also detected at 0.6 % concentration. Geosmin is a bicyclic alcohol known to be a product of certain bacteria, streptomyces and cyanobacteria. It is released when these microbes die (Izaguirre and Taylor, 2004) and has a perculiar earthly flavour and aroma. Geosmin can be obtained from sesquiterpenes like farnesyl pyrophosphate (Dionigi *et al*., 1992).

There are significant differences in chemical constituents of volatile oils of *C. pentandra* stembark and heartwood and other oils from the Bombacaceae family. Venkatesh *et al*., (2015) reported palmitic acid (41.54 %) and myristic acid (10.95 %) as the most prominent constituents in the stembark oil *Cullenia exarillata* Robyns. Flower essential oil of *Eriotheca longitubulosa* A. Robyns was dominated by α-farnesene (28.03 %), germacrene-D (25,68 %), *E*-ocimene (9.87 %) and toluene (6.92 %) (Courtois *et al*., 2009). *Ceiba pentandra* L. flower essential oil showed sabinene (20.8 %), (*E*,*E*)-*α*-farnesene (20.3 %), 2-hexenal (8.9 %), *α*-pinene (7.1 %), *α*-copaene (5.5 %) and 1,8-cineole (5.3 %) as the most abundant components while flower oil of *Adansonia digitata* L. contained 3-methylbutanol (16.3 %), dimethyl disulphide (10.3 %) and benzaldehyde (7.8 %) as major elements (Pettersson *et al*., 2004). All these compounds were absent in the present study except germacrene-D (0.7 %) and *α*-pinene (3.2 %) found in low quantity in the stembark and heartwood respectively.



geosmin

<u>4.34</u>



vanillin

<u>4.35</u>

Figure 4.7: ***C. pentandra*** **essential oil components**

Table 4.14: Chemical Constituents of *Ceiba pentandra* Essential Oils

Constituents	RI	Percentage compositions (%)	
		Stem bark	Heartwood
Methylcyclohexane	718	-	0.4
2-Methyl-2-pentanol	723	-	1.9
3-methyl-3-pentanol	744	-	1.1
1-Methylcyclopentan-1-ol	793	-	1.2
Hexanal	799	0.7	2.5
Octane	800	-	0.8
Styrene	890	-	0.7
Nonane	900	1.5	-
2-Ethoxyethyl acetate	903	-	11.3
α-Pinene	930	-	3.2
3-Octanone	983	0.8	-
2-Pentylfuran	987	-	0.7
1-Octanal	1002	-	0.8
Nonanal	1103	1.1	7.3
Decanal	1204	-	2.5
2-Undecanone	1290	-	0.8
β-Bourbonene	1380	0.7	-
β-Elemene	1386	18.5	-
Vanillin	1391	1.7	-
Geosmin	1410	-	0.6
β-Caryophyllene	1416	28.7	
Geranyl acetone	1444	0.8	4.5
(*E*)-β-Farnesene	1449	1.1	-
α-Humulene	1452	4.2	-

Allo-aromadendrene	1457	0.5	-
γ-Muurolene	1471	1.5	-
Germacrene D	1477	0.7	-
ar-Curcumene	1487	-	1.2
Bicyclogermacrene	1492	2.3	-
α-Muurolene	1495	7.8	-
γ-Cadinene	1509	1.6	-
Cubebol	1511	1.0	-
δ-Cadinene	1514	0.8	-
α-Bulnesene	1515	-	2.5
Germacrene-D-4-ol	1573	2.4	-
Caryophyllene oxide	1575	4.8	-
10-epi- γ-Eudesmol	1628	-	0.8
τ-Cardinol	1638	0.9	-
α-Eudesmol	1651	-	21.1
α-Cardinol	1652	1.0	-
Phytone	1837	1.9	-
(5*E*,9*E*)-Farnesyl acetone	1904	-	3.0
Methyl palmitate	1921	1.4	-
Methyl stearate	2121	1.4	0.9
1-Docosanol	2484	1.0	7.0
Pentacosane	2500	0.8	-
Total Identified		28	22
Percentage identified		98.8	76.8

RI- Retention indices from the analyses value.

Table 4.15: Classes of Components Identified in *C. pentandra* Essential Oils

Classes	Stem bark		Heartwood	
	PC (%)	**NI**	**PC (%)**	**NI**
Monoterpene hydrocarbons	-	-	3.2	1
Oxygenated monoterpenes	0.8	1	4.5	1
Sesquiterpene hydrocarbons	75.6	12	3.7	2
Oxygenated sesquiterpenes	10.1	5	24.9	3
Diterpenoid	1.9	1	-	-
Non-terpene derivatives	10.4	9	40.5	16

PC- Percentage composition; NI- Number identified

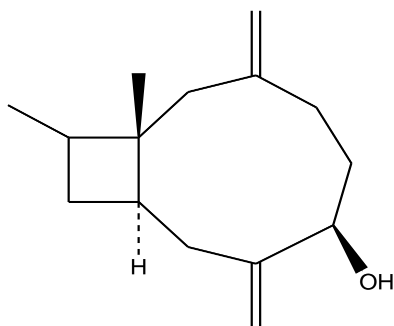
4.9 *Gliricidia sepium* Essential Oils Chemical Constituents

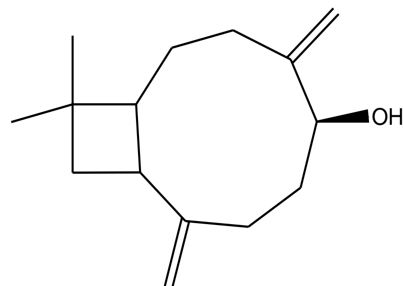
The GC-MS result of the volatile oils hydrodistilled from leaf and stem of *Gliricidia sepium* is given in Table 4.16. The leaf volatile oil was dominated by 34 non-terpene compounds equivalent to 66.8 % of the oil. These were aldehydes (42.9 %): (*E*)- and (*Z*)-hexadecantrienal (16.9 %, 4.9 %) and pentadecanal (16.0 %), cyclic alkanes (11.0 %) mainly methylcyclohexane (8.0 %), aromatic compounds (5.9 %), aliphatic alkanes (2.0 %), alcohols (3.6 %), ketones (0.6 %) and palmitic acid as the only fatty acid (0.8 %). Other compounds which were present in the volatile oil were phytone (4.5 %) phytol 0.9 % (diterpenoids), (*E*)-β-ionone 0.5 % that belongs to the class apocarotenoid an important material in perfumery, limonene 0.8 % (monoterpene), and other monoterpenoids specifically linalool (1.0 %), (*E*)-*p*-menthan-2-one (2.0 %), dendrolasin (0.7 %) and geranyl acetone (0.7 %), which are all odour impacting components. All the aromatic hydrocarbons present were important compounds in the synthesis of monoterpenes and sesquiterpenes namely toluene (2.7 %), ethylbenzene (1.0 %), *o*-xylene (1.0 %), *p*-xylene (0.4 %), hexyl benzoate (0.3 %) and (3*Z*)-hexenyl benzoate (0.5 %). The aromatic esters (3*Z*)-hexenyl benzoate and hexyl benzoate are flavour ingredient. These aromatic compounds may be present due to thermal degradation during distillation or by oxidation. The presence of pentadecanal, tridecanal, tetradecanal, hexadecanal and heptadecanal might have also added to the odour note of the leaf oil. The leaf volatile oil from Costa Rica analysed by Jasmine *et al*., (2017) using GC-MS and GC-FID showed presence of 96 constituents having pentadecanal 18.7 % (16.0 %), *trans*-phytol 7.8 % (0.9 %), methyl linolenate 6.0 % (absent) and nonanal 5.1 % (1.5 %) as prominent constituents when compared with the present study. On the contrary, Kaniampady *et al*., (2007) reported propylene glycol (25.1%), coumarin (18.2%), *Z*-3-hexanol (17.7%), β-farnesene (14.2%) and *E*-2-hexanol (6.5%) as the major constituents among the 16 components in the GC analysis of the leaf oil of *G. sepium* of Indian origin. Likewise, safrole (12.3%) and 2'-hydroxy-acetophenone (12.1%) were found to be the key constituents among the 80 volatile compounds from the Colombian leaf oil analysed by GC-MS and GC-FID (Clara *et al*., 2015).

Forty-three compounds equivalent to 93.3 % of the entire stem oil were recognised. There were 26 sesquiterpenes that constituted 63.2 % of the essential oil; 15 oxygenated

sesquiterpenes and 11 sesquiterpene hydrocarbons (Table 4.17). The most abundant constituents were (9*Z*)-docosenamide (18.0 %), humulene epoxide II (17.5 %), caryophyllene oxide (10.6 %) and α-cadinol (5.0 %). α-humulene (3.9 %) and caryophylla-4(12),8(13)-dien-5α-ol (3.8 %) were also detected in appreciable quantity while the only monoterpene hydrocarbon present is α-pinene in addition to (*E*)-myrtanol and geranyl acetone as oxygenated monoterpenes. The non-terpene derivatives detected include 5 aldehydes (6.5 %), 2 alcohols (0.9 %), 2 amides (18.6 %), 2 alkanes (0.6 %), 2 aromatic compounds (1.5 %) and 1 heterocyclic compounds (0.8 %). In contrast, Reddy and Jose (2010) reported 19 compounds with methyl-3-(*E*)-pentenyl ether (11.55 %), 3-methyl-2-butanol (10.65 %), 1-(1-ethoxyethoxy)-2-hexene (9.72 %), coumarin (8.07 %) and 2-decanol (8.97 %) as the key components among the identified from the stembark oil. All these compounds were not found in the present study.

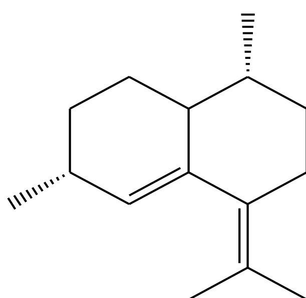
All the dominant constituents in the essential oil of the leaf were not detected in the stem oil and vice versa except methylcyclohexane (8.0 %, 0.2 %), phytone (4.5 %, 0.5 %) and (*Z*)-Hexadecatrienal (4.9 %, 0.9 %) respectively. Only eight compounds are common in both oils amidst 77 compounds identified all together. The chromatographic profile of the stem oil showed the presence of some rare sesquiterpenoids which were absent in the leaf oil. They were caryophylla-4(12),8(13)-dien-5β-ol **[4.36]**, caryophylla-4(12),8(13)-dien-5α-ol **[4.37]**, muurola-4,10(14)-dien-1α-ol **[4.38]**, salvial-4(14)-en-1-one **[4.39]**, germacra-4(15),5,10(14)-trien-1α-ol **[4.40]**, allocedrol **[4.41]**, Z-muurola-4(14),5-diene **[4.42]** and 14-hydroxy-9-epi-(*E*)-caryophyllene **[4.43]**. There are many non-terpene aromatic compounds and esters which are absent in the stem oil but identified in the leaf oil.

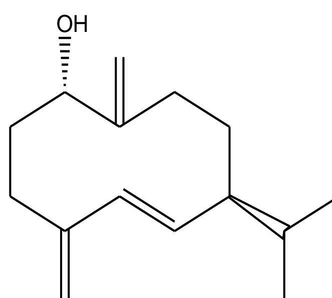


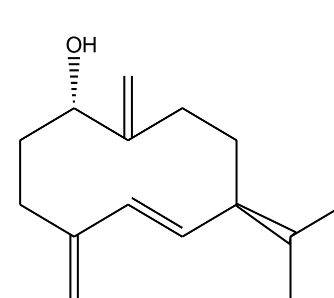


Caryophylla-4(12),8(13)-dien-5β-ol **Caryophylla-4(12),8(13)-dien-5α-ol**

4.36 **4.37**

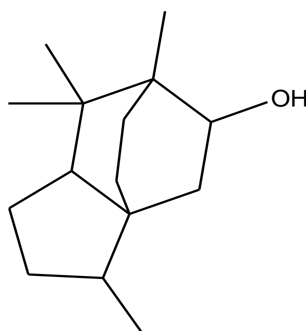


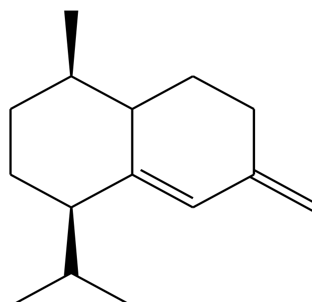


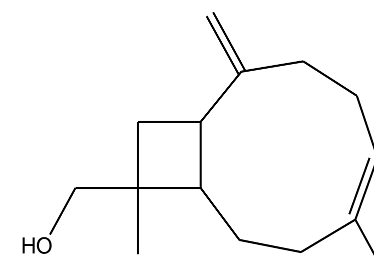


Muurola-4,10(14)-dien-1α-ol **Salvial-4(14)-en-1-one** **Germacra-4(15),5,10(14)-trien-1α-ol,**

4.38 **4.39** **4.40**







Allocedrol **Z-muurola-4(14),5-diene** **14-hydroxy-9-epi-(*E*)-caryophyllene**

4.41 **4.42** **4.43**

Figure 4.8: Sesquiterpene oxides in the *G. sepium* oil

Table 4.16: Chemical Compositions of *Gliricidia sepium* Essential Oils

Constituents	RI	Percentage compositions (%)	
		Leaves	Stem
Z-1,2-Dimethylcyclopentane	716	1.3	-
Methylcyclohexane	718	8.0	0.2
2-Methyl-2-pentanol	723	0.7	-
Ethylcyclopentane	726	0.5	-
3-Methyl-2-pentanol	745	0.4	-
2-Methylheptane	754	0.3	-
Toluene	760	2.7	0.5
3-Methylheptane	763	0.2	-
Z-1,3-Dimethylcyclohexane	777	0.6	-
E-1,3-Dimethylcyclohexane	780	0.3	-
2-Hexanone	786	0.6	-
1-Methylcyclopentanol	792	0.7	-
Octane	798	0.5	-
Hexanal	800	0.8	0.5
Z-1,4-Dimethylcyclohexane	807	0.4	-
Ethylcyclohexane	832	0.3	-
Ethylbenzene	856	1.0	-
1-Hexanol	864	0.4	-
o-Xylene	866	1.0	-
p-Xylene	867	0.4	-
Nonane	903	-	0.4
α-Pinene	930	-	0.4
1-Octen-3-ol	977	0.8	-
2-Pentylfuran	987	-	0.8
2-Ethylhexanol	1025	-	0.4

Limonene	1027	0.8	-
Linalool	1098	1.0	-
Nonanal	1103	1.5	0.7
Decanal	1204	0.5	-
E-*p*-Menthan-2-one	1208	2.0	-
E-Myrtanol	1260	-	0.5
β-Bourbonene	1381	-	1.4
β-Elemene	1386	-	0.3
β-Caryophyllene	1416	-	1.8
Geranyl acetone	1444	0.7	0.3
α-Humulene	1452	-	3.9
Z-Muurola-4(14),5-diene	1471	-	0.4
(*E*)- β-Ionone	1475	0.5	-
Germacrene D	1478	-	0.3
β-Selinene	1486	-	0.7
α-Selinene	1492	-	0.7
α-Muurolene	1495	-	0.3
Tridecanal	1508	0.5	-
δ-Cadinene	1514	-	0.6
(3*Z*)-Hexenyl benzoate	1568	0.5	-
Dendrolasin	1570	0.7	-
Spathulenol	1573	-	1.9
Hexyl benzoate	1576	0.3	-
Caryophyllene oxide	1579	-	10.6
Salvial-4(14)-en-1-one	1589	-	1.9
allo-Cedrol	1592	-	0.6
Humulene epoxide II	1606	-	17.5

Tetradecanal	1609	-	0.3
Muurola-4,10(14)-dien-1α-ol	1622	-	0.4
Caryophylla-4(12),8(13)-dien-5α-ol	1629	-	3.8
Caryophylla-4(12),8(13)-dien-5β-ol	1633	-	1.4
τ-Cadinol	1638	-	0.7
τ-Muurolol	1640	-	1.7
δ-Cadinol	1643	-	1.1
α-Cadinol	1652	-	5.0
Selin-11-en-4α-ol	1655	-	2.8
14-Hydroxy-9-epi-(*E*)-Caryophyllene	1667	-	1.5
Cadalene	1669	-	0.5
Germacra-4(15),5,10(14)-trien-1α-ol	1686	-	1.9
Pentadecanal	1711	16.0	4.1
Hexadecanal	1813	0.5	-
Phytone	1837	4.5	0.5
(*Z*)-Hexadecatrienal	1882	4.9	0.9
(*E*)-Hexadecatrienal	1887	16.9	-
Heptadecanal	1915	0.9	-
Palmitic acid	1953	0.8	-
Phytol	2102	0.9	-
(9*Z*)-Octadecenamide	2349	-	0.6
1-Docosanol	2484	0.6	0.5
Heptacosane	2700	1.0	-
(9*Z*)-Docosenamide	2754	-	18.0
Total compounds identified		42	43
Percentage identified		77.9	93.3

RI- Retention indices from the analyses value.

Table 4.17: Classes of Components Identified in *G. sepium* Essential Oils

Classes	Leaf		Stem	
	PC (%)	NI	PC (%)	NI
Monoterpene hydrocarbons	0.8	1	0.4	1
Oxygenated monoterpenes	3.7	3	0.8	2
Sesquiterpene hydrocarbons	-	1	10.4	10
Oxygenated sesquiterpenes	0.7	-	52.8	15
Diterpenoids	5.4	2	0.5	1
Apocarotenoids	0.5	1	-	-
Non-terpene derivatives	66.8	37	28.4	14

PC- Percentage composition; NI- Number identified

4.10 *Annona muricata* Essential Oils Chemical Constituents

The GC-MS analysis results of the leaf, branches and stem bark essential oils of *Annona muricata* are displayed in Table 4.18. The leaf oil is made up of 43 compounds accounting for 91.6 % of the total oil content with greater amount being sesquiterpene hydrocarbons-21 compounds (81.0 %). Other classes of compounds identified were 6 oxygenated sesquiterpenes (8.2 %), 1 apocarotenoid (0.2%), 4 monoterpene hydrocarbons (0.9%), 2 oxygenated diterpenes ((0.5%) and 9 non-terpene derivatives (0.9%) (Table 4.19). The most aboundant components are (*E*)-caryophyllene (28.2%), germacrene A (9.6 %), δ-cadinene (9.6 %), β-elemene (8.3 %) and α-muurolene (6.5 %). Most of the identified constituents in the leaf oil are isomers like germacrene A, B and D; α and β selinene; β, δ and γ elemene; (*E*) and (*Z*)- caryophyllene; δ and γ- cadinene as well as cadinene (δ and γ) and muurolol (α and τ). Comparing this volatile oil with the oil from Badagry in Nigeria (Owolabi *et al*., 2013), 19 constituents were identified having greater proportion of eugenol (30.2%) and (*E*)-caryophyllene (38.9%). Additional major compounds in the present study were δ-cadinene (6.0 %), caryophyllene oxide (5.0 %) and α-humulene (4.3%). Other constituents reported that are absent in this oil were β-pinene, γ-terpinene, terpinen-4-ol, α-terpineol and eugenol. The leaf oil from Brazil confirmed the presence of spathulenol (14.3 %), bicyclogermacrene (9.8 %) and linalool (8.7 %) (Moreira *et al*., 2002). Although bicyclogermacrene was also found in the current study, the other two compounds were not present. β-Caryophyllene was found as a dominant constituent in Cameroonean leaf volatile oil while β-pinene (20.6 %), ρ-mentha-2,4(8)-diene (9.8 %), germacrene D (18.1 %), β-elemene (9.1 %) and α-pinene (9.4 %) were prominent components in the leaf oil from Vietnam (Thang *et al*., 2013). δ-Cadinene (13.6 %), epi-α-cadinol (8.4 %) and α-cadinol (8.3 %) were most abundant in the leaf oil from Benin Republic (Kossouoh *et al*., 2007). Gyesi *et al*., (2019) gave account of essential oil extracted from Ghanian fruit pulp and leaves of *A. muricata*. δ-Cadinene, α-cadinol, τ-cadinol and β-caryophyllene were reported to be among the most abundant constituents and they were also detected in the present study in appreciable quantities while other dominant constituents like 3-(octadecyloxy) propyl ester and octadecane were absent. α–Murolene (10.64 %), the second most abundant component was identified as a minor component (0.3 %) in the leaf oil. Jirovetz *et al*.,

(1998) also reported pulp volatile oil from Cameroon to be rich in aliphatic acid esters mainly 2-hexenoic acid methyl ester and 2-hexenoic acid ethyl ester.

The volatile oil hydrodistilled from the branches gave 28 volatile constituents equivalent to 92.8 % of the whole oil. There were 25.3 % alkanes mainly tetracosane (23.5 %), 3.1 % alcohols, 7.8 % saturated aldehydes dominated by pentadecanal (2.9 %) and 12.5 % unsaturated aldehydes namely (*Z*)-hexadecatrienal (6.5 %), (*E*)-hexadecadienal (4.3 %) and heptadecenal (1.7 %). The oil also contains 28.4 % of 1-tricosene (alkane), 4.7 % palmitic acid (unsaturated fatty acid) and 6.7 % toluene (benzene derivative). The terpenes found in the oil were α-pinene (0.2 %) and limonene (1.2 %) as monoterpene hydrocarbons, and linalool (0.6 %) and geranyl acetone (0.2 %) as oxygenated monoterpenes. β-Caryophyllene (0.4 %) - a sesquiterpene hydrocarbon, caryophyllene oxide (1.3 %) and (5*Z*,9*E*)-farnesyl acetone (0.4 %) - oxygenated sesquiterpenes were also identified. Apart from palmitic acid, the compounds mentioned above possess odour characters which are commonly found in plant essential oil (Sarkic and Stappen, 2018).

Forty-six compounds were detected in the stem bark essential oil corresponding to 95.7 % of the total oil. The oil contains 19 terpenes and 27 non terpenes. The non terpenes were mainly fatty acids dominated by palmitic acid (29.8 %) and oleic acid (7.1 %), followed by aliphatic aldehydes such as n-nonanal and low concentration of alcohols, ketone, ester, alkanes and aromatic compounds. Caryophyllene oxide (10.0%), β-caryophyllene (7.5 %) and limonene (7.3 %) were dominant terpenes in the stem bark oil.

There is a marked difference between the chemical profiles of the essential oils extracted from the three different parts of *A. muricata* as given in Table 4.19. The leaf oil was dominated by sesquiterpene hydrocarbons (81.0 %) while the oil from the branches was mainly non-terpenes (88.5 %) with low percentage of terpenes (4.3 %), whereas there were more non-terpenes (58.7%) than terpenes (36.7 %) in the stembark oil. None of the fatty acids found in the stem oil was present in the other oil samples except palmitic acid (4.7 %) identified in noticeable amount in the branches oil. A total of 93 constituents were identified in the oils out of which 4 compounds were common to all, 6 were regular in both leaf and stem bark oils, while 15 compounds appear only in branches and stembark oils.

Table 4.18: Chemical Composition of *Annona muricata* Essential Oils

		Percentage Compositions (%)		
Conctituents	RI	Leaf	Branches	Stem bark
Z-1,3-Dimethylcyclopentane	716	-	0.1	-
Methylcyclohexane	718	-	0.7	0.2
2-Methyl-2-pentanol	723	-	0.8	0.4
3-Methyl-3-pentanol	745	-	0.5	0.2
Toluene	760	-	6.7	2.2
1-Methylcyclopentanol	792	-	0.7	0.3
Octane	800	-	0.3	0.6
Hexanal	801	-	0.5	1.1
Heptanal	901	-	-	0.5
(2*E*)-Hexenal	851	0.1	-	-
Ethylbenzene	874	0.1	-	-
o-Xylene	884	0.2	-	-
p-Xylene	908	tr	-	-
α –Pinene	932	0.3	0.2	0.1
Camphene	945	0.4	-	0.9
(3*E*)-Hexen-2-one	962	-	-	0.3
1-(1-Methyl-cyclopentyl)-ethanone	964	0.3	-	-
1-Octen-3-ol	975	tr	-	0.4
6-Methyl-5-hepten-2-one	981	tr	-	-
Myrcene	986	-	-	1.4
Isobutyl 2-methylbutanoate	1000	-	-	0.4
Octanal	1002	-	-	0.6
p-Cymene	1020	tr	-	-
Limonene	1026	0.2	1.2	7.3
1,8-cineole	1030	-	-	0.5
2-Methylbutylbutyrate	1038	-	-	0.3
(*E*)- β –Ocimene	1043	-	-	0.3
Linalool	1097	-	0.6	1.2
2-Methybutyl-2-methylbutyrate	1100	-	-	0.5

n-Nonanal	1106	0.1	2.9	5.8
Decanal	1204	-	1.1	0.9
(*3Z*)-Hexenyl-2-methylbutanoate	1233	0.1	-	0.7
δ –Elemene	1340	0.4	-	-
α –Copaene	1375	0.7	-	-
β –Bourbonene	1388	0.5	-	-
β –Elemene	1389	8.3	-	-
(*Z*)-Caryophyllene	1408	0.1	-	-
α –Gurjunene	1411	0.4	-	-
β –Caryophyllene	1416	-	0.4	7.5
(*E*)-Caryophyllene	1421	28.2	-	-
γ –Elemene	1429	0.2	-	-
Geranyl acetone	1444	-	0.2	0.4
(*E*)- β –Farnesene	1449	-	-	0.3
α –Humulene	1451	3.8	-	0.9
Geranyl acetone	1455	0.2	-	-
allo-Aromadendrene	1458	0.7	-	-
(*E*)-β-Farnesene	1460	0.9	-	-
γ-Muurolene	1475	1.0	-	-
Germacrene D	1478	2.8	-	-
β-Selinene	1483	0.2	-	-
α –Selinene	1492	0.5	-	-
Bicyclogermacrene	1494	3.6	-	-
α –Muurolene	1500	6.5	-	-
Germacrene A	1503	9.6	-	-
Isocaryophyllene oxide	1547	-	-	1.9
Dodecanoic acid	1555	-	-	0.2
γ-Cadinene	1512	2.6	-	-
δ-Cadinene	1523	9.6	-	-
Germacrene B	1553	0.2	-	-
Caryophyllene oxide	1580	2.7	1.3	10.0

Humulene epoxide II	1606	-	-	0.4
Tetradecanal	1610	-	0.4	-
Caryophylla-4(12),8(13)-dien-5β –ol	1634	-	-	1.3
1-*epi*-Cubenol	1629	0.9	-	-
τ-Cadinol	1642	2.0	-	0.4
τ-Muurolol	1644	0.6	-	0.3
α-Muurolol	1649	0.3	-	-
α-Cadinol	1657	1.7	-	1.0
14-hydroxy-9-epi-(*E*)-Caryophyllene	1667	-	-	0.6
Pentadecanal	1711	-	2.9	-
Myristic acid	1754	-	-	1.3
Phytone	1839	0.4	-	-
1-Hexadecanol	1876	-	0.4	-
(*Z*)-Hexadecatrienal	1882	-	6.5	-
(*E*)-Hexadecatrienal	1886	-	4.3	-
Heptadecenal	1889	-	1.7	-
(5*Z*,9*E*)-Fernesyl acetone	1904	-	0.4	-
Palmitic acid	1957	-	4.7	29.8
(*E,E*)-Geranyl linalool	2090	0.1	-	-
1-Octadecanol	2079	-	0.7	-
Linoleic acid	2123	-	-	2.0
Oleic acid	2129	-	-	7.1
1-Tricosene	2296	-	28.4	-
Tricosane	2300	-	-	1.1
Tetracosane	2400	-	23.5	0.3
Pentacosane	2500	-	0.7	1.1
Hexacosane	2600	-	-	0.3
Heptacosane	2700	-	-	0.4
Total Identified		43	28	46
Percentage identified		91.6	92.8	95.7

RI- Retention indices from the analyses value. tr-trace

Table 4.19: Classes of Components Identified in *A. muricata* Essential Oils

Classes	Leaf		Branches		Stem bark	
	PC (%)	**NI**	**PC (%)**	**NI**	**PC(%)**	**NI**
Monoterpene hydrocarbons	0.9	4	1.4	2	10.3	5
Oxygenated monoterpenes	-	-	0.8	2	2.1	3
Sesquiterpene hydrocarbons	81.0	21	0.4	1	8.7	3
Oxygenated sesquiterpenes	8.2	6	1.7	2	15.9	8
Apocarotenoids	0.2	1	-	-	-	-
Non-terpene derivatives	0.9	9	88.5	21	58.7	27

PC- Percentage composition; NI- Number identified

4.11 Antioxidant Activity

The antioxidant activity of the volatile oils was evaluated using DPPH free-radical scavenging assay which measures the ability of the essential oils to donate proton or electron to free-radicals. This is characterised by their IC_{50} values (concentrations at which 50 % of DPPH free-radicals are reduced) obtained by plotting the values of percentage inhibitions of the radicals against the concentations of the volatile oils (APPENDIXES 28-55). The antioxidant potentials of the tested essential oils increase as the concentration of the oils increases. The IC_{50} values are given in Table 4.20.

Essential oils extracted from *A. muricata* leaf (43.22 µg/ mL), *T. stans* seed (33.03 µg/ mL) and stem (6.44 µg/ mL) as well as the stem (32.02 µg/ mL) and fruit (29.39 µg/ mL) of *D. repens* gave better antioxidants activity than the reference antioxidants, butylated hydroxyanisole and α-tocopherol with IC_{50} values of 45.11 µg/ mL and 81.58 µg/ mL respectively. The lower the IC_{50} values the higher the scavenging ability. Among the essential oils, *T. stans* stem essential oil exhibited the highest antioxidant activity while the oils obtained from the two *Plumeria* species showed the least activities.

The antioxidant properties of essential oils are related to their chemical constituents. It was earlier reported that essential oils dominated by monoterpenes posssess good antioxidant activity (Edziri *et al* 2010). Most of the essential oils with high percentage of oxygenated monoterpenes with alcohol, carbonyl (aldehyde or ketone) and ester functional groups demonstrated high antioxidant activities (Dhifi *et al*., 2016). Previous studies also showed that presence of oxygenated monoterpenes like carvacrol, α-terpineol and linalool in essential oils of plants could be responsible for antioxidant potential of the oil (Bicas *et al* 2011). Although these facts could not justify the radical-scavenging ability of all the oils that showed good antioxidant activity in this study, it reflected in the activity of the essential oil of *Tecoma stans* stem and seed. Among *Tecoma stans* essential oils examined, only the stem and seed oils contained monoterpene hydrocarbons and also have a large percentage of oxygenated monoterpenes (Table 4.3). Presence of linalool and α-terpineol as prominent component of the stem and seed oils of *T. stans* might have contributed to the high radical-scavenging activity of the two oils. Other oil also showed moderate activity but lower than the synthetic antioxidants. Conversely, *Annona muricata* leaf which possessed the least

quantity of monoterpene hydrocarbons, no oxygenated monoterpenes and good proportion of sesquiterpene hyrocarbons (81.0 %) among the essential oils studied in the plant showed better scavenging ability than oils from other parts. The essential oil of *Annona muricata* leaf from Ghana gave IC_{50} value of 244.8±3.2 µg/mL in DPPH assay (Gyesi *et al*., 2019). *D. repens* stem volatile oil that showed good antioxidant activity also lacked both monoterpene hydrocarbons and oxygenated monoterpenes. The fruit oil with 1.1 % and 0.9 % monoterpene hydrocarbons and oxygenated monoterpenes respectively also showed good activity.

Z. zanthoxyloides leaf and stembark oils demonstrated moderate radical-scavenging property. Previous work on antioxidant activity of *Z. alatum* from Himalaya reported high scavenging activity for the crude methanol extract and acetone fraction of *Z. alatum* while the essential oil scavenging activity was relatively low (Guleria *et al*., 2013).

The volatile oils hydrodistilled from the leaf and flower of *T. stans*, *D. repens* twig, *D. regia* leaf and root, *C. pentandra* stem bark, *G. sepium* stem and *A. muricata* stem bark and branches showed moderate antioxidant activity.

Table 4.20: IC_{50} Values indicating free radical-scavenging activity of the Essential oils

Plant	Part used	Inhibition of $DPPH^+$ (IC_{50}) (µg/ mL)
Tecoma stans	Leaf	168.62
	Seed	33.03
	Stem	6.44
	Flowers	104.94
Plumeria acuminata	Leaf	695.00
	Flowers	441.09
	Root	581.58
P. rubra	Leaf	528.23
	Flower	359.05
	Stem	639.00
	Root	299.17
Delonix regia	Twig	139.15
	Flowers	254.12
	Root	445.00
Duranta repens	Leaf	140.41
	Fruits	29.39
	Root	118.72
	Stem	32.02
Z. zanthoxyloides	Leaf	212.29
	Stem bark	431.02
Ceiba pentandra	Stem bark	109.68
	Heartwood	295.56
Gliricidia sepium	Leaf	84.26
	Stem	142.15

Annona muricata	Leaf	43.22
	Branches	98.15
	Stem bark	101.67
α-Tocopherol		81.58
BHA		45.11

4.12 Brine shrimp lethality effects of Essential oils

The data from the cytotoxicity study of the essential oils from the nine medicinal plants are given in Table 4.21. The results showed that the volatile oils from the leaves of *Zanthoxylum zanthoxyloides*, *Annona muricata* and *Plumeria acuminata* have LC_{50} values of 5.8992 ppm, 6.1518 ppm and 6.9070 ppm respectively, while *Delonix regia* twig essential oil had LC_{50} of 9.3046 ppm. Actually, all the plant oils were toxic, having LC_{50} values less than 100 ppm but the toxicity of *Plumeria rubra* stem (89.8190 ppm), *Gliricidia sepium* leaf oil (79.6717 ppm), *Tecoma stans* stem and flower oil (79.1023 ppm and 62.2700 ppm) and *P. acuminata* root oil 61.2245 (ppm) were the lowest. There was little or no information from literature about the biotoxicity of the essential oils considered in this study except *A. muricata* leaf and some genus *Zanthoxylum* that were tested against human cancer cell lines. The leaf oil of *A muricata* showed remarkable *in-vitro* cytotoxic effect against MCF-7 cells (breast cell line), at 100 ppm with 99.2 % mortality (Owolabi *et al*., 2013),

There were many reports which showed the toxicity of the genus *Zanthoxylum* as they are used in traditional medicine in cancer treatment. These reports cover not just the essential oils, but also other extracts from different parts of the plants. These include toxicity of *Z. rhesta* bark extract against *Artemia salina* (Islam *et al*., 2001); inhibition of DNA synthesis in HL-60 cells by dipetalline and alloxanthoxyletin isolated from *Z. americanum* (Ju *et al*., 2001); induced alterations in albumin, activity of *Z. leprieurii* and *Z. zanthoxyloides* fruit essential oils against MCF-7 (breast cancer), PC-3 (prostate cancer), WRL-68 (liver cancer) and $CACO_2$ (colon cancer) (Misra *et al*., 2013) cell lines; toxicity of *Z. nitidum* leaf, stem and fruit essential oils against MCF-7 (human breast adenocarcinoma), HGC-27 (human stomach carcinoma), Hep-2 (hepatocellular carcinoma), A-549 (human lung adenocarcinoma epithelial) and HeLa (cervical cancer) (Tuyen *et al*., 2021); and alkaline phosphatase levels in mice using *Z. naranjillo* hexane extract (Rodrigues *et al.*, 1998) and selective toxicity of *Z. rhoifolium* Lam leaf essential oil against human tumour cells (Da Silva *et al*., 2017). Various degrees of toxicity associated with the administration of aqueous extract of *Duranta repens* on the liver, blood cells, kidney, lipid profile and oxidative stress indicators using wistar rats was reported by Amadi *et al.,* (2018). In most cases, there is

link between the cytotoxicity and antitumor properties of plant essential oils or other extracted phytochemicals (Krishnaraju *et al*., 2005).

Some components of essential oil described as cytotoxic substances against tumour cells were δ and β-elemene, β-caryophyllene, caryophyllene oxide, α-humulene and phytol (Hammami *et al*., 2015; Alencar *et al*., 2018). Presence of these compounds as dominant constituents in the essential oil could improve the oil's cytotoxicity. The toxicity of the essential oils may not be attributed only to their main components, synergistic effect of both major and minor constituents also contribute to the overall activity of the oil (Dhifi *et al*., 2016). For instance, *ceiba pentandra* stembark oil dominated by β-caryophyllene (28.7 %), β-elemene (18.5 %), caryophyllene oxide (4.8 %) and α-humulene (4.2 %) was not as toxic as the *Z. zanthoxyloides* leaf oil containing only caryophyllene oxide (27.0 %) and humulene epoxide II (8.0 %) among its prominent constituents. Phytol showed cytotoxic potential against cancer cell lines from breast, cervical, lung, prostate adenocarcinoma, melanoma and colorectal, therefore, its presence in appreciable quantity in the essential oils of *D. regia* root, *P. rubra* leaf and *P. acuminata* leaf could have contributed to the high toxicity of the essential oils (Sakthivel *et al*., 2018).

Comparing the toxicity of the volatile oils extracted from stem bark and leaf of *Z. zanthoxyloides*, the leaf oil (LC_{50}= 5.8992 ppm) with higher toxicity consisted of 35 % oxygenated sesquiterpenes, 21.2 % monoterpene hydrocarbons and 29.7 % non-terpenes. The stem bark oil that was characterised by larger percentage of monoterpenes (43.3 %) than the sesquiterpenes (9.6 %) and 46.6 % non-terpenes have higher LC_{50} value of 47.7079 ppm. Moreover, *Ceiba pentandra* stem bark oil which was mainly sesquiterpenes (75.6 %) displayed high toxicity (LC_{50} = 25.9185 ppm), but not as high as *Z. zanthoxtloides* leaf oil. *Annona muricata* leaf oil that was made up of 81.0 % sesquiterpene dominated by (*E*)-caryophyllene (28.2 %) showed higher toxicity (6.1518 ppm) than other parts studied which composed higher percentage of non terpenes. *P. acuminata* leaf oil, which was the most toxic among the parts examined from genus *Plumeria* (6.9070 ppm) was made up of 22.5 % monoterpenes, 10.0 % sesquiterpenes and 22.5 % non-terpenes. Also toxic was the flower oil (23.1982 ppm) with good percentage of monoterpenes (19.1 %) and 39.0 % non-terpenes while the root oil with the highest LC_{50} value of 61.2245 ppm among the three *P.*

acuminata volatile oils was chiefly made up of 47.6 % oxygenated sesquiterpenes mainly β-eudesmol (43.0 %) and 49.8 % non-terpenes. In the same manner, the leaf oil of *P. rubra* (LC_{50} = 12.5636 ppm) was more toxic than other parts examined followed by the flower oil (LC_{50} = 24.8631 ppm) and lastly the stem volatile oil (LC_{50} = 89.8190 ppm).

D. repens essential oils have LC_{50} between 12.56-35.50 ppm, the oils were largely composed of non-terpene compounds (above 90 % of the total oil in each case) except the leaf oil having 74.2 % non-terpenes which showed the least toxicity among them. Among *Delonix regia* oils, the root comprising of 2.8 % monoterpenes, 2.1 % sesquiterpenes, 13.4 % diterpenoids and 75 % non-terpenes was the most toxic of all the oils (LC_{50} = 0.0000 ppm), there was 96.7 % mortality of the shrimps at 10 μg/mL concentration. This is followed by the twig essential oil (LC_{50} of 9.3016 ppm) that was likewise dominated by non-terpenes (97.6 %) with (9Z)-Docosecenamide (51.6 %) as the most prominent. The least toxic among them, flower oil was having heptacosane (40.2 %) and (9Z)-docosecenamide as its major constituents.

Gliricidia sepium stem essential oil that was more toxic than the leaf oil was quantitatively made up of terpenes, mainly oxygenated sesquiterpenes (52.8 %) followed by 10.9 % sesquiterpene hydrocarbons. There were only 29.4 % non-terpenes while the leaf oil was dominated by non-terpenes (91.0 %).

Critical observation of the chemical constituents of each of these essential oils with respect to their lethal concentrations gave an irregular relationship. Some with more non-terpenes gave lower toxicity while some have higher toxicity. In addition, the oils with greater proportion of sesquiterpene hydrocarbons exhibited higher toxicity; *T. stans* seed (13.5 %) *C. pentandra* leaf (76.5 %) and *A. muricata* (81.0 %) than other parts of the same plant. Thus, the toxicities of the essential oils examined were likely due to synergistic effect of their chemical components regardless of their percentage compositions.

Table 4.21: Brine Shrimp Lethality Assay Result

Plant/Part used	LC_{50} (ppm)	LC limit (%)	UC limit (%)	CL (95 %)
Tecoma stans				
Leaves	24.8631	13.200	42.4256	0.1542
Seed	13.6016	4.1913	24.2901	0.2255
Stem	79.1023	48.1870	127.7710	0.1118
Flowers	62.2700	36.0488	103.8487	0.1067
Plumeria acuminata				
Leaves	6.9070	1.1274	14.6368	0.2970
Flowers	23.1982	10.5660	41.7706	0.1514
Root	61.2245	33.8675	104.6401	0.1106
P. rubra				
Leaves	12.5636	2.8246	27.8879	0.2070
Stem	89.8190	37.6330	207.1217	0.1843
Flower	24.8631	13.200	42.4256	0.1542
Root	47.7079	24.4775	84.5752	0.1223
Delonix regia				
Twig	9.3046	2.9185	16.7713	0.2699
Flowers	10.5256	3.9204	18.3657	0.2504
Root	0.0000	104827	0.8056	9.4506
Duranta repens				
Leaves	35.4997	15.2889	67.3516	0.1428
Stem	14.4237	7.4120	23.6496	0.2010
Fruits	12.5636	2.8246	27.8879	0.2070
Root	17.7164	10.9088	27.3801	0.1536
Z. zanthoxyloides				
Leaves	5.8992	0.7573	12.4225	0.3428
Stembark	47.7079	24.4775	84.5752	0.1223
Ceiba pentandra				
Stembark	25.9185	16.3460	40.9119	0.1363
Heartwood	89.8190	67.6330	107.1217	0.1843
Gliricidia sepium				
Leaves	79.6717	28.0301	203.9742	0.2275
Stem	38.7081	20.5002	66.9387	0.1200
Annona muricata				
Leaves	6.1518	0.9387	12.0221	0.3632
Branches	19.7159	4.2947	46.0847	0.2189
Stembark	27.1501	45.1873	78.3340	0.1449

LC-Lower confidence; UC-Upper confidence; LC_{50}- Concentration at which there was 50 % mortality; CL- Confidence limit

4.13 Antimicrobial Activity

The results of the antimicrobial assay were subjected to statistical analysis using two-way ANOVA if there are significant difference between the activities of the essential oils, and also compared with the activity of the standard drugs.

4.13.1 Antimicrobial activity of *Tecoma stans*

The antimicrobial activities of *Tecoma stans* volatile oils are presented in Table 4.22. Both *E. coli* and *S. aureus* were susceptible to the leaf oil at 100 µL/mL, although weak inhibition zones were observed, but resistant to the stem, seed and flower oils. The oils exhibited moderate antifungal activity with zones of inhibition ranging from 1.4 to 8.1 mm for 100 µL/mL concentration while no activity was observed at low concentration (1 µL/mL). Ethanol extract of *T. stans* flower reported by Sowjanya and Srinivasa, (2017) exhibited moderate to high antimicrobial activities against both Gram-positive and Gram-negative bacteria (zones of inhibition range of 9.33 - 20.33 mm).

Comparing the activities of *T. stans* essential oils (one with another) using two-way ANOVA (APPENDIX 56), there was no significant difference in their activity against each of the test organisms ($P > 0.05$). On the other hand, there was significant difference between the activity of gentamycin and the essential oils ($P < 0.001$).

Table 4.22: Antimicrobial Activity of *T. stans*

Essential oils	Conc (µL/mL)	Zones of Inhibition (mm)				
		S. aureus	*E. coli*	*C. albicans*	*F. solani*	*A. niger*
Leaf	1	0.0a	0.0a	0.0a	0.0a	0.0a
	10	0.0a	0.0a	2.3a	2.1a	2.1a
	100	1.3a	1.7a	7.8a	4.1a	6.1a
Stem	1	0.0a	0.0a	0.0a	0.0a	0.0a
	10	0.0a	0.0a	2.2a	1.6a	1.8a
	100	0.0a	0.0a	6.1a	3.9a	4.0a
Seed	1	0.0a	0.0a	2.1a	2.1a	0.0a
	10	0.0a	0.0a	4.0a	3.9a	0.0a
	100	0.0a	0.0a	6.2a	6.0a	1.4c
Flower	1	0.0a	0.0a	3.9a	1.9a	3.9a
	10	0.0a	0.0a	6.0a	3.9a	6.0a
	100	0.0a	0.0a	8.1a	6.1a	8.1a
+ve control		11.5b	9.0b	21.0b	10.3b	10.5b
-ve control		0.0	0.0	0.0	0.0	0.0

Mean followed by different letters in the same column indicate there was significant difference between the mean ($p < 0.001$) while mean followed by the same letter indicate no significant difference between the mean ($p > 0.005$)
+ve control- Gentamycin for antibacterial assay, Ketoconazole for antifungal assay
-ve control- DMSO

4.13.2 Antimicrobial activity of *Plumeria acuminata*

The activities of *Plumeria acuminata* essential oils against the microorganisms tested in this study are reported in Table 4.23 and Table 4.24. In the pilot study, the plants' essential oils were active against the tested bacteria (Plate 4.1)**,** and also demonstrated high antifungal activity against *C. albicans* (Plate 4.2). Therefore, *P. acuminata* essential oils were further tested against more bacteria strains (*S. typhi, L. adecarboxylata, M. morganii, C. freundii* and *K. pneumoniae*). *M. morganii*, *C. freundii* and *K. pneumoniae* were resistant to gentamycin (10 µL/ mL) but susceptible to the leaf and flower essential oils even at 1µL/ mL (zones of inhibition varying from 2.0 to 6.4 mm). The leaf oil also exhibited good antifungal activity against *Candida albicans* with 15.6 mm zone of inhibition at 1 µL/ mL but no activity against *A. niger* even at 100 µL/ mL. *S. typhi* and *K. pneumoniae* were resistant to the root oil, while moderate zones of inhibition range of 1.9 - 12.0 mm were observed against *C. freundii*, *M. morganii* and *L. adecarboxylata*. Among the fungi, only *F. solani* was susceptible to the root oil. The flower volatile oil also displayed good activity against the fungus *C albicans* and moderate activity against *F. solani*. Like the leaf oil, *A. niger* was resistant to the flower and the root oil. Highest zone of inhibition was observed in the leaf essential oil of *P. acumunata* for both bacteria and fungi. Statistical analysis (APPENDIX 57) showed no significant difference ($p < 0.001$) in the antimbacterial activities of the leaf and flower oils, the root and flower oils (except for *E. coli* and *K. pneumoniae*) and gentamycin and the essential oils (in their activities against *L. adecarboxylata* and *K. pneumoniae*), the activity of gentamycin and those of the essential oils against other organisms were significantly different ($p > 0.05$). The antifungal activities of the essential oils and ketoconazole were significantly different (APPENDIX 58).

In an earlier report, the flower essential oil of the *P. alba* showed antibacterial potential against Gram-negative bacteria (Zahid *et al*., 2010) but not as active as the flower oil in the present study. However, good antifungal property was reported and the activity was compared with the standard drug, griseofulvin (Kumari *et al*., 2012). *P. alba* methanol extract as well as the isolated fraction from the extract demonstrated significant antimicrobial activity (Radha *et al*., 2008). The leaf and petal methanol extracts also showed good antibacterial property (Gupta *et al*., 2008; Syakira and Brenda, 2010).

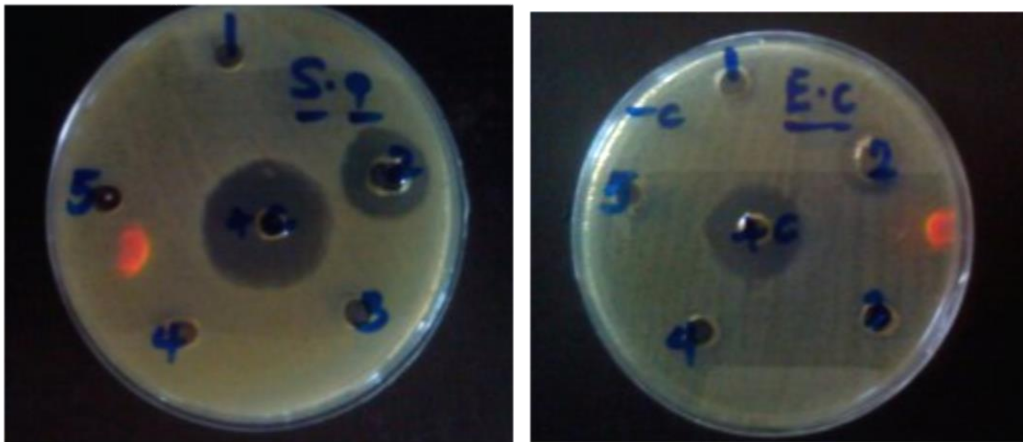


Plate 4.1: Antibacterial activity of *P. acuminata* leaf essential oil (labelled 2) in the pilot study, c- gentamycin (S.a- *S. aureus*; E.c- *E. coli*)

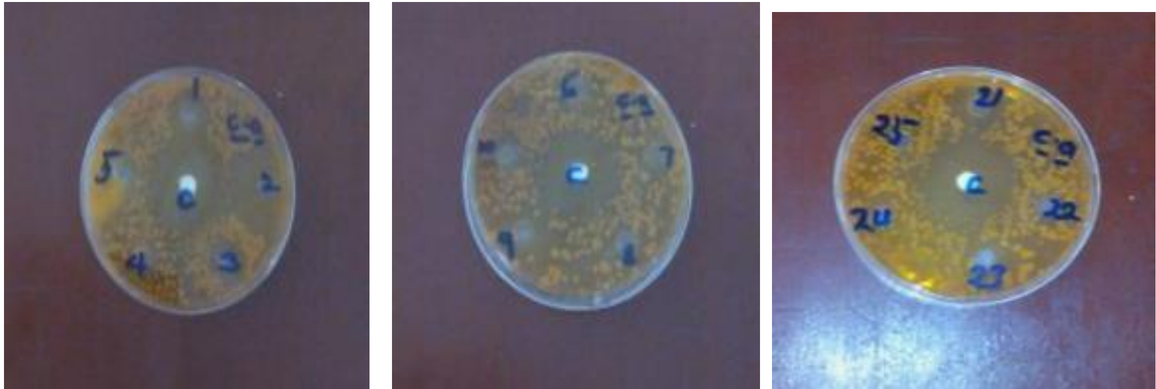


Plate 4.2: Inhibitory zones of *P. acuminata* essential oils against *Candida albicans* in the Pilot study (label 2, leaf; label 9, root, label 23, flower)

Table 4.23: Antibacterial Activity of *P. acuminata*

Samples	Conc. (µL/mL)	Zones of Inhibition (mm)						
		E. coli	*S. typhi*	*S. aureus*	*L. adecarb*	*M. morganii*	*C. freundii*	*K. pneumo*
Leaf oil	1	1.7a	0.0a	3.9a	3.9a	6.4a	2.3a	2.0a
	10	4.0a	2.1a	6.0a	6.1a	8.2a	7.9a	4.0a
	100	6.1a	4.1a	8.1a	10.1a	12.0a	12.0a	6.1a
Root oil	1	2.3a	0.0a	1.6a	1.9a	3.9a	0.0a	0.0a
	10	6.4a	0.0a	3.9a	3.9a	6.0a	1.9a	0.0aa
	100	12.1a	0.0a	5.9a	6.0a	8.1a	4.1a	0.0a
Flower oil	1	0.0a	0.0a	0.0a	1.9a	3.8a	3.9a	4.0 b
	10	1.8c	1.9a	1.5a	3.8a	5.9a	6.0a	6.0b
	100	4.0c	4.1a	4.0a	5.9a	7.9a	8.1a	8.1b
Gentamycin (10)		9.0b,a	12.5b	11.5b	8.0a,b,	0.0b	0.0b,a	0.0a
DMSO		0.0	0.0	0.0	0.0	0.0	0.0	0.0

Mean followed by different letters in the same column indicate there was significant difference between the mean ($p < 0.001$) while mean followed by the same letter indicate no significant difference between the mean ($p > 0.005$)

Table 4.24: Antifungal Activity of *Plumeria acuminata*

Samples	Concentrations (µL/mL)	Zones of Inhibition (mm)		
		C. albicans	*A. niger*	*F. solani*
Leaf oil	1	15.6a	0.0a	0.0a
	10	18.1a	0.0a	4.1a
	100	22.1a	0.0a	7.9a
Root oil	1	0.0b	0.0a	0.0b
	10	0.0b	0.0a	1.8b
	100	0.0b	0.0a	4.0b
Flower oil	1	8.0c	0.0a	0.0b
	10	10.1c	0.0a	2.1b
	100	12.0c	0.0a	6.0b
Ketoconazole (200µg)		21.0d	10.5b	10.3c
DMSO		0.0	0.0	0.0

Mean followed by different letters in the same column indicate there was significant difference between the mean ($p < 0.001$) while mean followed by the same letter indicate no significant difference between the mean ($p > 0.005$)

4.13.3 Antimicrobial activity of *Plumeria rubra*

Antimicrobial activities demonstrated by *P. rubra* essential oils are displayed in Table 4.25. Unlike the *P. acuminata* oils, all the essential oil samples were inactive against *E. coli* while low activity was observed against *S. aureus* (diameter of inhibition zones range from 1.8 - 2.0 mm). Besides *F. solani* that was resistant to the flower essential oil, the fungi were susceptible to the oils with variable zones of inhibition between 1.9 - 8.1 mm. The leaf volatile oil was more active than the oils from other organs of the plant. There was no significant difference ($p > 0.05$) in the activity of leaf and stem oils, leaf and root oils, leaf and flower oils, stem and root oils as well as flower and root oils against *S. aureus*, *E. coli* and *C. albicans* while the activities of the essential oils compared with the activities of the standard drugs were significantly different, $p < 0.001$ (APPENDIX 59).

Two different *P. rubra* flower oils from China reported by Liu *et al* (2012) exhibited good antimicrobial activities. They showed significant zones of inhibition against both Gram-positve and Gram-negative as well as fungus, *Aspergillus niger*. The difference observed in the activities of the volatile oils of these *Plumeria* species is as a result of variation in their chemical profiles due to pedological factors, edaphic and climatic stress (Barra, 2009)

Other studies on the plant's extract have also been documented. Ethyl acetate and chloroform leaf extracts of *P. rubra* leaves displayed good antibacterial and antifungal activities while petroleum ether and carbon tetrachloride extracts exhibited no activity (Jarin *et al*., 2008). Baghel *et al*., (2010) also gave account of the antibacterial activity of the chloroform, ethyl acetate, ethanol and aqueous extracts of the leaf. Some of the isolated compounds from the stem bark also demonstrated antibacterial and antifungal activities (Kuigoua *et al*., 2010). Likewise, extracts from other *Plumeria* species have shown good antimicrobial activity (Rasool *et al*., 2008; Sibi *et al*., 2012).

Table 4.25: Antimicrobial Activity of *Plumeria rubra*

Essential oils	Conc (µL/mL)	Zones of Inhibition (mm)				
		S. aureus	*E. coli*	*C. albicans*	*F. solani*	*A. niger*
Leaf	1	0.0a	0.0a	0.0a	4.1a	4.0a
	10	0.0a	0.0a	2.1a	6.0a	5.9a
	100	2.0a	0.0a	5.9a	8.0a	8.1a
Stem	1	0.0a	0.0a	0.0a	0.0a	0.0b
	10	0.0a	0.0a	2.1a	2.0a	0.0b
	100	0.0a	0.0a	4.2a	4.0a	2.0b
Flower	1	0.0a	0.0a	3.9a	0.0b	4.0a
	10	0.0a	0.0a	5.9a	0.0b	5.9a
	100	1.9a	0.0a	8.0a	0.0b	7.9a
Root	1	0.0a	0.0a	0.0a	0.0c,a,b	0.0c,b,a
	10	0.0a	0.0a	4.1a	0.0c,a,b	2.2c,b,a
	100	1.8a	0.0a	6.0a	1.9c,a,b	6.0c,b,a
+ve control		11.5b	9.0b	21.0b	10.3d	10.5d
-ve control		0.0	0.0	0.0	0.0	0.0

Mean followed by different letters in the same column indicate there was significant difference between the mean ($p < 0.001$) while mean followed by the same letter indicate no significant difference between the mean ($p > 0.005$)

+ve control- Gentamycin for antibacterial assay, Ketoconazole for antifungal assay

-ve control- DMSO

4.13.4 Antimicrobial activity of *Delonix regia*

The activities of *Delonix regia* twig, flower and root volatile oils against the tested bacteria and fungi are shown in Table 4.26. The bacteria were resistant to the oils but the fungi were all susceptible. Moderate activity was observed at the highest concentration (100 µL/mL) while no activity was detected at low concentration (1 µL/mL). Statistical analysis (APPENDIX 60) showed no significant difference between the activities of twig and flower oils as well as twig and root oils ($p > 0.05$). The activity of the standard drugs was significantly different from the activities of the essential oils ($p < 0.001$)

In contrast, crude extracts from various organs of *Delonix regia* as reported in the literature were active against both bacteria and fungi. The bark and flowers extracts showed broad-spectrum antibacterial and antifungal activities (Salem 2013; Ahmad and Aquil 2003). Also the essential oil from the wood gave antibacterial and antifungal activity at the range 13.0-21.0 mm (Salem *et al.,* 2014).

Table 4.26 Antimicrobial Activity of *D. regia*

Essential oils	Conc	Zones of Inhibition (mm)				
	(µL/mL)	*S. aureus*	*E. coli*	*C. albicans*	*F. solani*	*A. niger*
Twig	1	0.0a	0.0a	0.0a	0.0a	0.0a
	10	0.0a	0.0a	2.2a	2.1a	2.0a
	100	0.0a	0.0a	4.3a	4.1a	4.0a
Flower	1	0.0a	0.0a	0.0 a	0.0a	0.0a
	10	0.0a	0.0a	2.1a	0.0a	1.9a
	100	0.0a	0.0a	4.1a	3.9 a	4.0a
Root	1	0.0a	0.0a	0.0a	1.9a	0.0a
	10	0.0a	0.0a	2.2a	4.1a	0.0a
	100	0.0a	0.0a	5.7a	6.0 a	1.8a
+ve control		11.5b	9.0b	21.0b	10.3b	10.5b
-ve control		0.0	0.0	0.0	0.0	0.0

Mean followed by different letters in the same column indicate there was significant difference between the mean ($p < 0.001$) while mean followed by the same letter indicate no significant difference between the mean ($p > 0.005$)

+ve control- Gentamycin for antibacterial assay, Ketoconazole for antifungal assay

-ve control- DMSO

4.13.5 Antimicrobial activity of *Duranta repens*

The results of antimicrobial assay of *D. repens* leaf, stem, fruit and root essential oils are presented in Table 4.27. *E. coli* was resistant to all the volatile oils but weak inhibitory zone of about 2.0±0.0 mm was observed against *S aureus.* The root and stem oils showed no activity against *E. coli*. The fruit oil was the most active against all the fungi with the inhibitory zone range of 4.1 -8.5 mm, but not as active as the reference antifungi drug i.e ketoconazole (diameter of inhibition zones range from 10.3 - 21.0 mm). The leaf, stem and root oils presented low or no activities against the tested fungi. *F. solani* was resistant to the leaf and root and low concentration of the stem oil while *A. niger* was resistant to the stem and root oils as well as low concentration of the leaf oil. On the other hand, comparing the activity of the leaf and stem oil using two-way ANOVA (APPENDIX 61), there was no significant difference ($p > 0.05$). Likewise, no significant difference was observed in the activity of leaf and fruit oil against *S. aureus*, *E. coli* and *C. albicans* ($p > 0.05$) as well as activity of the leaf and root oils against all the microbes (except *E. coli*, $p < 0.001$).

There was no information on the antimicrobial activity of the *D. repens* essential oils in the literature except the crude extracts from different organs. The plant extacts from diverse geographical areas demonstrated potent antimicrobial activity. Among them were leaf crude extract of Nigeria origin, stem and fruit extracts from Bangladesh, methanol extracts of leaves, stem and roots from India (Jayalakshmi *et al*., 2011; Sharma *et al*., 2012; Sikarwar *et al*., 2014; Ogbuagu *et al*., 2015). Therefore, the crude extracts from the plant are more active than the essential oil.

Table 4.27: Antimicrobial Activity of *D. repens*

Essential oils	Conc (µL/mL)	Zones of Inhibition (mm)				
		S. aureus	*E. coli*	*C. albicans*	*F. solani*	*A. niger*
Leaf	1	0.0a	0.0a	3.9a	0.0a	0.0a
	10	0.0a	0.0a	5.5a	0.0a	0.0a
	100	1.8a	0.0a	7.5a	0.0a	2.1a
Stem	1	0.0a	0.0a	2.3a	0.0a	0.0a
	10	0.0a	0.0a	3.8a	0.0a	0.0a
	100	0.0a	0.0a	6.0a	2.0a	0.0a
Fruit	1	0.0a	0.0a	4.1a	4.0 b	3.9b
	10	0.0a	0.0a	6.3a	5.8 b	6.1b
	100	2.0a	0.0a	8.0a	8.3b	8.5b
Root	1	0.0a	0.0a	0.0b	0.0a	0.0a
	10	0.0a	0.0a	0.0b	0.0a	0.0a
	100	0.0a	0.0a	2.0b	0.0a	0.0a
+ve control		11.5b	9.0b	21.0c	10.3c	10.5c
-ve control		0.0	0.0	0.0	0.0	0.0

Mean followed by different letters in the same column indicate there was significant difference between the mean ($p < 0.001$) while mean followed by the same letter indicate no significant difference between the mean ($p > 0.005$)

+ve control- Gentamycin for antibacterial assay, Ketoconazole for antifungal assay

-ve control- DMSO

4.13.6 Antimicrobial activity of *Zanthoxylum zanthoxyloides*

The pilot study showed that *Z. zanthoxyloides* volatile oils exhibited low activity against both *E. coli* and *S. aureus*. The stem and leaf oils gave weak inhibition zones of 1.9±0.1 and 2.3±0.4 mm against *S. aureus* respectively at 100 µL/ mL. The two oils exhibited moderate antifungal activity against the fungi except *A. niger* which was resistant to the stembark essential oil. Table 4.28 presents the antimicrobial activities of the stembark and leaf of *Z. zanthoxyloides* essential oils. Comparing the activities of the oils and positive control using two-way ANOVA (APPENDIX 62), there was significant difference between the activities of *Z. zanthoxyloides* essential oils and the standard drugs ($p < 0.001$) while no significant difference was observed between the activity of the leaf oil and stem bark oil against *S. aureus*, *E. coli* and *A. niger* ($p > 0.05$).

Misra *et al.,* (2013) reported the relationship between the chemical constituents and antimicrobial properties of essential oils using *Z. leprieuni* and *Z. zanthoxyloides* fruit essential oils, shows that *Z. leprieuni*, dominated by a monoterpene hydrocarbon i.e. *E*-β-ocimene (77.36 %) showed poor antimicrobial activity while *Z. zanthoxyloides*, with high percentage of oxygenated monoterpenes (69.05%) majorly β-citronellol (40.00 %) and geraniol (9.00%) displayed a remarkable antimicrobial activity, in agreement with earlier suggestion made by Knobloch *et al*., (1986) that terpenoids interrupt the membrane of bacteria in addition to the flow of electron during ATP synthesis. In this study, the stembark oil with high percentage of monoterpene hydrocarbons (42.0 %) displayed better activity than the leaf oil dominated by oxygenated sesquiterpenes. Odebiyi and Sofowora, (1979) reported good antimicrobial activity of *Z. zanthoxyloides* crude extract against *S. aureus* and *E. coli*. Organic extract of the root bark had also been documented as a source of broad-spectrum antimicrobial agents (Ynalvez *et al*., 2012).

The leaf essential oil of *Z. alatum* from Himalaya (dominated by linalool 30.58 %, 2-decanone 20.85 %, β-fenchol 9.43 %, 2-tridecanone 8.86 % and β-phellandrene 5.99 %, inhibited the growth of both gram-negative and gram-positive including *S. aureus* and *E. coli* with the diameter of zones of inhibition range of 15.0-21.0 mm while the methanol extract was not active (Guleria *et al*., 2013). *Z. monogynum* Leaf essential oil dominated by citronellol (43.03 %), farnesol (32.96 %) and citronellal (9.57 %) displayed good

antimicrobial activity (Da Silva *et al*., 2017). Furthermore, *Z. nitidum* leaf, fruit and branches essential oils as well as *Z. armatum* leaf oil respectively dominated by limonene, n-pentadecane, 2-undecanone and bornyl acetate also demonstrated good antimicrobial activity (Negi *et al*., 2012; Tuyen *et al*., 2021).

Table 4.28: Antimicrobial Activity of *Z. zanthoxyloides*

Essential oils	Conc (µL/mL)	Zones of Inhibition (mm)				
		S. aureus	*E. coli*	*C. albicans*	*F. solani*	*A. niger*
Leaf	1	0.0a	0.0a	0.0a	0.0a	3.5a
	10	0.0a	0.0a	2.2a	2.1a	6.2a
	100	2.3a	0.0a	4.1a	6.1a	7.9a
Stem bark	1	0.0a	0.0a	3.8b	2.1a	0.0b
	10	0.0a	0.0a	6.3b	3.8a	0.0b
	100	1.9a	0.0a	8.1b	5.9a	0.0b
+ve control		11.5b	9.0b	21.0c	10.3b	10.5c
-ve control		0.0	0.0	0.0	0.0	0.0

Mean followed by different letters in the same column indicate there was significant difference between the mean ($p < 0.001$) while mean followed by the same letter indicate no significant difference between the mean ($p > 0.005$)
+ve control- Gentamycin for antibacterial assay, Ketoconazole for antifungal assay
-ve control- DMSO

4.13.7 Antimicrobial activity of *Ceiba pentandra*

The activity of the stem bark and heartwood of *C. pentandra* essential oils against the tested fungi and bacteria is given in Table 4.29. *E. coli* was resistant to both oils while only stem bark (100 µL/ mL) exhibited weak activity with zone of inhibition of 2.0 mm against *S. aureus*. Inhibitory zone range of 1.9 - 4.3 mm were observed against the fungi which was quite lower than the activity demonstrated by ketoconazole (10.3 - 21.0 mm), the synthetic drug. *A. niger* was resistant to the heartwood oil. There was no significant difference ($p > 0.05$) between the activities of the two oils while their activities were significantly different from the standard drugs when subjected to statistical analysis using two-way ANOVA (APPENDIX 63). Thus, the essential oils from *C. pentandra* heartwood and stem bark are not as active as the standard drugs used in this study.

Good antibacterial potential of the stem bark methanol extract against *E. coli* and *S. aureus* has been reported (Ur-Rehman *et al*., 2017). Moderate antifungal activities of organic crude extracts of *C. pentandra* flower, stem wood, stem bark, leaf, root wood and root bark were also reported (Jan *et al*., 2017).

Table 4.29: Antimicrobial Activity of *C. pentandra*

Essential oils	Conc (μL/mL)	Zones of Inhibition (mm)				
		S. aureus	*E. coli*	*C. albicans*	*F. solani*	*A. niger*
Stembark	1	0.0a	0.0a	0.0a	0.0a	0.0a
	10	0.0a	0.0a	2.1a	1.9a	2.2a
	100	2.0a	0.0a	3.9a	4.3a	4.1a
Heartwood	1	0.0a	0.0a	0.0a	0.0a	0.0a
	10	0.0a	0.0a	2.1a	1.9a	0.0a
	100	0.0a	0.0a	4.1a	4.0a	0.0a
+ve control		11.5b	9.0b	21.0b	10.3b	10.5b
-ve control		0.0	0.0	0.0	0.0	0.0

Mean followed by different letters in the same column indicate there was significant difference between the mean ($p < 0.001$) while mean followed by the same letter indicate no significant difference between the mean ($p > 0.005$)

+ve control- Gentamycin for antibacterial assay, Ketoconazole for antifungal assay

-ve control- DMSO

4.13.8 Antimicrobial activity of *Gliricidia sepium*

The results obtained from antimicrobial assay of *G. sepium* leaf and stem oils are presented in Table 4.30. The bacteria were resistant to the essential oils while the fungi, especially *Fusarium solani* were susceptible with significant zones of inhibition varying from 2.0 - 8.1 mm (Plate 4.3). Only the stem oil was active against *C. albicans* and *A. niger*. The stem oil showed higher acvtivity than the leaf oil, although statistical analysis showed no significant difference ($p > 0.05$) between their activities except for *C. albicans* (APPENDIX 64). Many oxygenated sesquiterpenes that were found in the stem oil were absent in the leaf oil and this could have contributed to the reason *C. albicans* was more susceptible to the stem oil. The activities of the oils and standard drugs, gentamycin and ketoconazole were significantly different ($p < 0.001$).

Alternatively, Jose and Reddy (2010) reported that *G. sepium* stembark and leaf essential oils have significant activity against *S. aureus* and *E. coli*. This was also supported by the use of the plant bark decoction in Guatemala and Costa Rica as an antibacterial agent. However, this is contrary to the observation from this study. The difference in their activity may be ascribed to the disparity in thier chemical compositions as a result of variation in geographical origin of the plants.

Antimicrobial assay of the plant's leaf extract has also been reported. Ethanol leaf extract demonstrated good activity against *S. aureus* and *E. coli* (Abulude and Adebote, 2009). Salud *et al.,* (2007) as well gave account of antimicrobial activity of chloroform and methanol extracts of *G. sepium* bark as potent antibacterial agents.

Table 4.30: Antimicrobial Activity of *G. sepium*

Essential oils	Conc (µl/mL)	Zones of Inhibition (mm)				
		S. aureus	*E. coli*	*C. albicans*	*F. solani*	*A. niger*
Leaf	1	0.0a	0.0a	0.0a	2.0a	0.0a
	10	0.0a	0.0a	0.0a	3.9a	0.0a
	100	1.8a	0.0a	0.0a	6.0a	0.0a
Stem	1	0.0a	0.0a	2.1a	0.0a	0.0a
	10	0.0a	0.0a	4.3b	3.8a	0.0a
	100	0.0a	0.0a	8.1b	6.1a	1.9a
+ve control		11.5b	9.0b	21.0c	10.3b	10.5b
-ve control		0.0	0.0	0.0	0.0	0.0

Mean followed by different letters in the same column indicate there was significant difference between the mean ($p < 0.001$) while mean followed by the same letter indicate no significant difference between the mean ($p > 0.005$)
+ve control- Gentamycin for antibacterial assay, Ketoconazole for antifungal assay
-ve control- DMSO

Plate 4.3 : Zones of Inhibition of *G. sepium* essential oil against *F. solani*
(11_1 & 11_2- Stem essential oil; 18_1 & 18_2- Leaf essential oil; C- Ketoconazole)

4.13.9 Antimicrobial activity of *Annona muricata*

Essential oils extracted from the leaf, branches and stem bark of *Annona muricata* showed insignificant antibacterial activity but better activity was observed against all the tested fungi. The leaf and stem oils have similat activity while the stem bark oil had low activity (Table 4.31). *Candida albican* was the most succeptible among the fungi. Dominance of sesquiterpene hydrocarbons in the leaf essential oil could likely be responsible for the better antifungal activity showed by the oil when compared with the other two essential oils of the plant. In addition, (*E*)-Caryophyllene (28.2 %), δ-Cadinene (9.6 %) and β –Elemene (8.3 %) which predominated the leaf oil were absent in the other two oil samples. Statistical analysis of the inhibitory zones showed no significant difference ($p > 0.05$) between the activities of the oils while the activity of the standard drugs was significantly different ($p < 0.001$) when compared with the oils' activities (APPENDIX 65).

The fruit essential oil of the plant has recently been reported to have good antimicrobial activity (Badrie and Schauss, 2009). Extracts from various parts of the plant also displayed moderate antibacterial and antifungi activities (Pathak *et al*., 2010; León-Fernández *et al*., 2019).

Table 4.31: Antimicrobial Activity of *A. muricata*

Essential oils	Conc (µL/mL)	Zones of Inhibition (mm)				
		S. aureus	*E. coli*	*C. albicans*	*F. solani*	*A. niger*
Leaf	1	0.0a	0.0a	4.0a	3.9a	4.1a
	10	0.0a	0.0a	6.1a	6.2a	5.9a
	100	2.0a	0.0a	7.9a	8.2a	8.0a
Branches	1	0.0a	0.0a	2.3a	2.2a	3.9a
	10	0.0a	0.0a	6.0a	4.2a	6.0a
	100	0.0a	0.0a	8.1a	5.8a	8.1a
Stem bark	1	0.0a	0.0a	2.1 a	0.0b	0.0b
	10	0.0a	0.0a	6.1a	2.0b	0.0b
	100	0.0a	0.0a	7.9a	4.0a	0.0b
Positive control		11.5b	9.0b	21.0b	10.3c	10.5c
Negative control		0.0	0.0	0.0	0.0	0.0

Mean followed by different letters in the same column indicate there was significant difference between the mean ($p < 0.001$) while mean followed by the same letter indicate no significant difference between the mean ($p > 0.005$)
+ve control- Gentamycin for antibacterial assay, Ketoconazole for antifungal assay
-ve control- DMSO

CHEPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

A total of 64 compounds were identified in *T. stans*, 104 in *P. acuminata*, 145 in *P. rubra*, 51 in *D. regia*, 72 in *D. repens*, 49 in *Z. zanthoxyloides*, 46 in *C. pentandra*, 76 in *G. sepium* and 89 in *A. muricata* essential oils. Many rare monoterpenes and sesquiterpenes were detected in the oils. For example, ishwarane, hinesol, mustakone, germacra-4(15),5,10(14)-trien-1α-ol, salvia-1α-ol, *Z*-cadina-1(16),4-diene, thujospan-2α-ol, pogostol to mention a few. Pronounced differences in the chemical components were observed among the volatile oils extracted from different organs of the same plant giving rise to variation in their odour, colour and biological activities. Many important aroma compounds which are of significant importance in perfumery and food industries were detected as major constituents in the oils.

The leaf essential oil of *A. muricata*, *Z. zanthoxyloides* and *P. acuminata* have been previously reported in Nigeria but there were marked differences in the chemical profiles of the oils and those reported in the present work. The essential oils studied in other parts of the world exhibited different chemical and biological properties from the oils in this study. The observed variations can be attributed to seasonal difference, geographical disparity, plant's maturity, extraction procedure, pedological factors, edaphic and climatic stress.

The IC_{50} values of the essential oils of *Annona muricata* leaf (43.22 µg/ mL), *Tecoma stans* seed (33.03 µg/ mL) and stem (6.44 µg/ mL) as well as *Duranta. repens* stem (32.02 µg/ mL) and fruit (29.39 µg/ mL) showed higher radical-scavenging ability than the standard compounds, α-tocopherol (81.58 µg/ mL) and BHA (45.11 µg/ mL). Other investigated oils showed scavenging activity (IC_{50} values between 84.26 and 695.00 µg/ mL) but not as

active as the synthetic antioxidant. The leaf essential oils of *Zanthoxylum zanthoxyloides*, *Annona muricata* and *Plumeria acuminata* exhibited strong toxicity against *Artemia salina* larvae with the LC_{50} values of 5.8992 ppm, 6.1518 ppm and 6.9070 ppm respectively. The lower the LC_{50}, the higher the cytotoxicity and the more the pharmacological activity of the plant extract. Besides, brine shrimp lethality assay also predicts anti-tumour properties of phytochemicals extracted from plant. Ethnobotanical review of these plants has unveiled their use in cancer chemotherapy and previous study on their extract confirmed their anticancer potentials. Hence, the present results support the traditional use of these plants in the treatment of tumour. Other examined oils also showed high cytotoxicity with LC_{50} values less than 100 ppm. The toxicty of the essential oils was likely due to synergistic effect of their chemical components.

Plumeria acuminata essential oils possessed high antimicrobial activity especially at 100 µl/ mL. The leaf oil demonstrated broad-spectrum activity against *Escherichia coli*, *Morganella morganii*, *Citrobacter freundii*, *Klebsiella pneumoniae, Salmonella typhi* and *Leclericia adecarboxylata* with inhibition values ranging between 1.7 and 12.1 mm displayed high antibacterial activity when compared with the standard drugs, gentamycin (8.0-12.5 mm) while the remaining volatile oils presented no or weak antibacterial potential. The high antimicrobial activities displayed by the *Plumeria* essential oils could be attributed to the presence of oxygenated terpenes like linalool, phytol and *Z*-β-farnesene, which were known as antimicrobial agents. Synergic effect of these antimicrobial agents might have also contributed to the oils' activities. The poor antimicrobial activity demonstrated by other volatile oils can be ascribed to antagonistic effect observed when the activity of different combined constituents was less than those of individual constituents. For example, some compounds like **γ**–terpinene and *p*-cymene (monoterpene hydrocarbons) found in these oils tend to produce an antagonistic effect with most active compounds by lowering their solubility, which leads to poor diffusion in the seeded media. All the volatile oils displayed moderate inhibitory effects against the assayed fungi except *Plumeria acumunata* root, *Duranta repens* leaf and stem with *Gliricidia sepium* leaf and stem that were inactive against *Aspergillus niger*.

5.2 Conclusion

Quantitative and qualitative characterisation of the essential oils of *Tecoma stans*, *Plumeria acuminata*, *Plumeria rubra, Delonix regia, Duranta repens, Zanthoxylum zanthoxyloides, Ceiba pentandra, Gliricidia sepium* and *Annona muricata* as well as their antioxidant, cytotoxicity, antimicrobial and antifungal activities were reported. The essential oils of the investigated plants were cytotoxic. The antioxidant properties displayed by *Annona muricata* leaf, *Tecoma stans* oils (seed and stem) and *Duranta. repens* oils (stem and fruit) as well as antimicrobial activities demonstrated by *Plumeria acuminata* essential oils support the folkloric claims of these plants.

5.3 Recommendations

The dominance of flavour and fragrance compounds in the essential oils of *Tecoma stans*, *Plumeria acuminata*, *Plumeria rubra*, *Ceiba pentandra*, *Gliricidia sepium* and *Annona muricata* suggest that they could be used as natural sources of flavour and fragrance agents. The cytotoxicity and antimicrobial activity of the leaf volatile oil of *Plumeria acuminata* suggest the oil could be of great importance to pharmaceutical industries in discovering of new antimicrobial medicine, cancer psychotherapy agents and food preservatives. The radical-scavenging activity demonstrated by *Tecoma stans* seed and stem, *Duranta repens* fruit and stem, as well as *Annona muricata* recommends their use as sources of natural antioxidants in food industry and could have application as therapeutic agent in stopping or decelerating oxidative stress associated with degenerative diseases.

5.4 Contributions to Knowledge

Lastly, this research has established the chemical constituents and biological activities of the examined plants' essential oils, which is the basic information that is essential to exploring their chemical and biological properties in pharmaceutical, food and cosmetic industries. The essential oils of *Tecoma stans*, *Plumeria rubra* (pink flower), *Delonix regia, Gliricidia sepium*, *Duranta repens* and *Ceiba pentandra* are being investigated in Nigeria for the first time.

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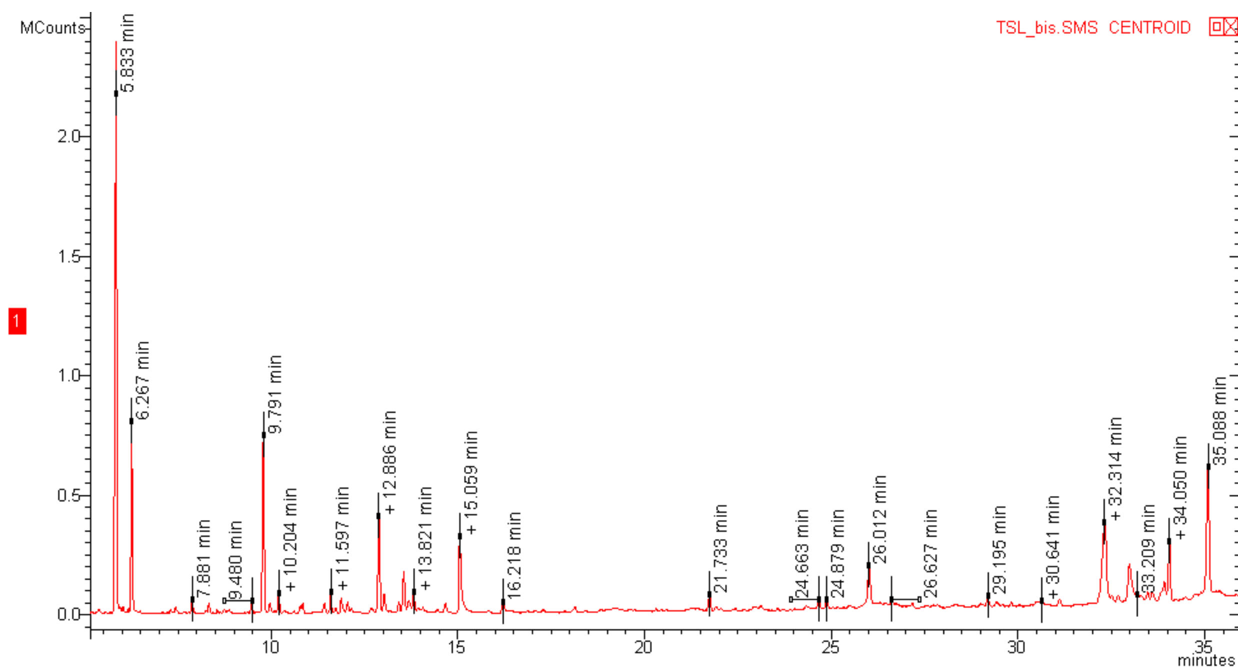
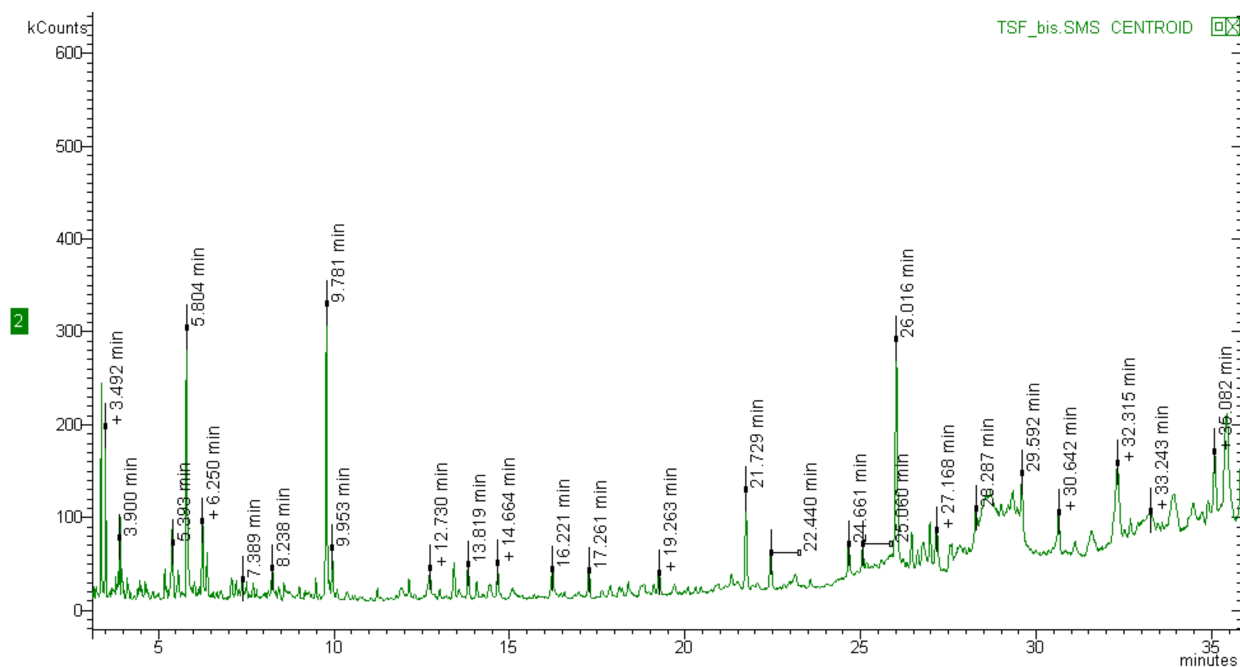
APPENDIX 1**:** Total Ion Chromatogram of *Tecoma stans* Leaf Essential oil



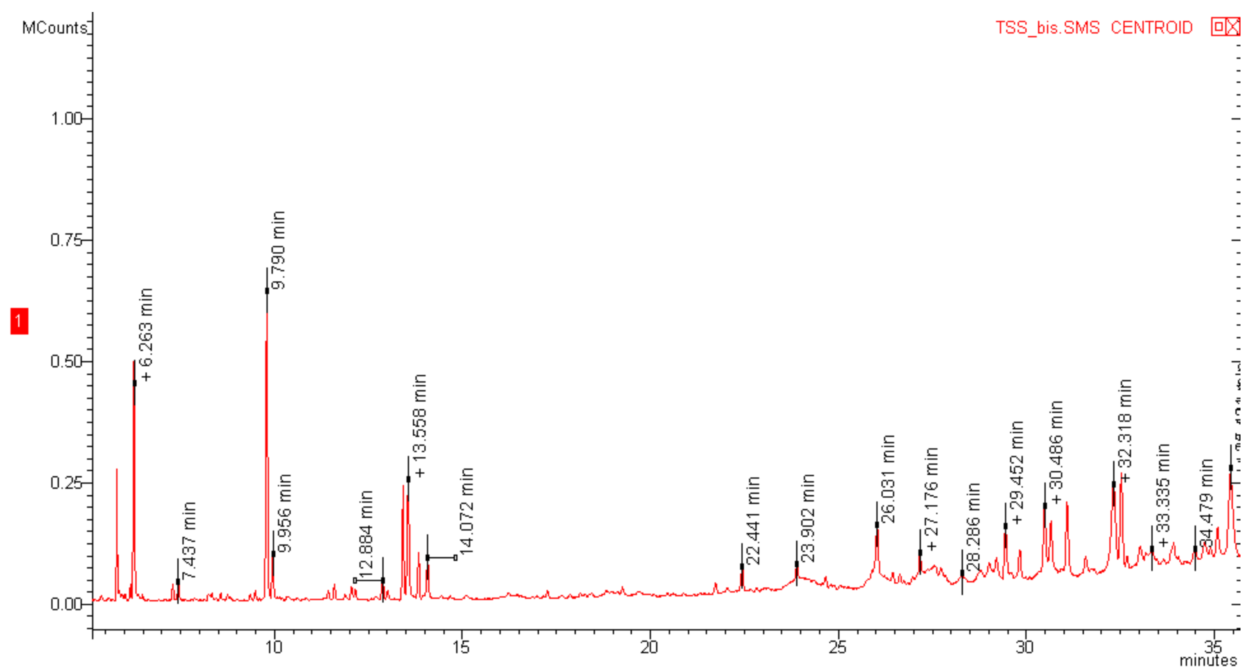
Capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 60 ºC (held for 10 min) heated to 220 ºC at 5 ºC/min at 70 eV.

APPENDIX 2: **Total Ion Chromatogram of *Tecoma stans* Flower Essential oil**



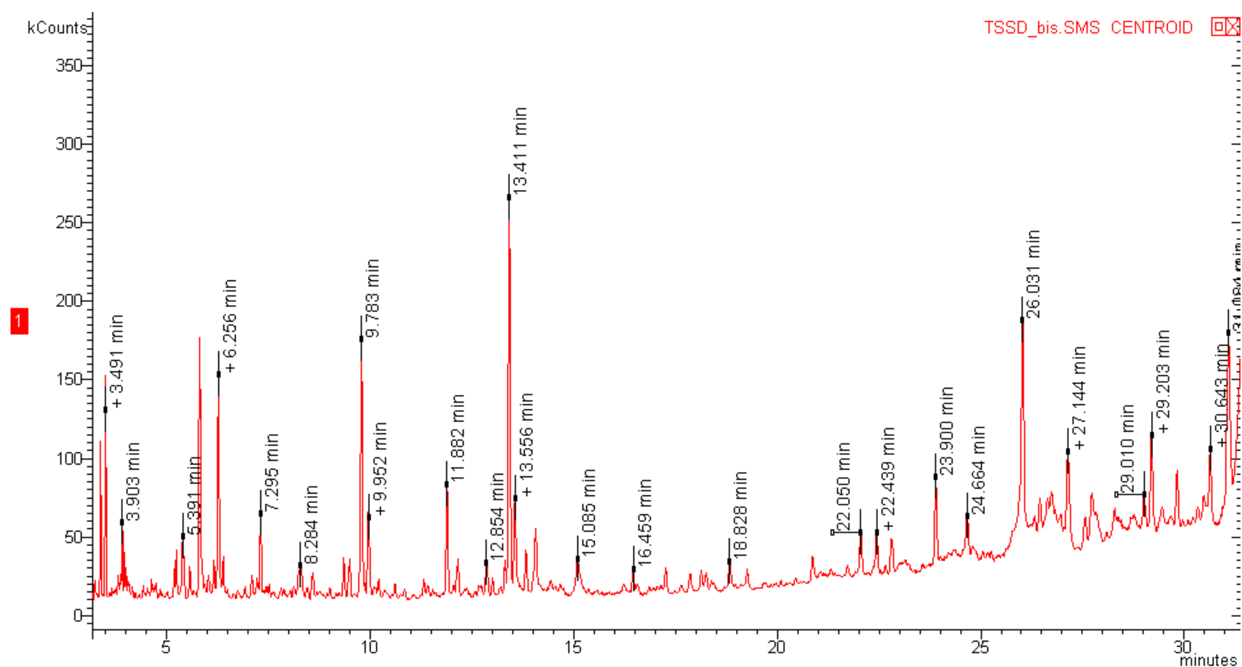
Capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 60 ºC (held for 10 min) heated to 220 ºC at 5 ºC/min at 70 eV.

APPENDIX 3: **Total Ion Chromatogram of *Tecoma stans* stem Essential oil**



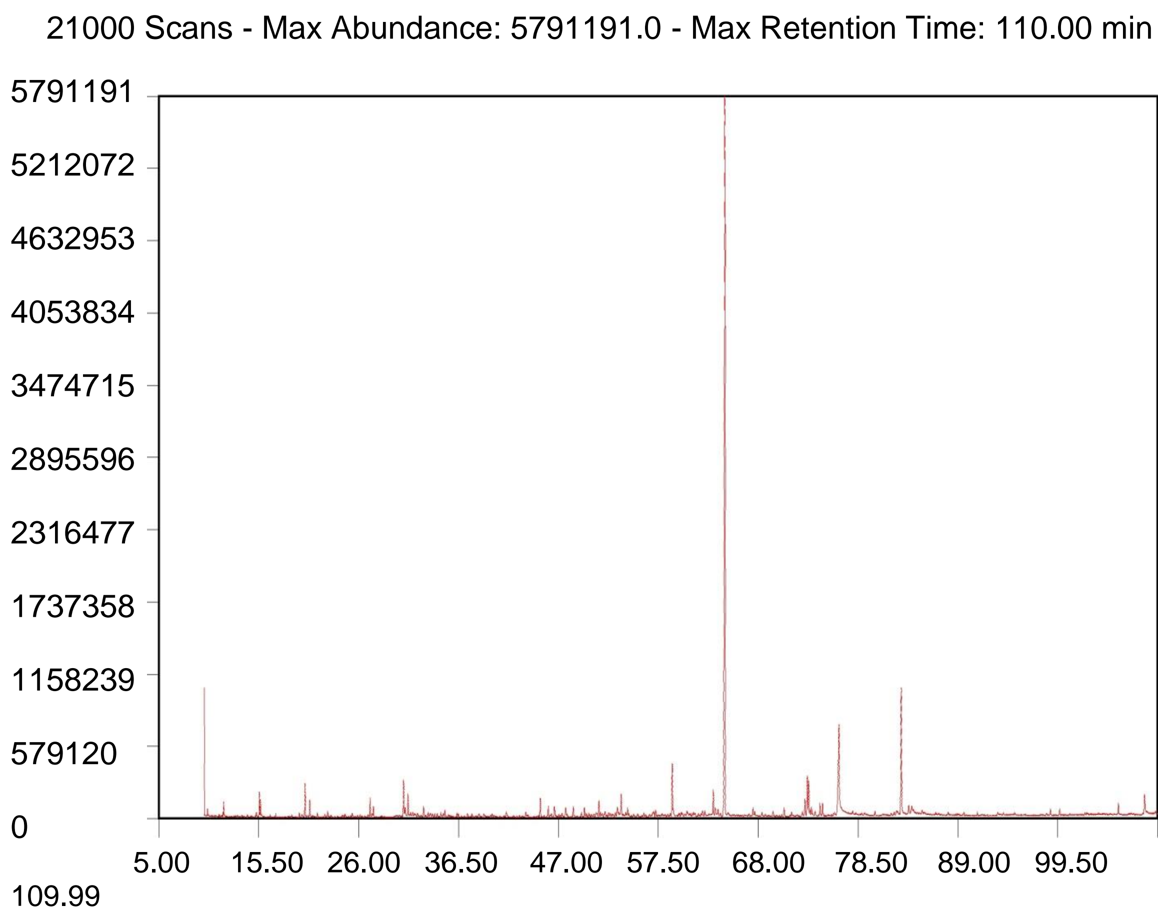
Capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 60 ºC (held for 10 min) heated to 220 ºC at 5 ºC/min at 70 eV.

APPENDIX 4: **Total Ion Chromatogram of *Tecoma stans* seed Essential oil**



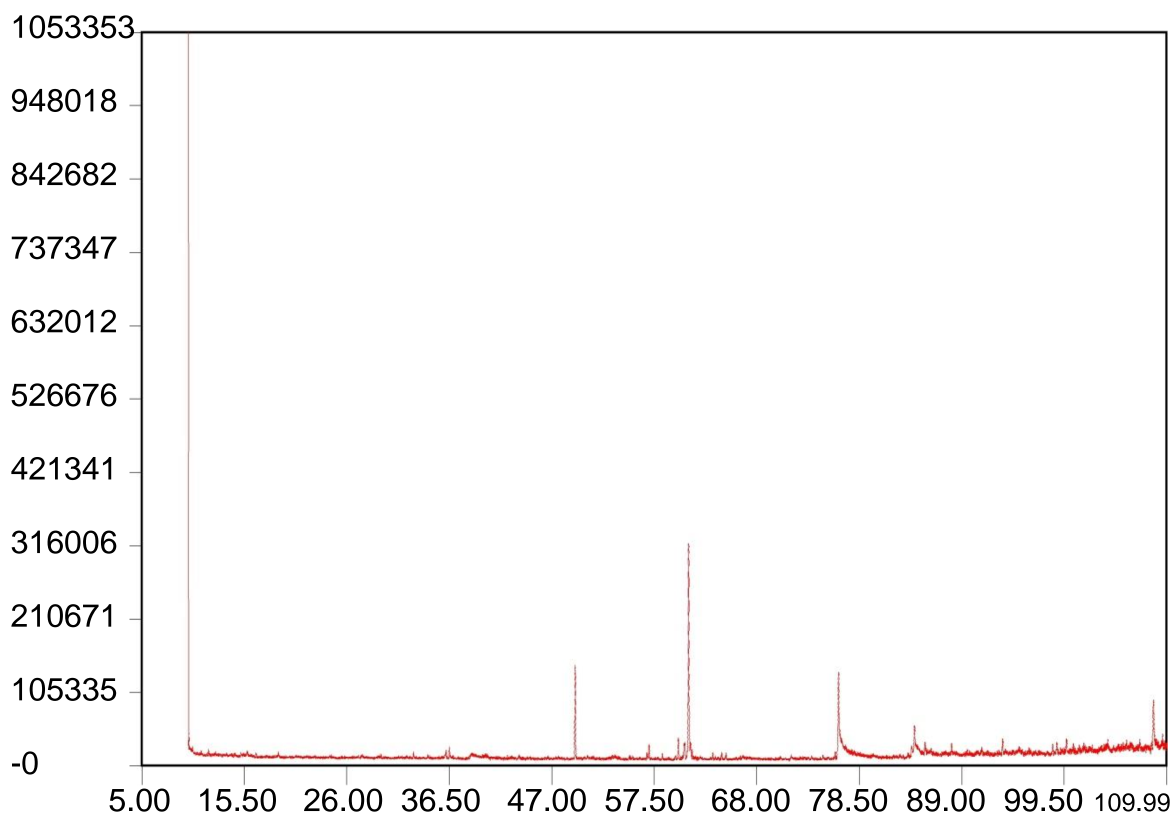
Capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 60 ºC (held for 10 min) heated to 220 ºC at 5 ºC/min at 70 eV.

APPENDIX 5: Gas Chromatogram of *Plumeria acuminata* leaf Essential oil



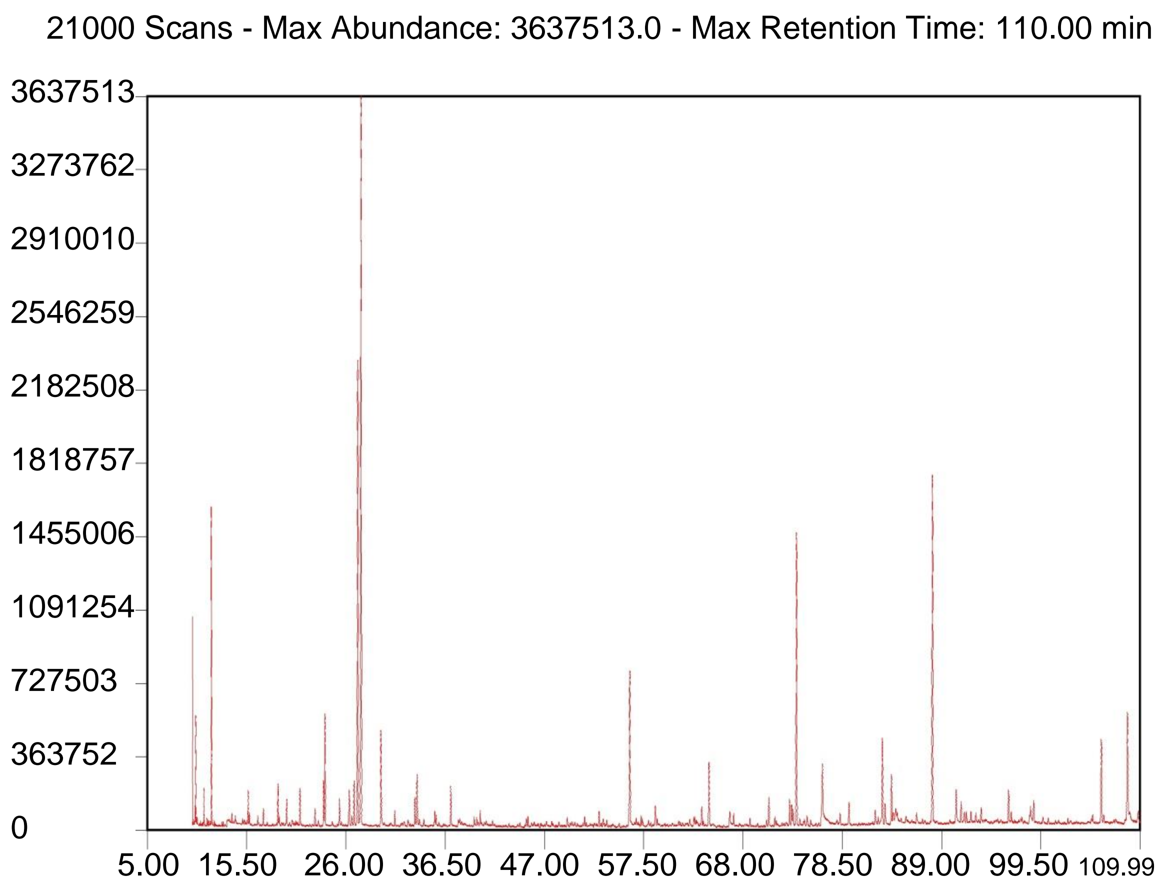
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ℃ (held for 10 min) heated to 260 ℃ at 2 ℃/min at 70 eV

APPENDIX 6: Gas Chromatogram of *Plumeria acuminata* root Essential oil



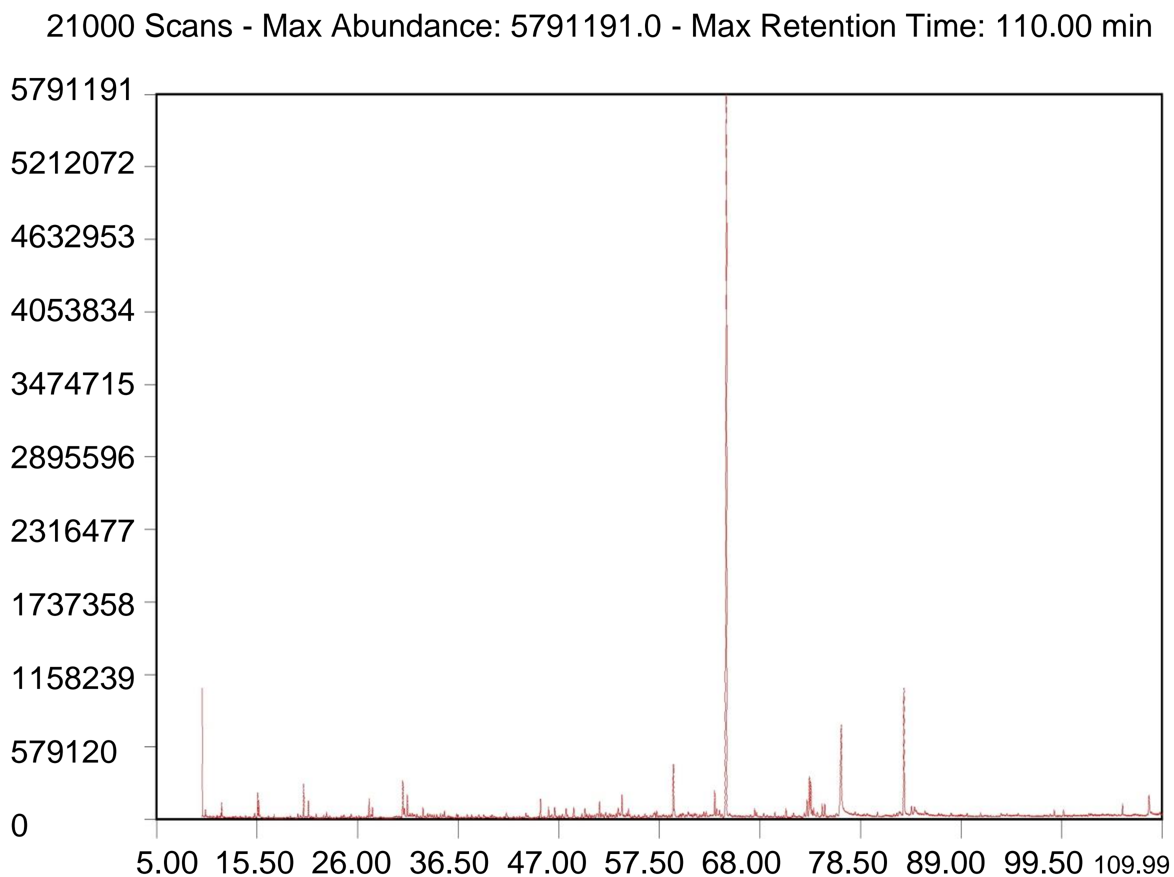
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 7: Gas Chromatogram of *Plumeria acuminata* flower Essential oil



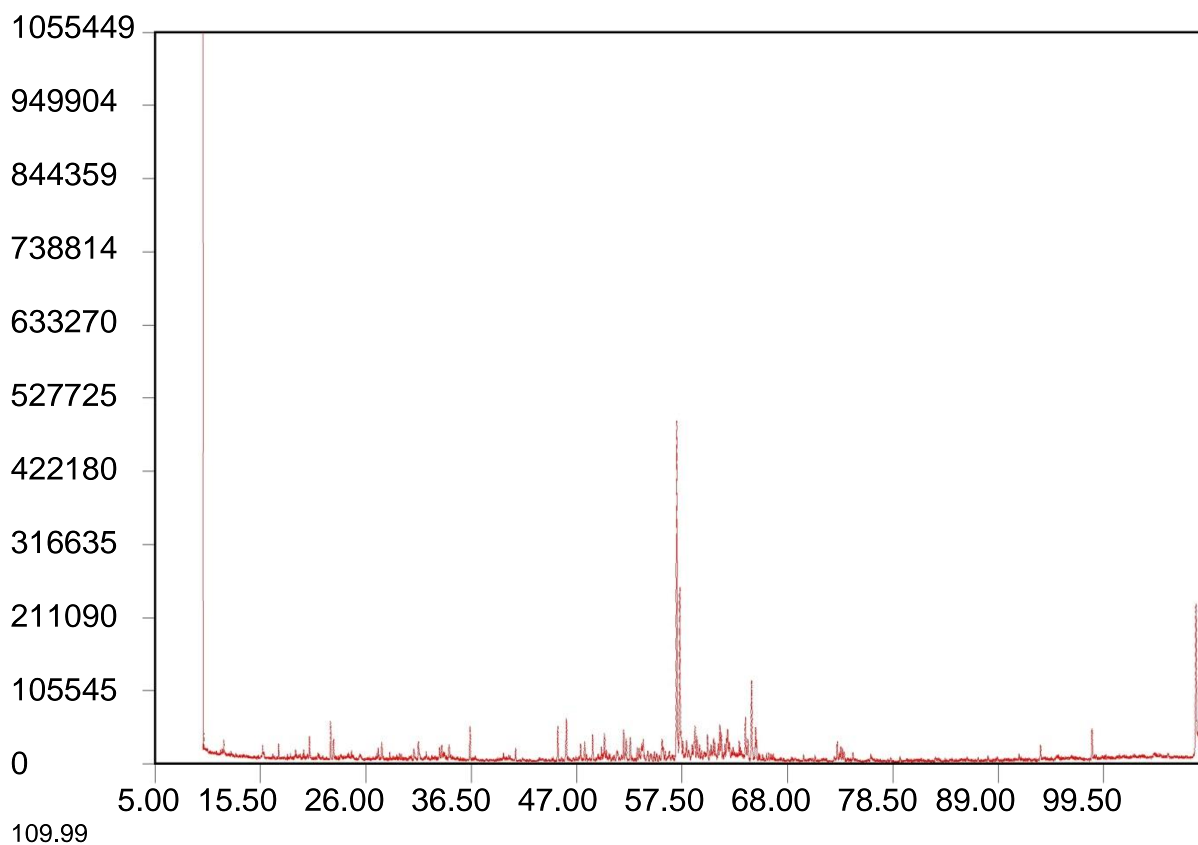
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 8: Gas Chromatogram of *Plumeria ruba* leaf Essential oil



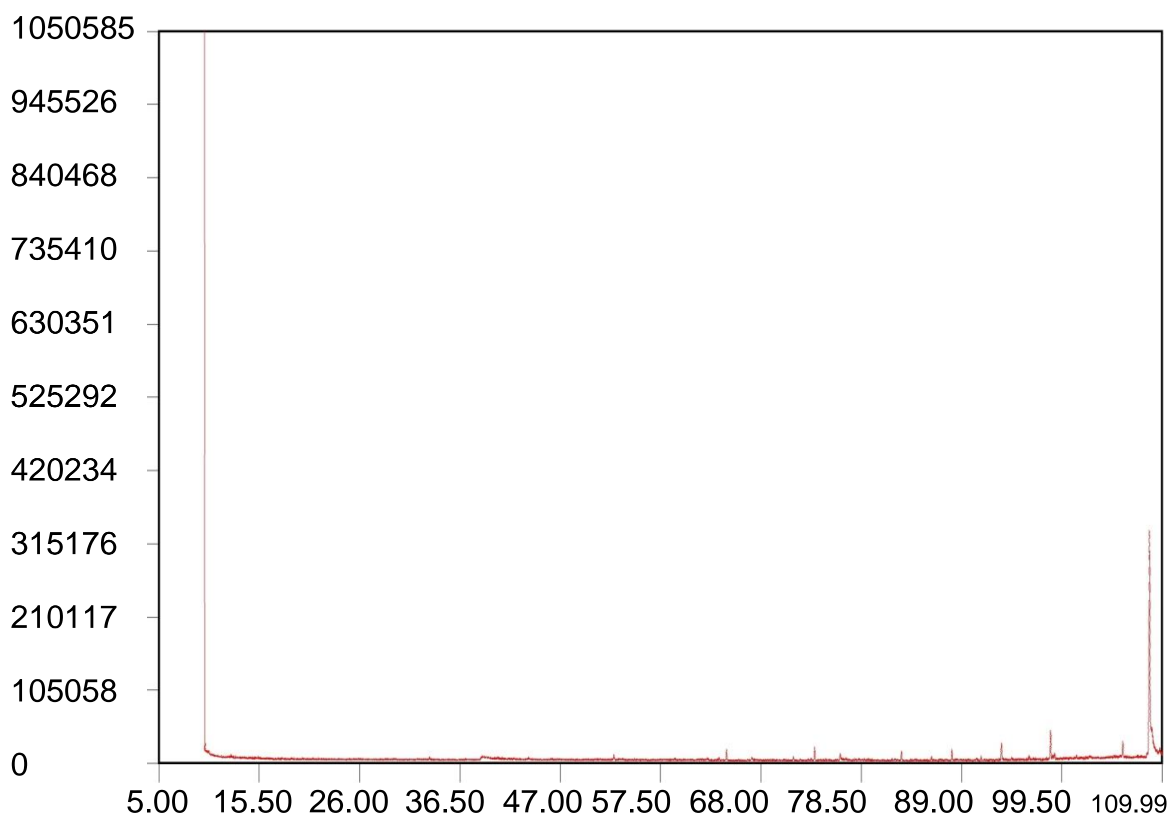
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 9: Gas Chromatogram of *Plumeria rubra* stem Essential oil



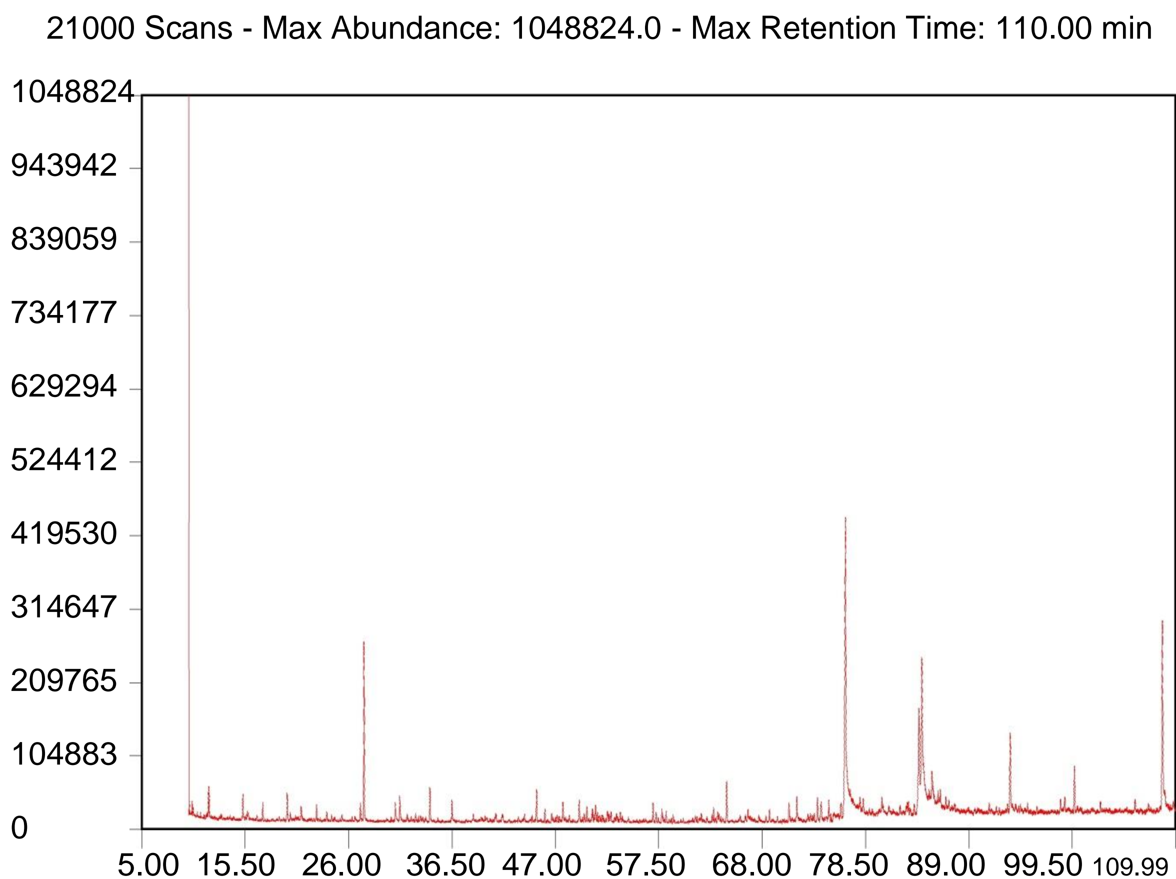
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 μm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 10: Gas Chromatogram of *Plumeria rubra* flower Essential oil



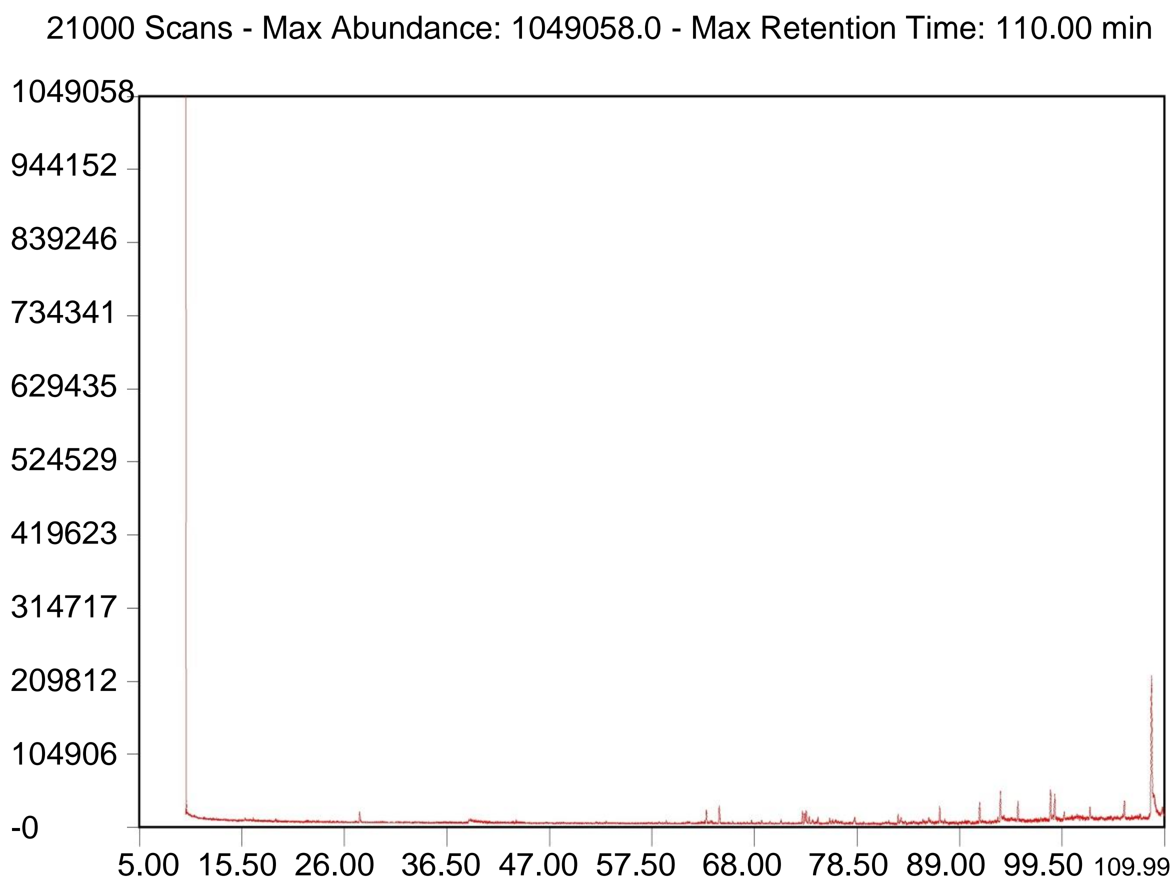
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 μm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 11: Gas Chromatogram of *Plumeria rubra* root Essential oil



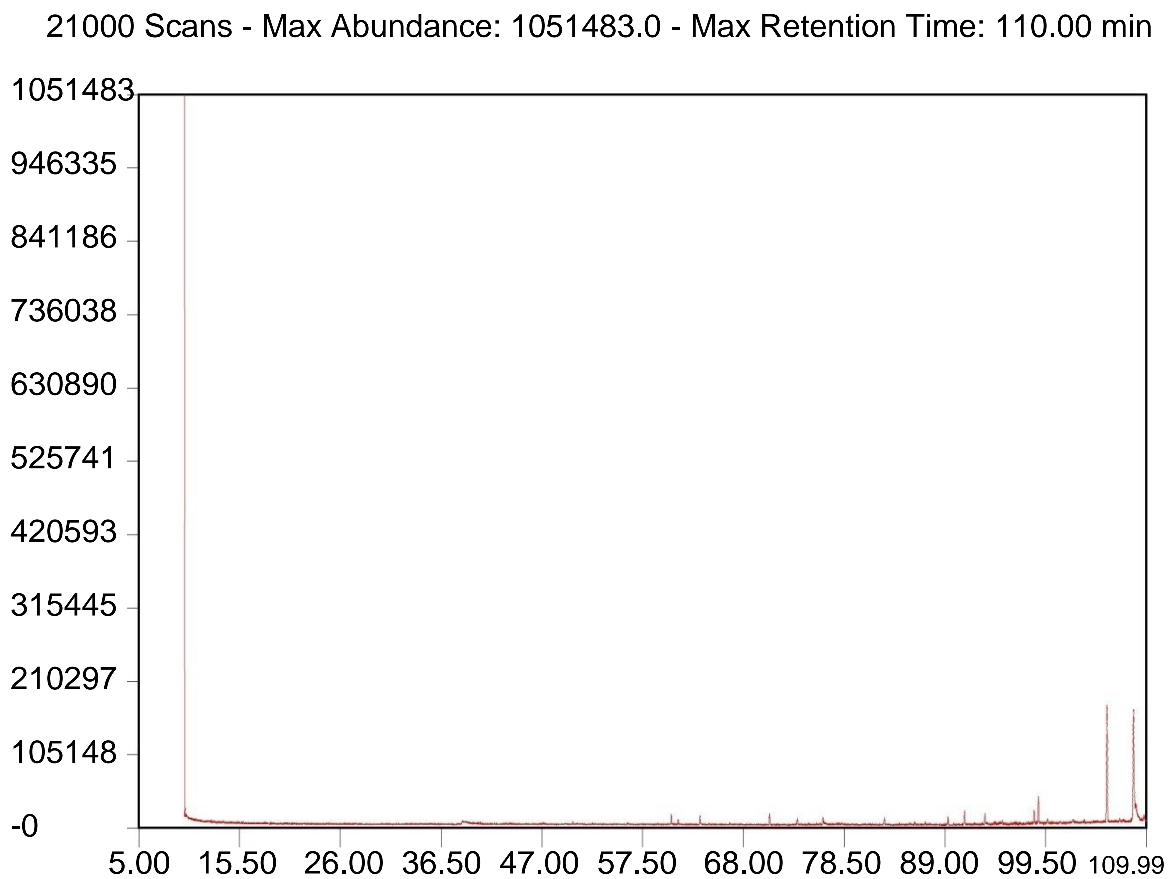
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 12: Gas Chromatogram of *Delonix regia* twig Essential oil



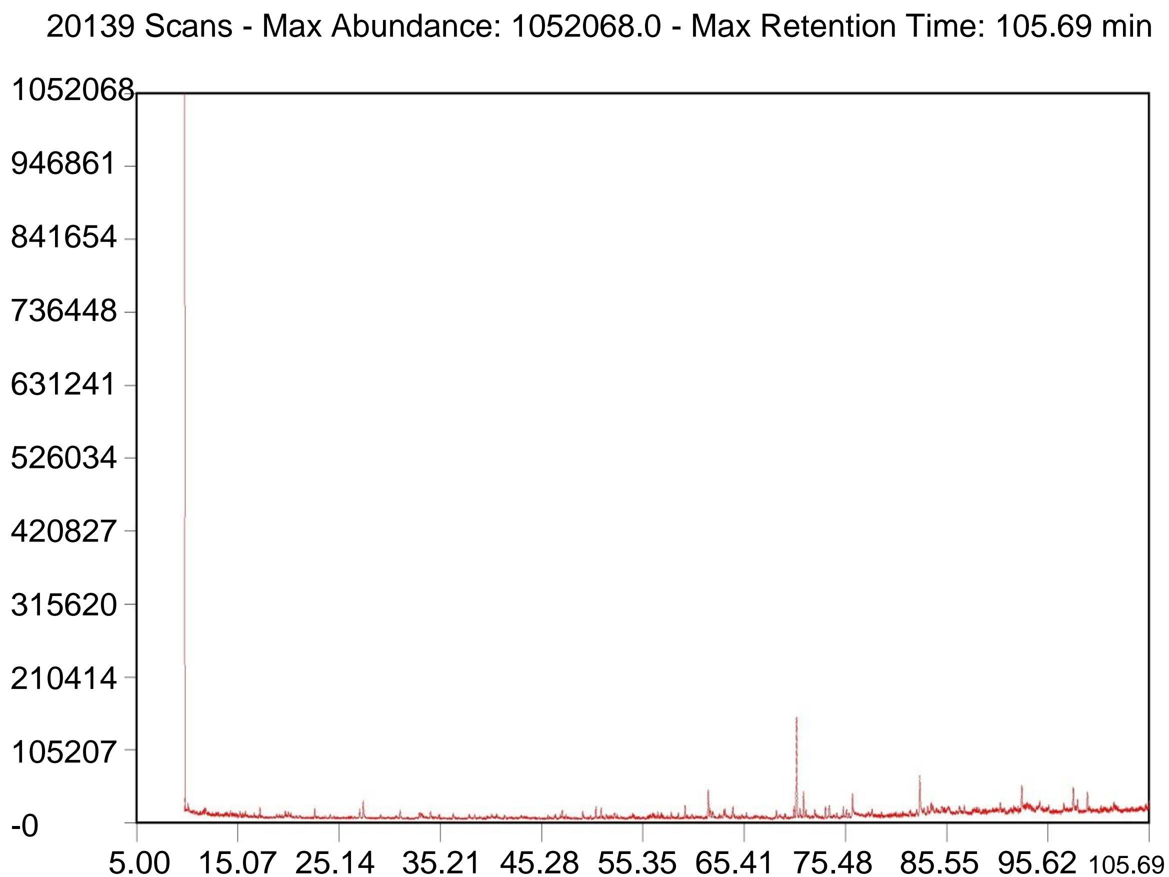
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 13: Gas Chromatogram of *Delonix regia* flower Essential oil



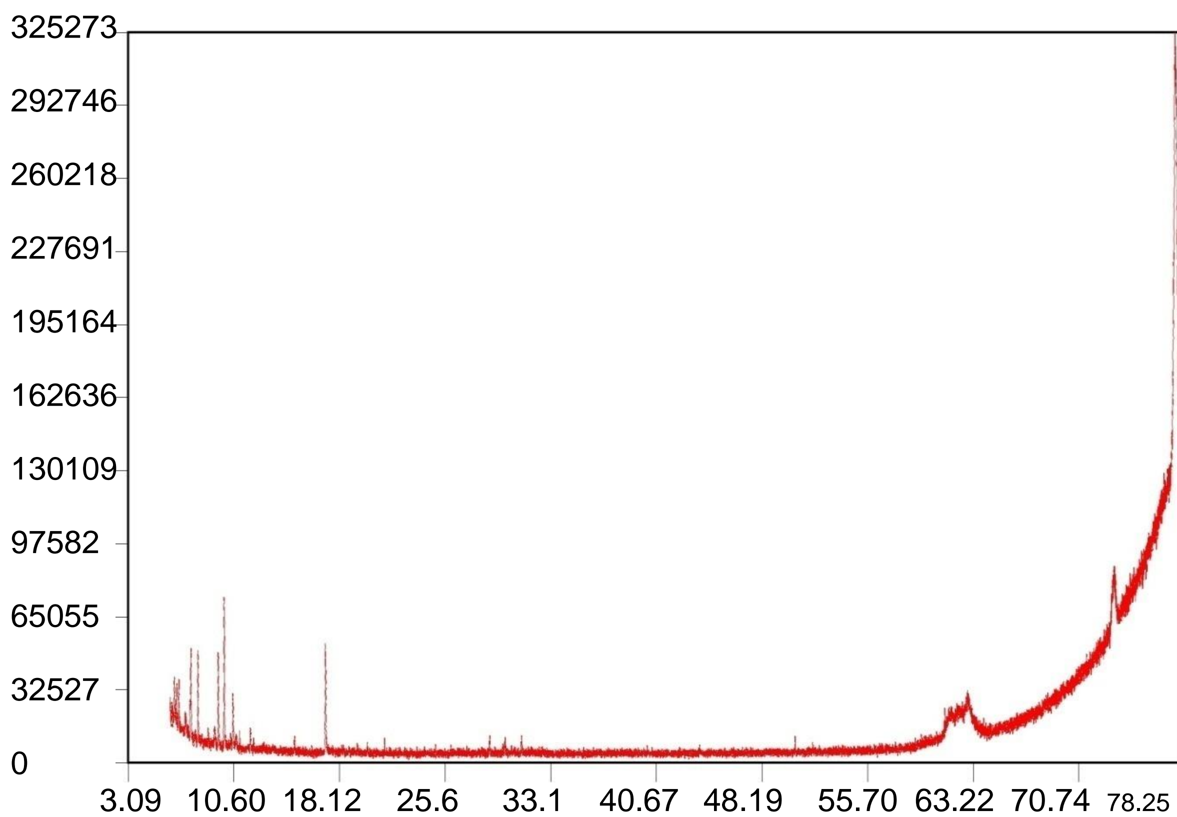
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 14: Gas Chromatogram of *Delonix regia* root Essential oil



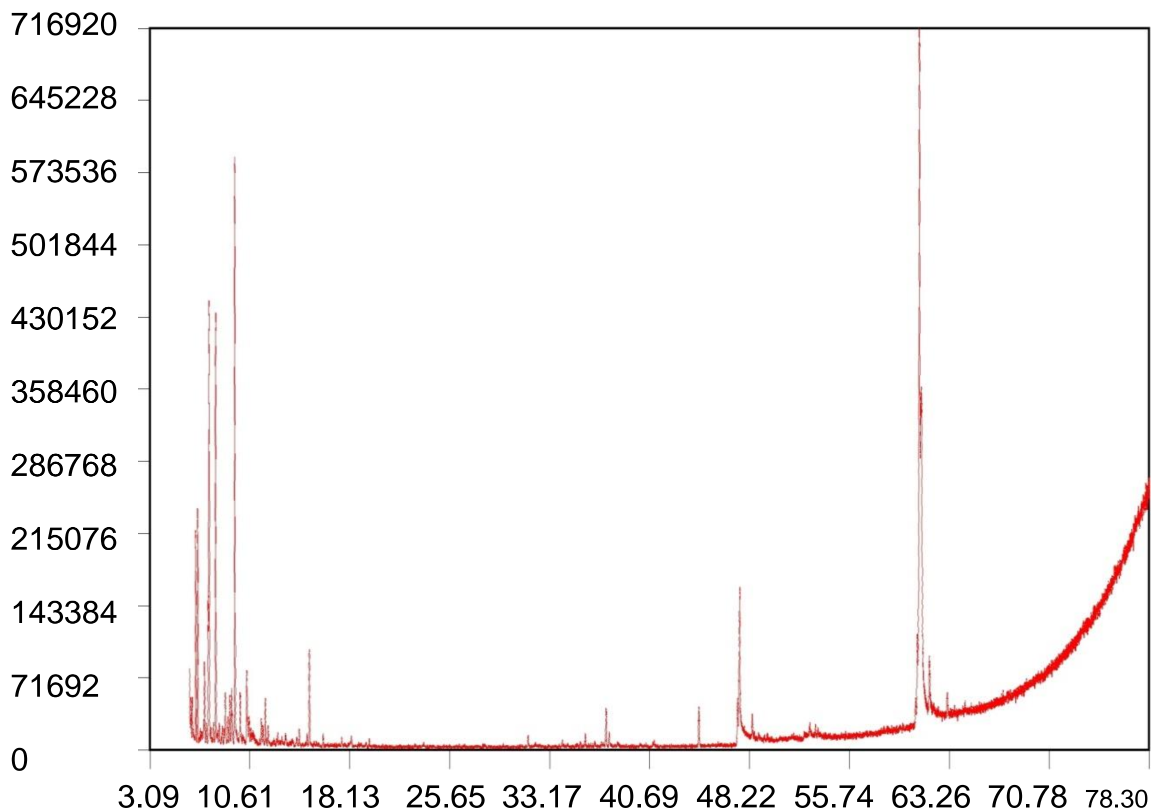
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 15: Gas Chromatogram of *Duranta repens* leaf Essential oil



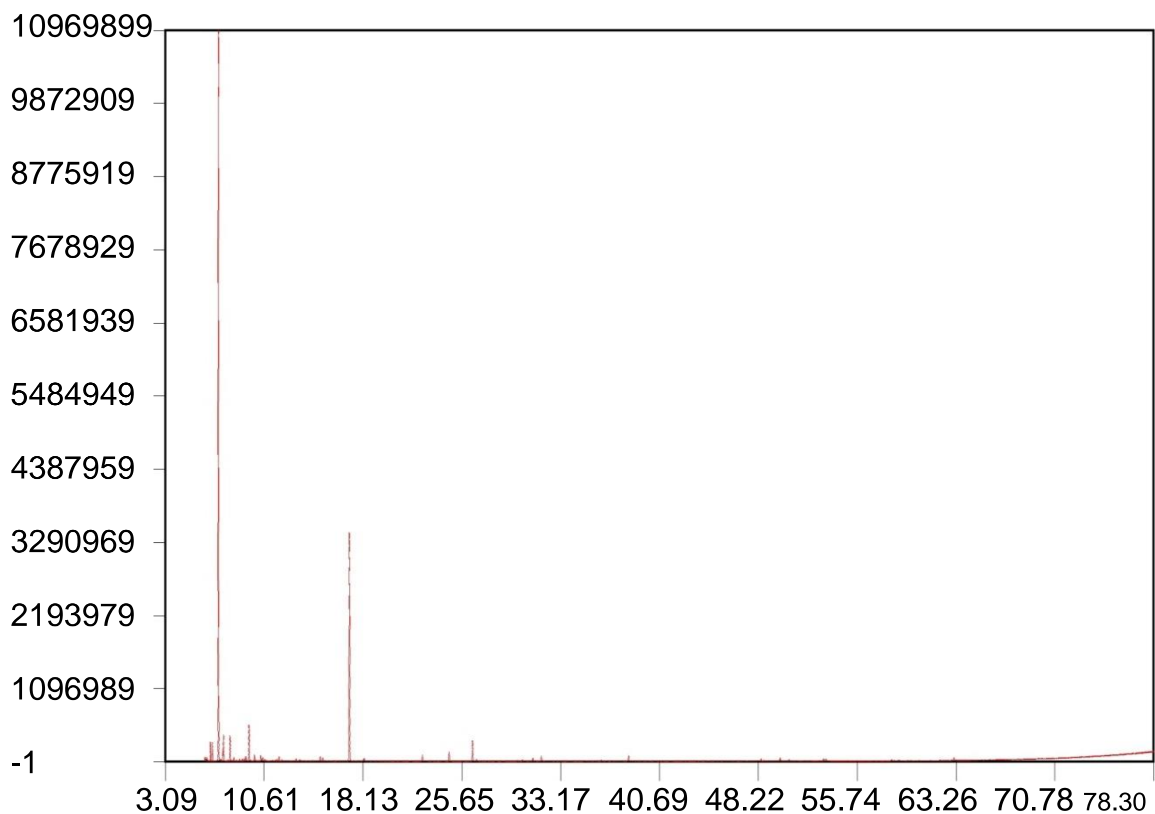
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 16: Gas Chromatogram of *Duranta repens* stem Essential oil



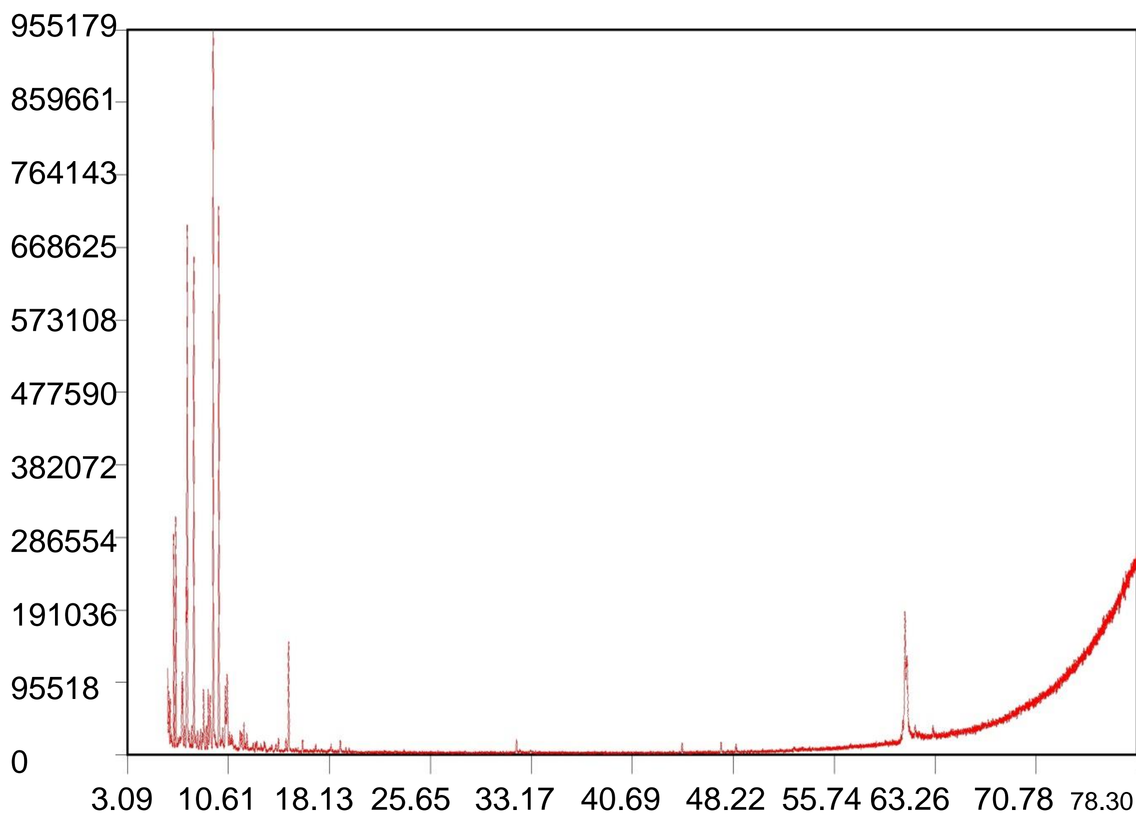
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 17: Gas Chromatogram of *Duranta repens* fruit Essential oil



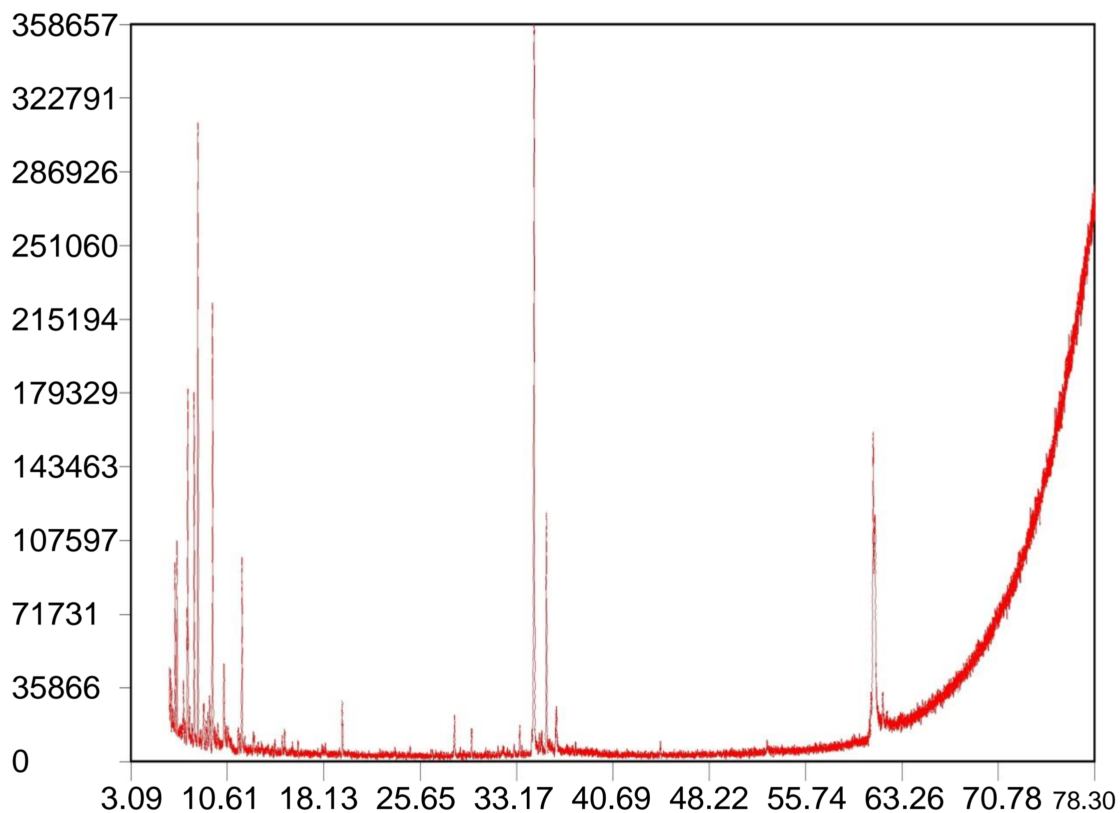
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 μm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 18: Gas Chromatogram of *Duranta repens* root Essential oil



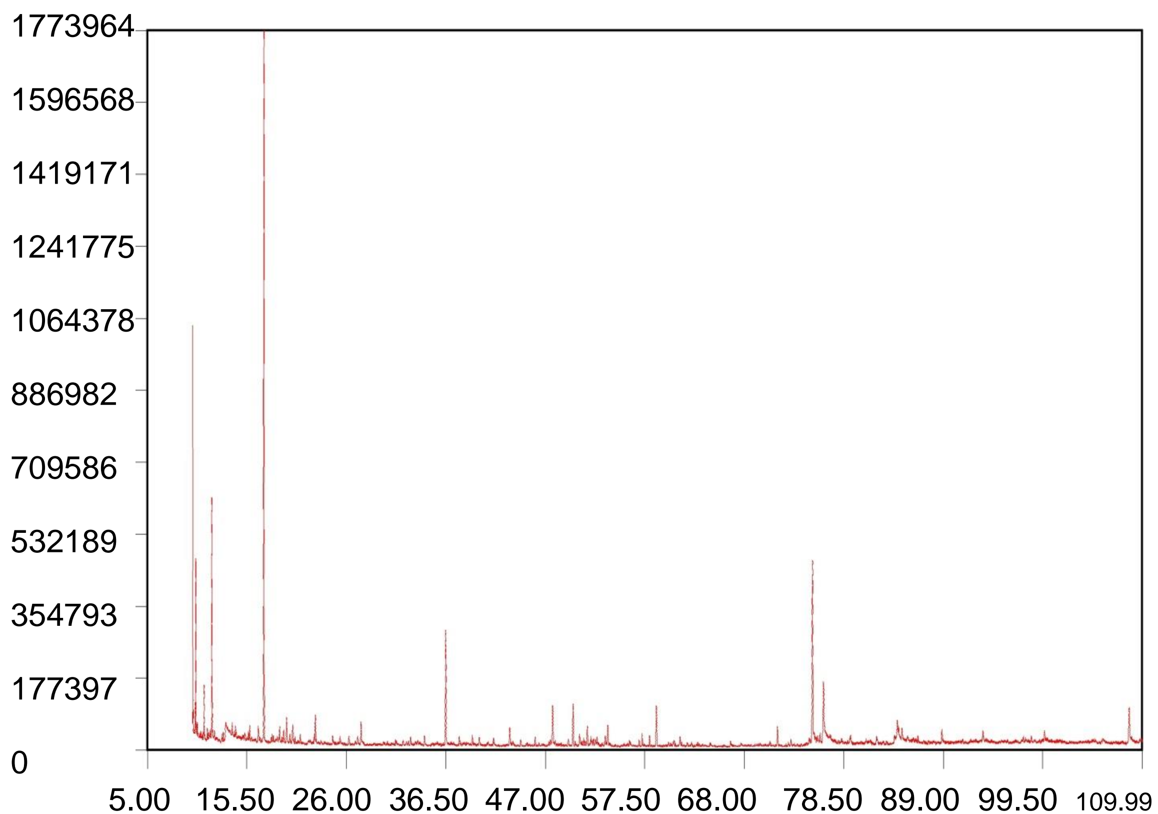
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 19: Gas Chromatogram of *Zanthoxylum zanthoxyloides* leaf Essential oil



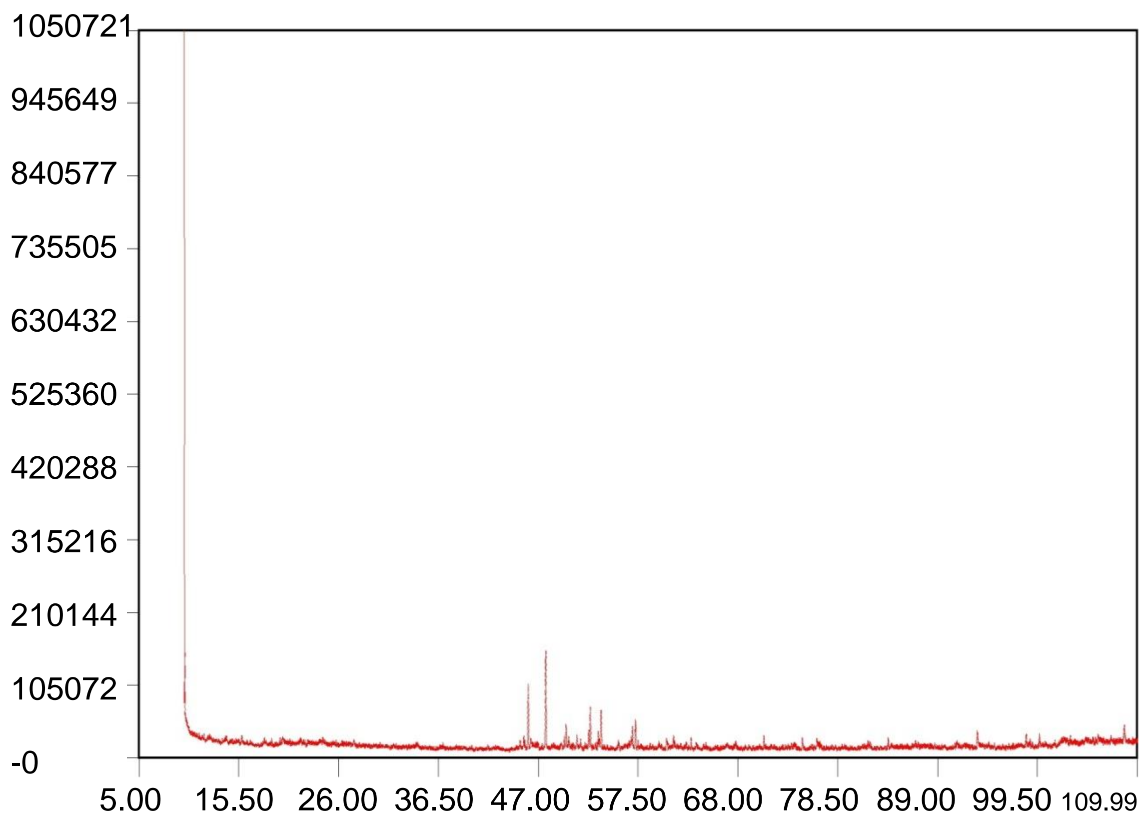
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 μm film thickness). Oven temperature: 50 ºC (held for 10 mins) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 20: Gas Chromatogram of *Z. zanthoxyloides* stem bark Essential oil



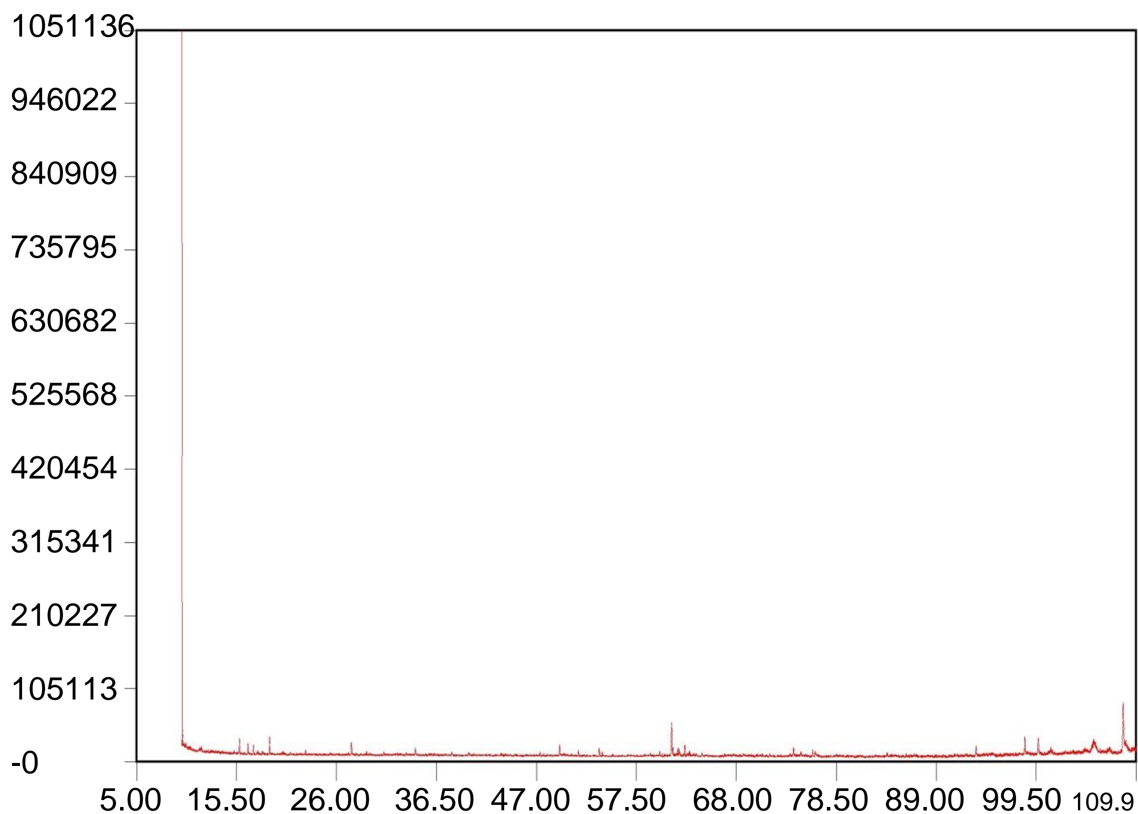
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 21: Gas Chromatogram of *Ceiba pentandra* stem bark Essential oil



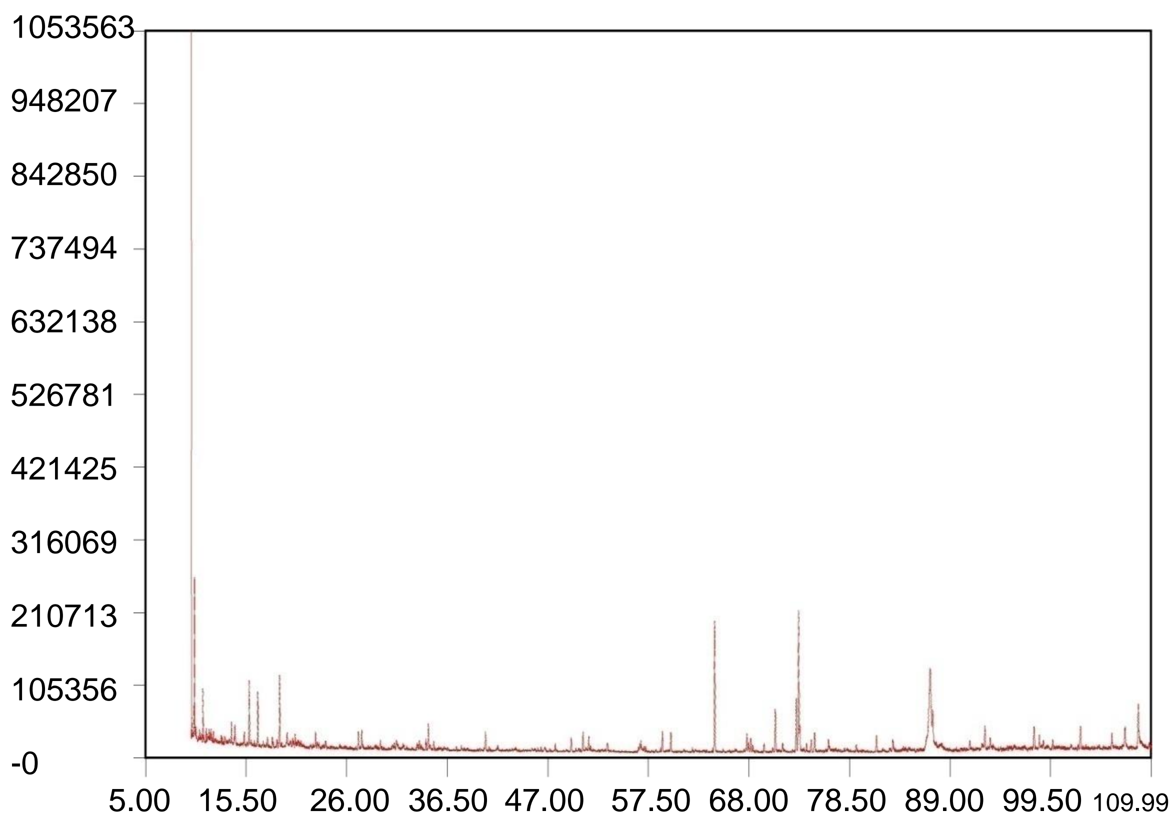
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 μm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 22: Gas Chromatogram of *Ceiba pentandra* Heartwood Essential oil



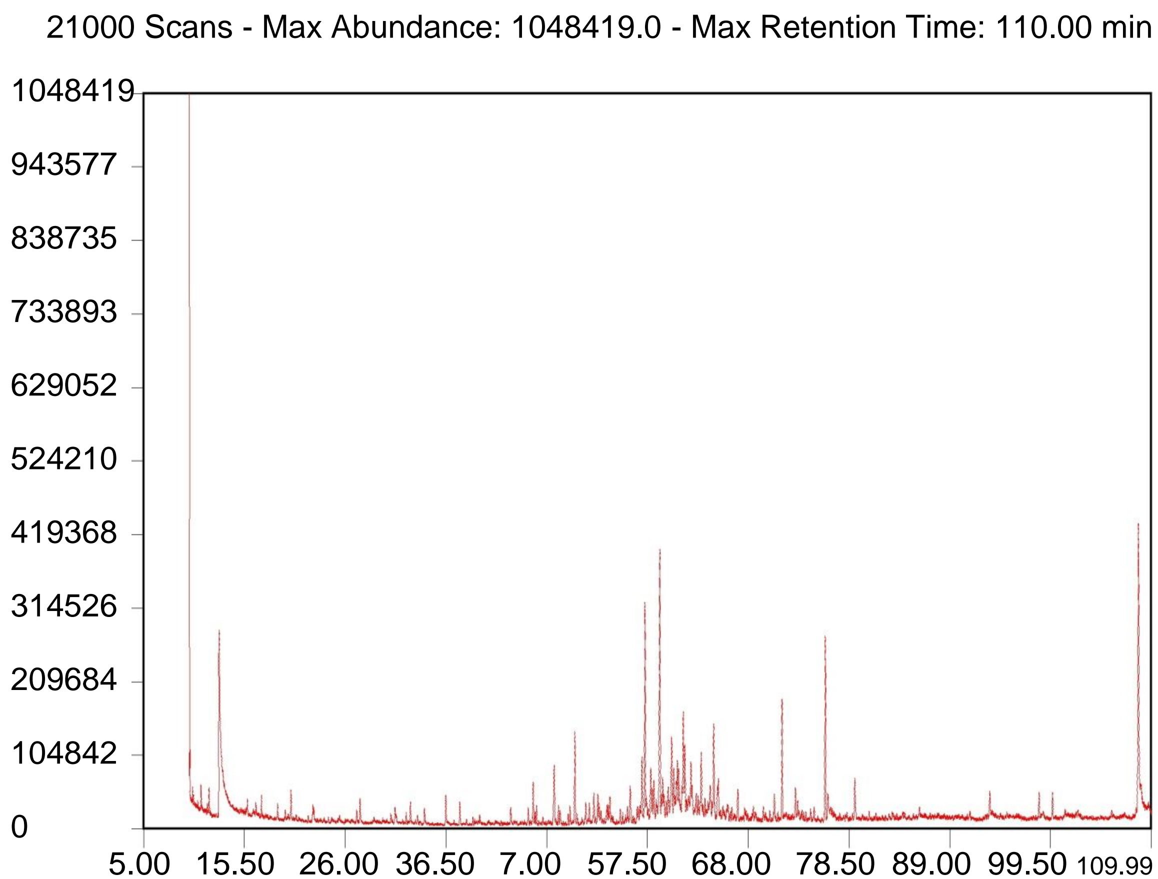
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 23: Gas Chromatogram of *Gliricidia sepium* leaf Essential oil



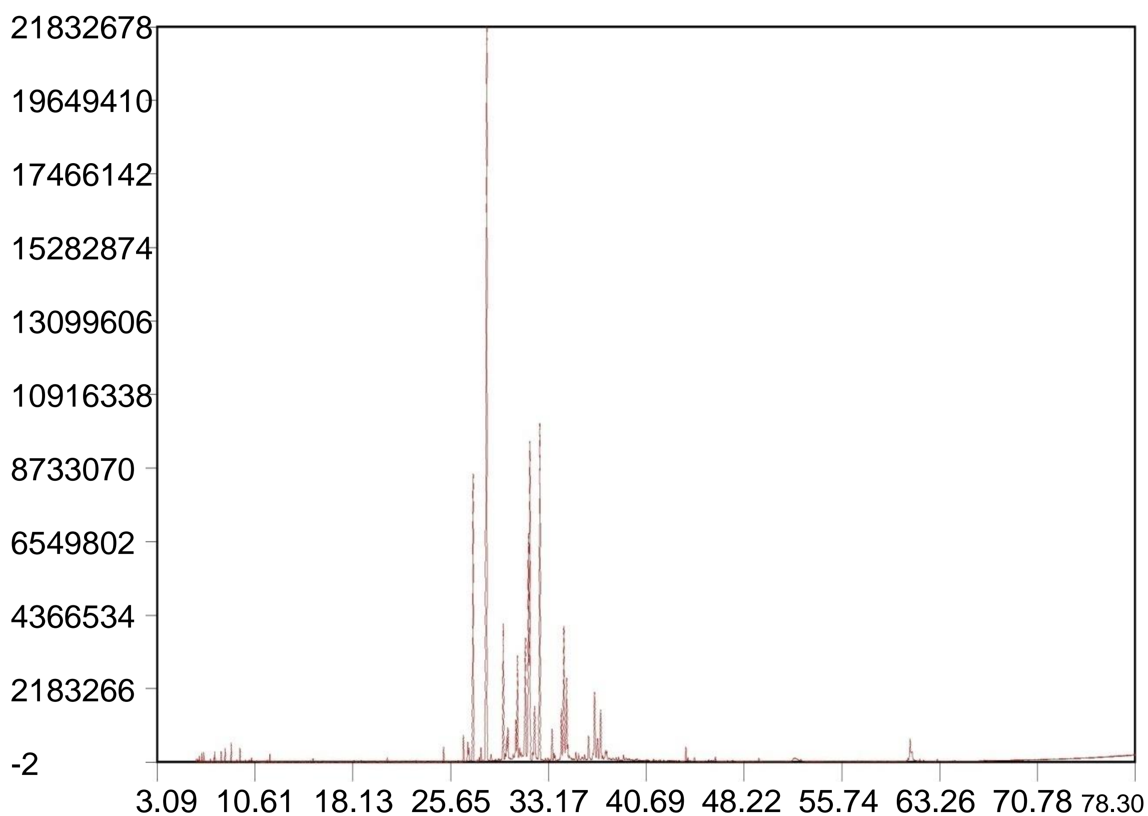
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 24: Gas Chromatogram of *Gliricidia sepium* stem Essential oil



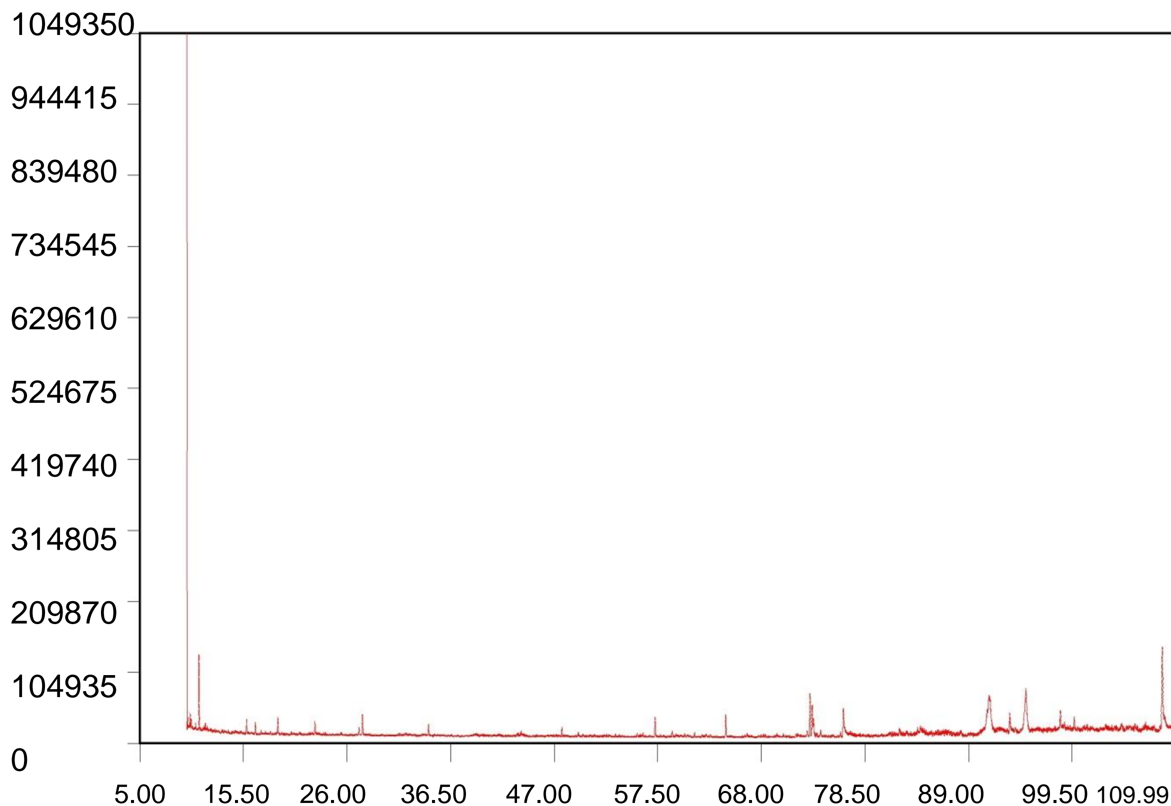
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 25: Gas Chromatogram of *Annona muricata* leaf Essential oil



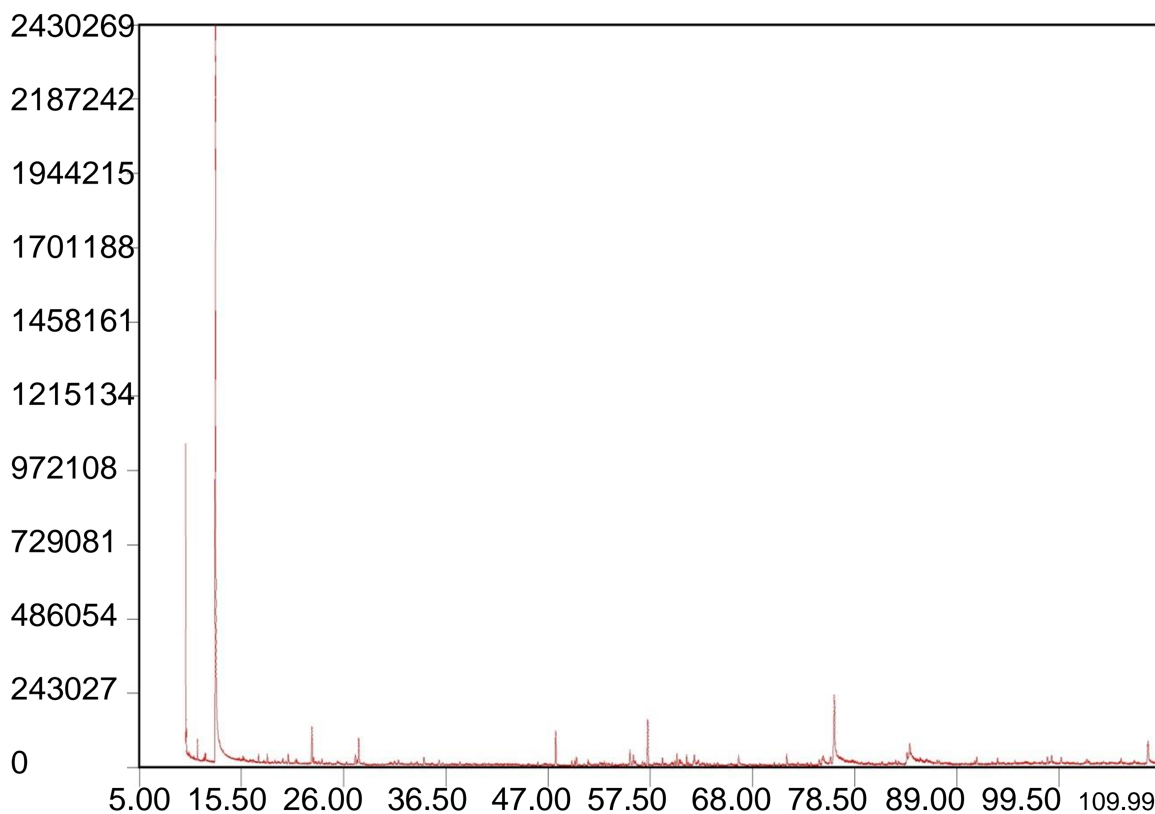
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 26: Gas Chromatogram of *Annona muricata* Branches Essential oil



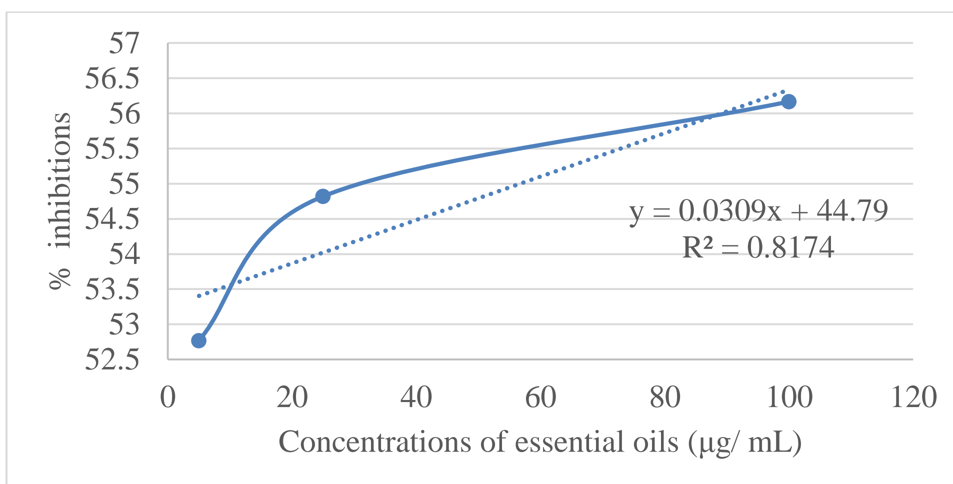
ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

APPENDIX 27: Gas Chromatogram of *Annona muricata* Stem bark Essential oil

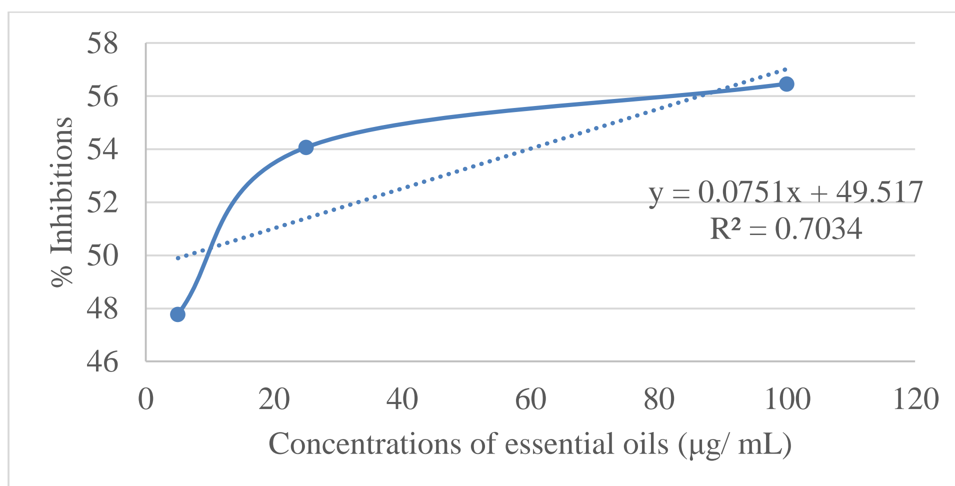


ZB-5 fused silica capillary column (30 m x 0.25 m x 0.25 µm film thickness). Oven temperature: 50 ºC (held for 10 min) heated to 260 ºC at 2 ºC/min at 70 eV

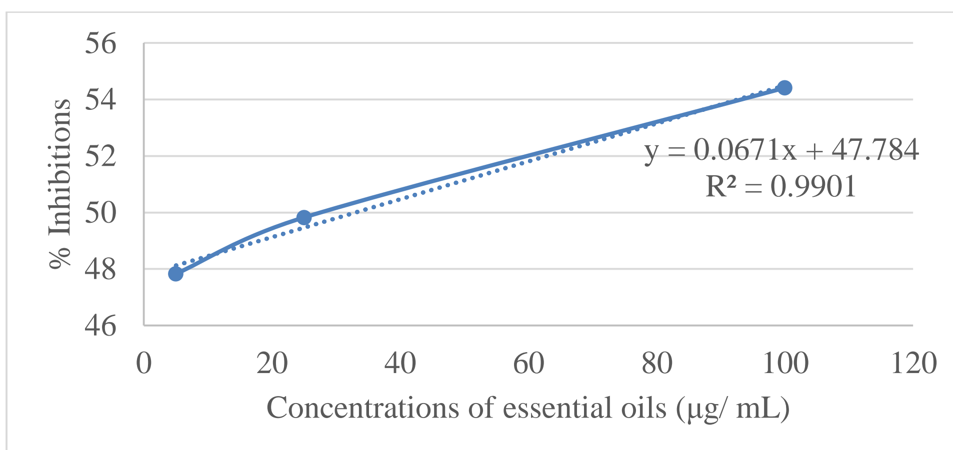
APPENDIX 28: Antioxidant activity of *Tecoma stans* leaf essential oil



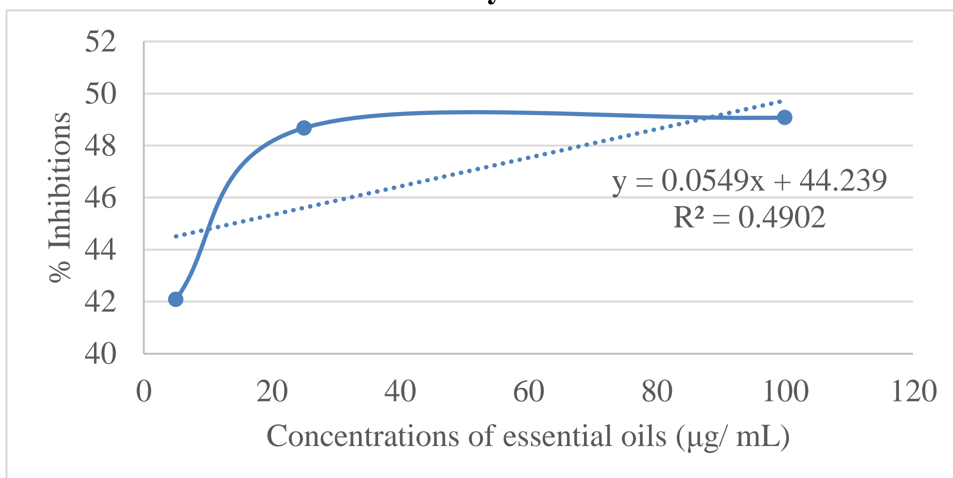
APPENDIX 29: Antioxidant activity of *Tecoma stans* stem essential oil



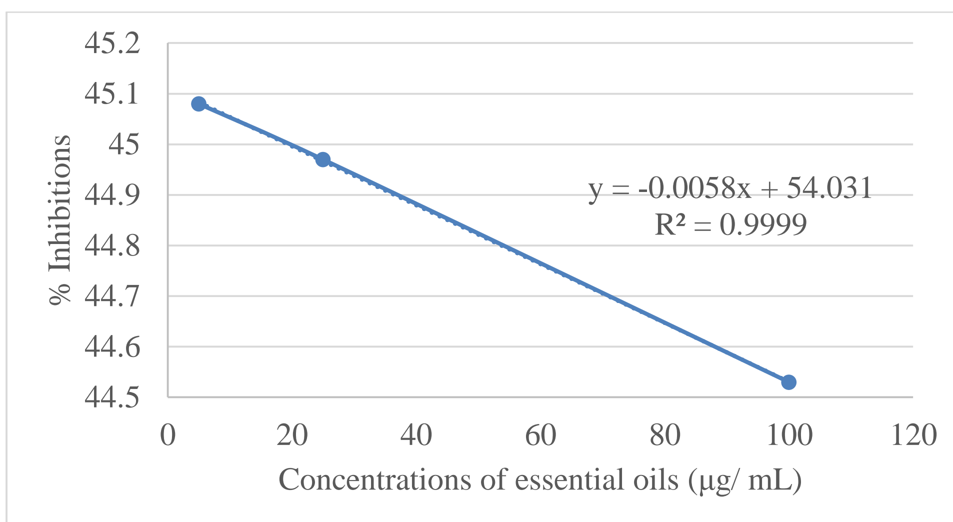
APPENDIX 30: Antioxidant activity of *Tecoma stans* seed essential oil



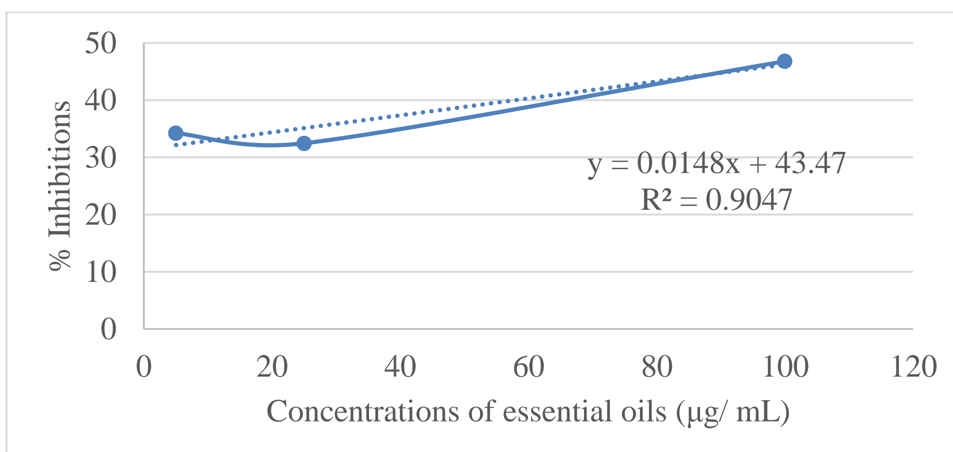
APPENDIX 31: Antioxidant activity of *Tecoma stans* flower essential oil



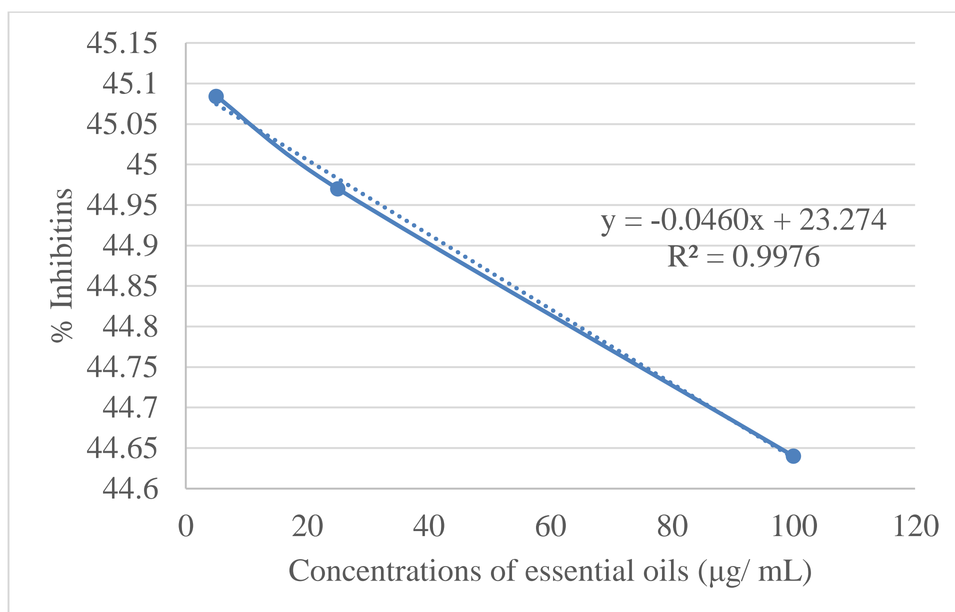
APPENDIX 32: Antioxidant activity of *Plumeria acuminata* leaf essential oil



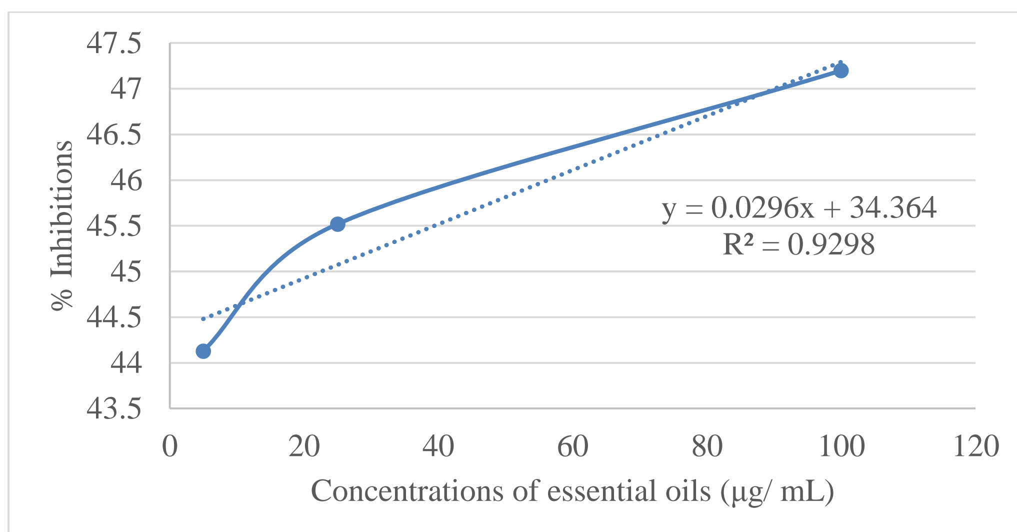
APPENDIX 33: Antioxidant activity of *Plumeria acuminata* flower essential oil



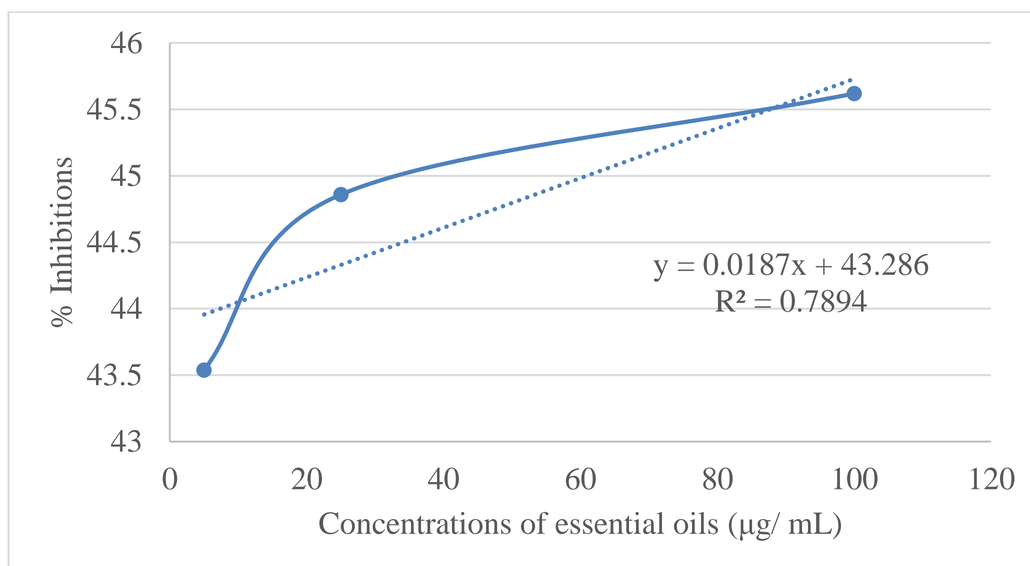
APPENDIX 34: Antioxidant activity of *Plumeria acuminata* root essential oil



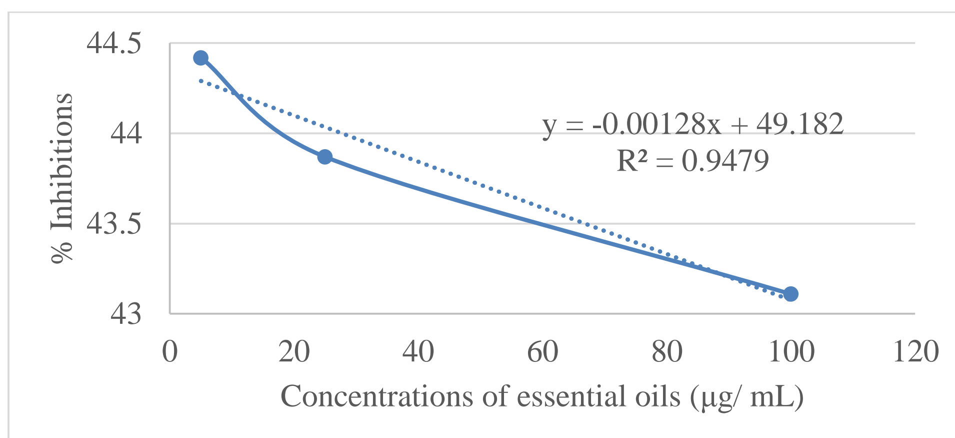
APPENDIX 35: Antioxidant activity of *Plumeria rubra* leaf essential oil



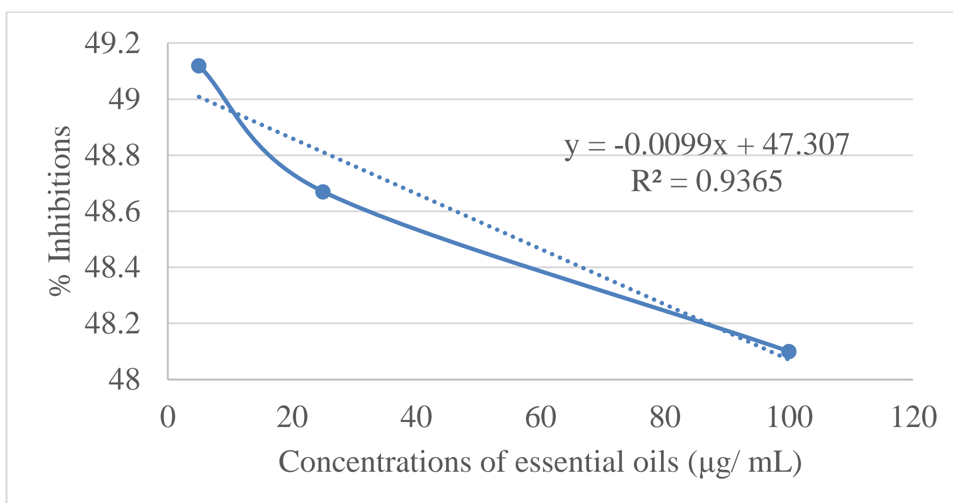
APPENDIX 36: Antioxidant activity of *Plumeria rubra* flowers essential oil



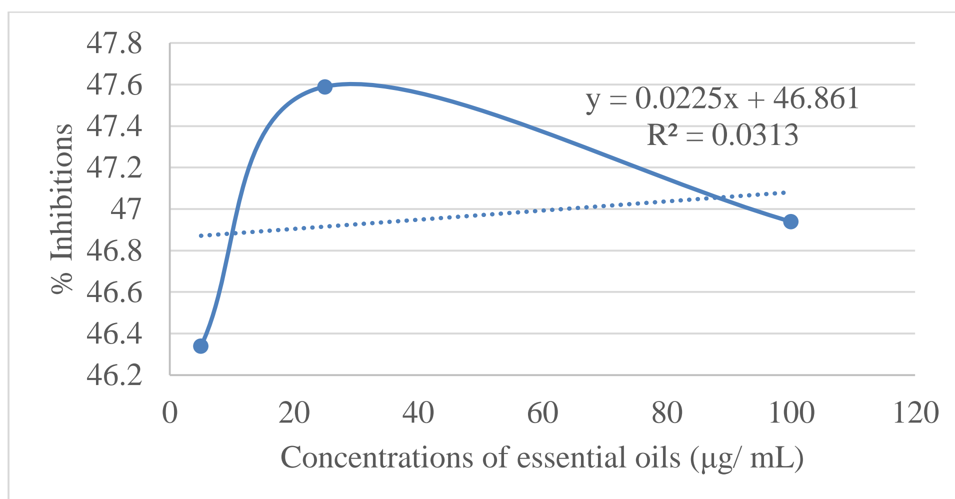
APPENDIX 37: Antioxidant activity of *Plumeria rubra* stem essential oil



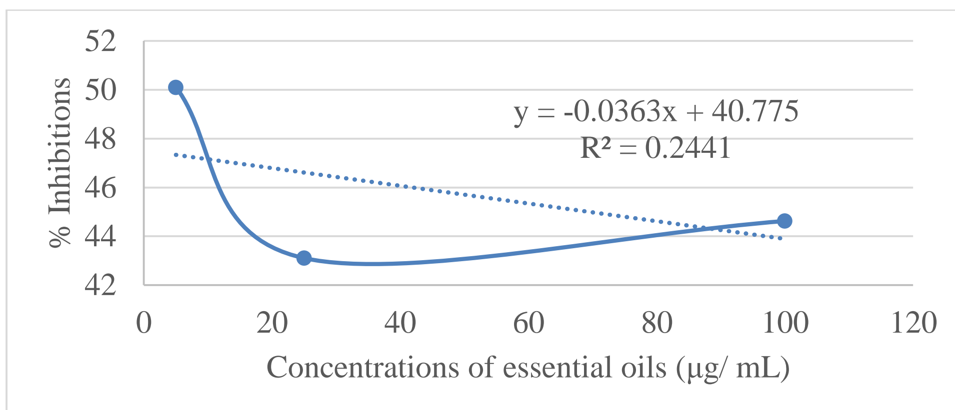
APPENDIX 38: Antioxidant activity of *Plumeria rubra* root essential oil



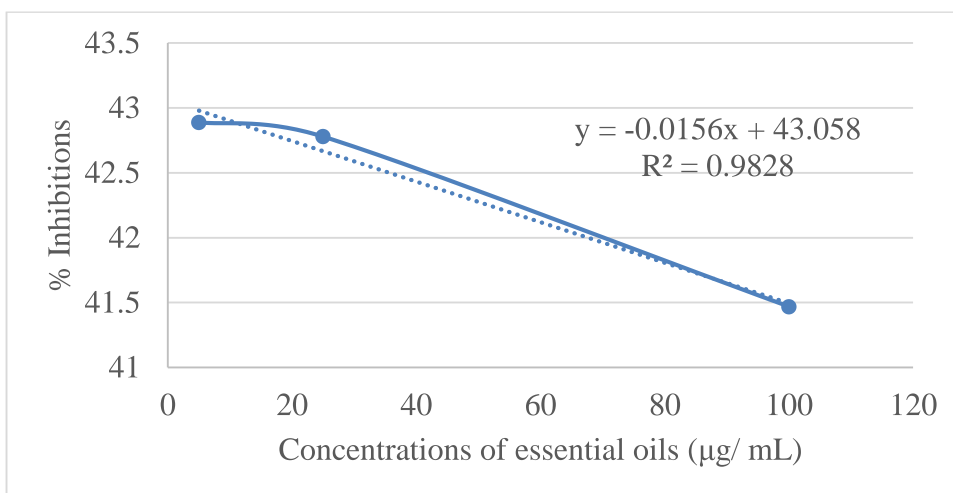
APPENDIX 39: Antioxidant activity of *Delonix regia* twig essential oil



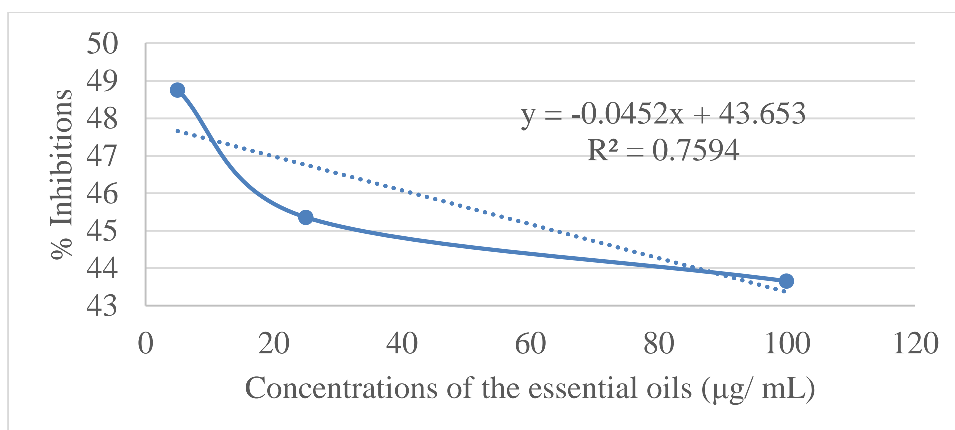
APPENDIX 40: Antioxidant activity of *Delonix regia* flower essential oil



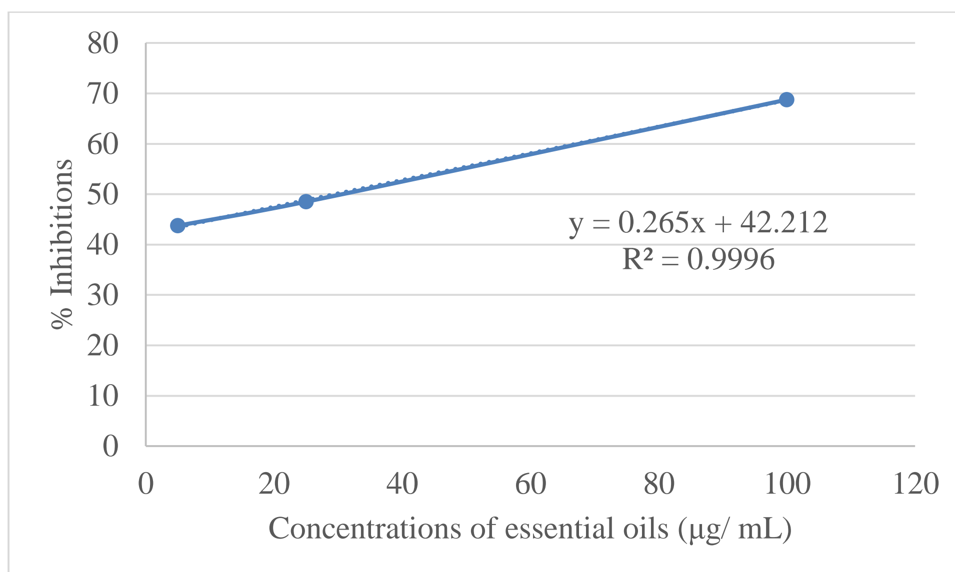
APPENDIX 41: Antioxidant activity of *Delonix regia* root essential oil



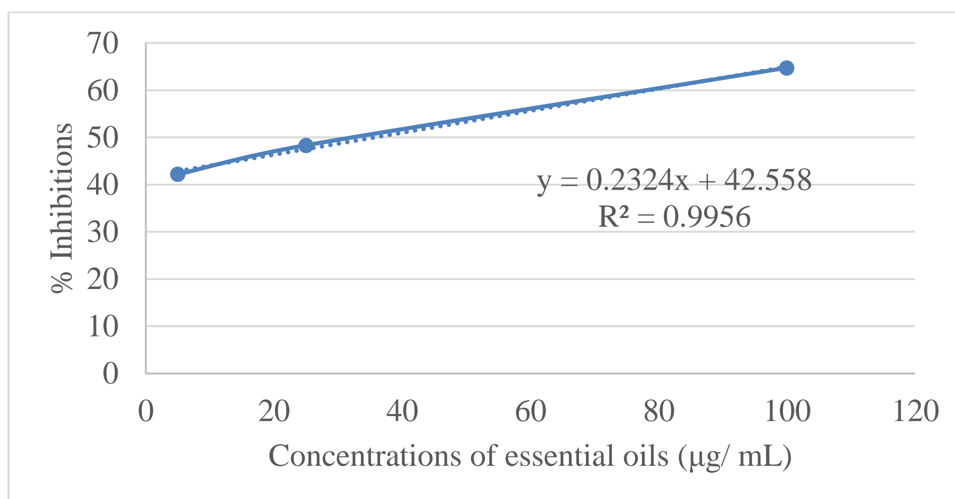
APPENDIX 42: Antioxidant activity of *Duranta reens* leaf essential oil



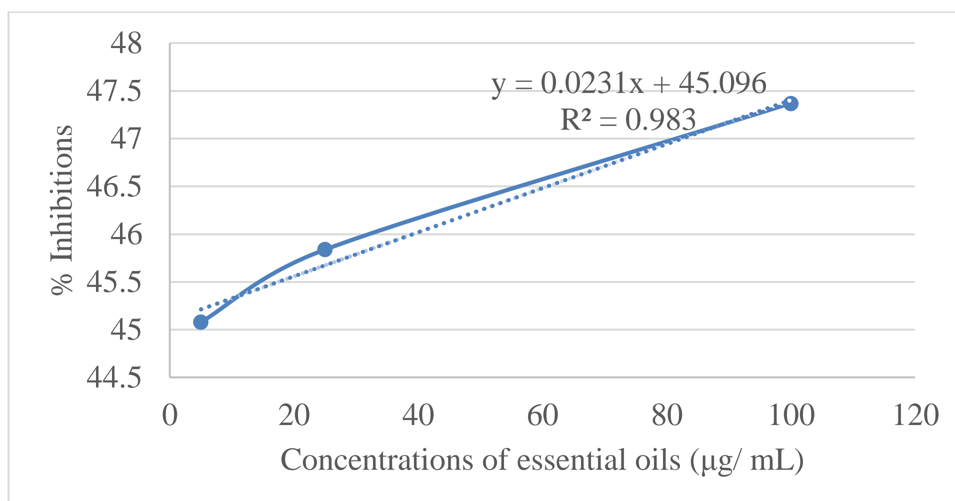
APPENDIX 43: Antioxidant activity of *Duranta repens* fruits essential oil



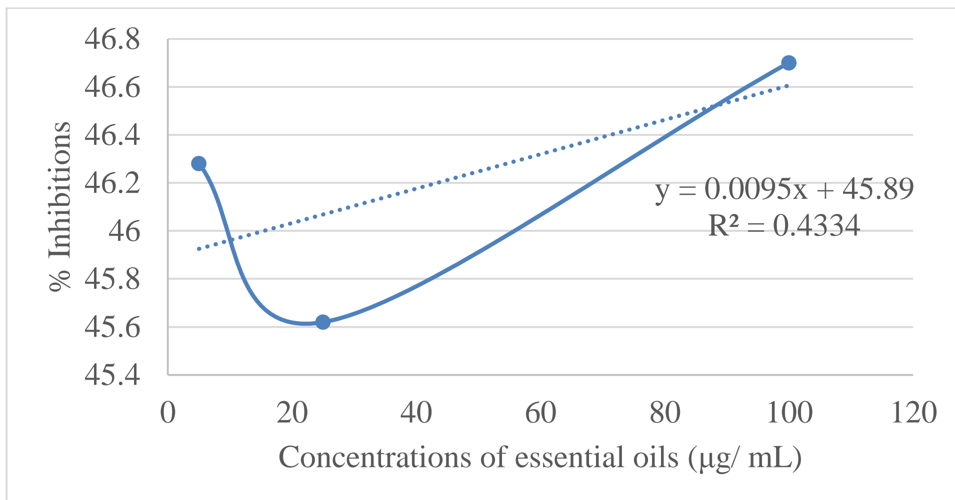
APPENDIX 44: Antioxidant activity of *Duranta repens* stem essential oil



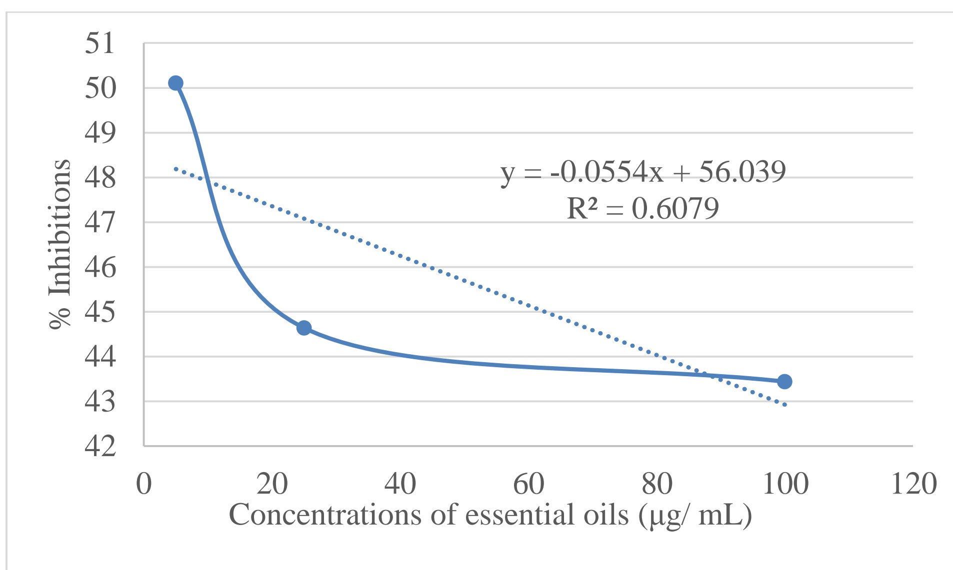
APPENDIX 45: Antioxidant activity of *Zanthoxylum zanthoxyloides* leaf essential oil



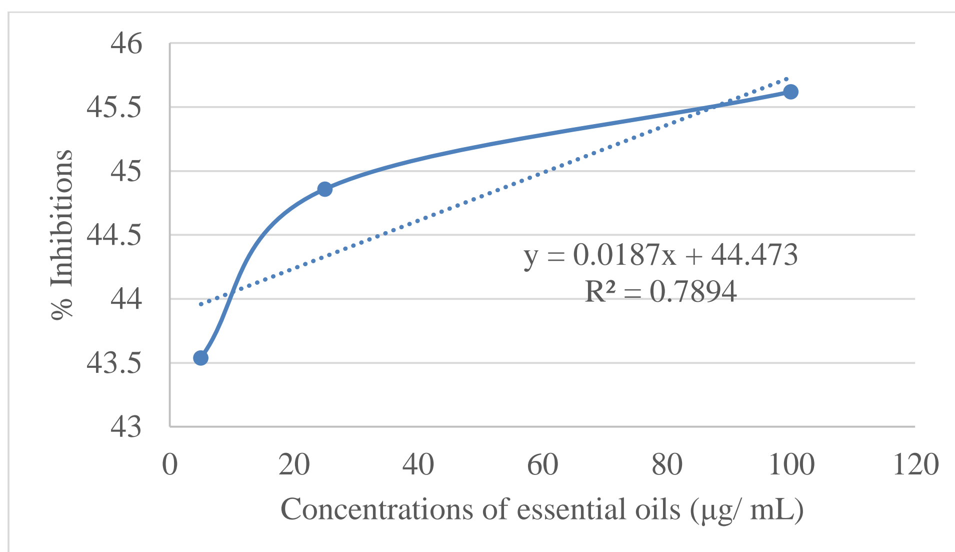
APPENDIX 46: Antioxidant activity of *Zanthoxylum zanthoxyloides* stem bark essential oil



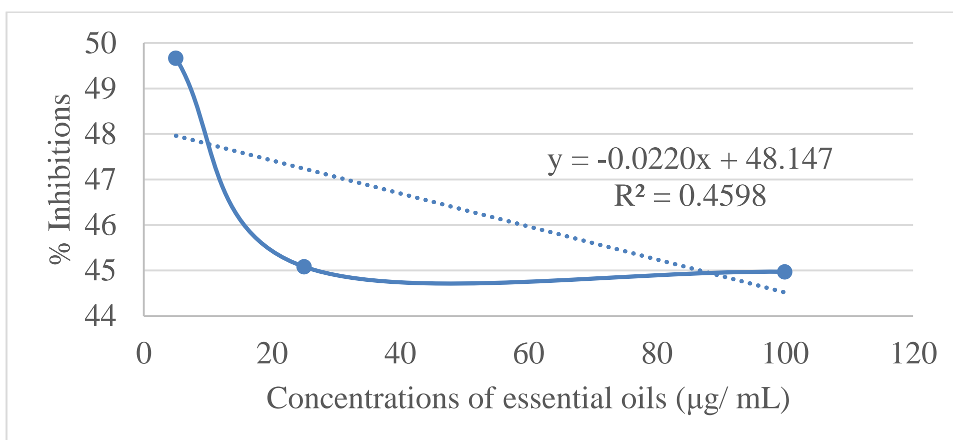
APPENDIX 47: Antioxidant activity of *Ceiba pentandra* stem bark essential oil



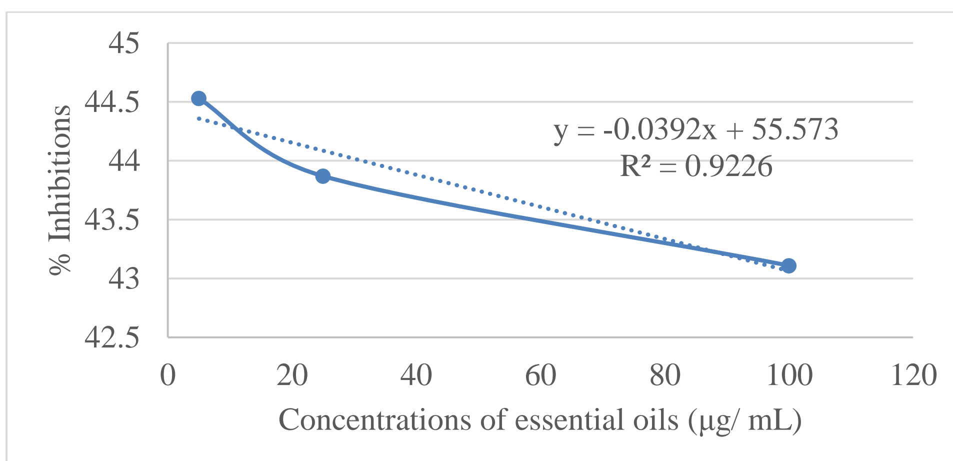
APPENDIX 48: Antioxidant activity of *Ceiba pentandra* heartwood essential oil



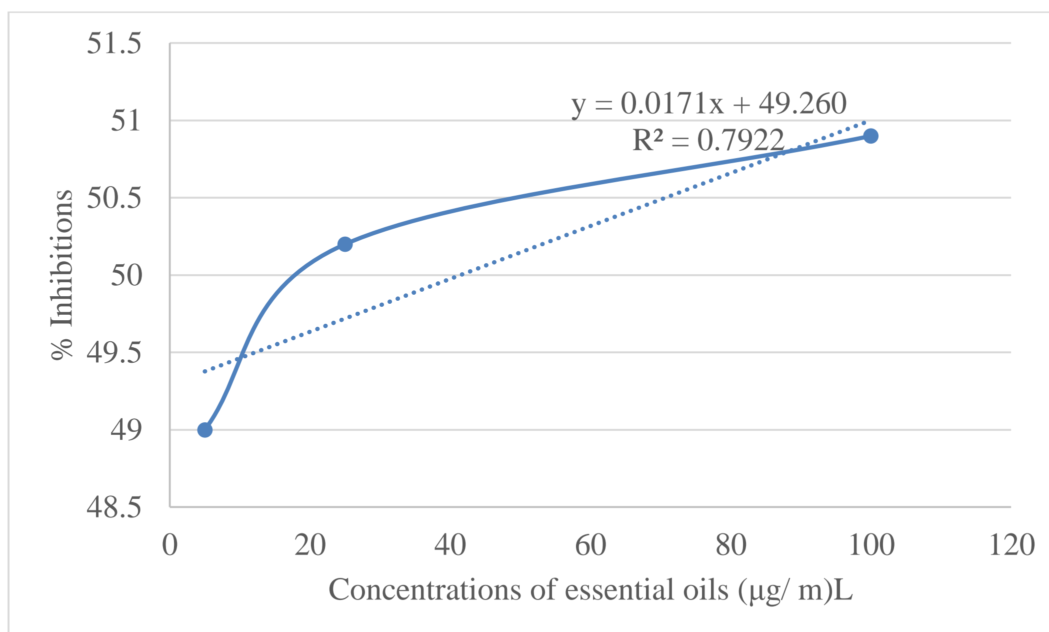
APPENDIX 49: Antioxidant activity of *Gliricidia sepium* leaf essential oil



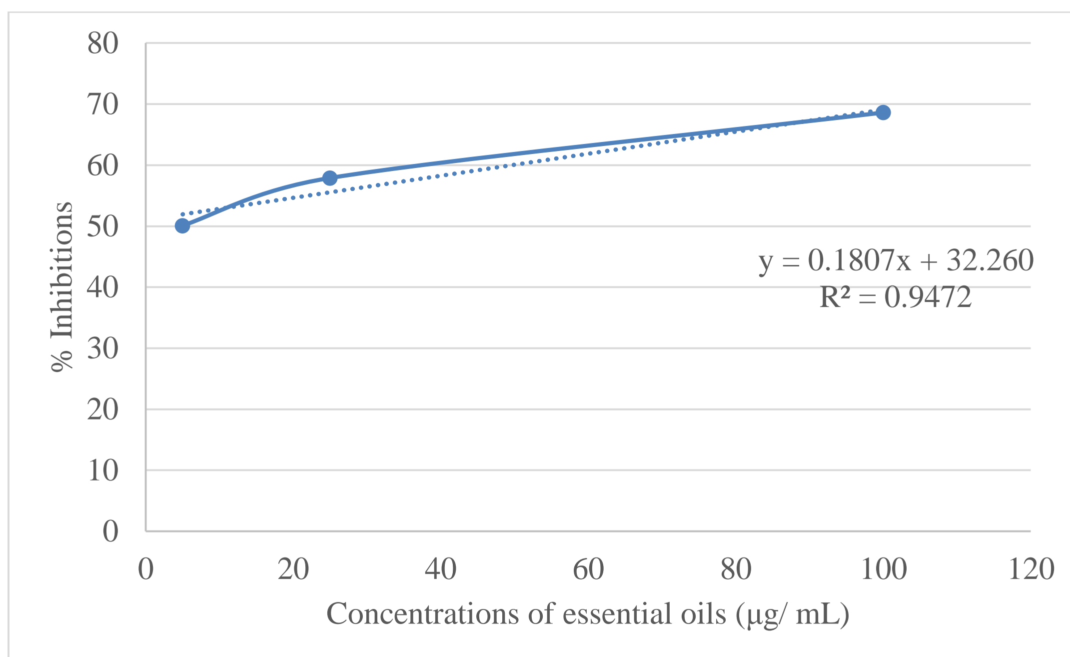
APPENDIX 50: Antioxidant activity of *Gliricidia sepium* stem essential oil



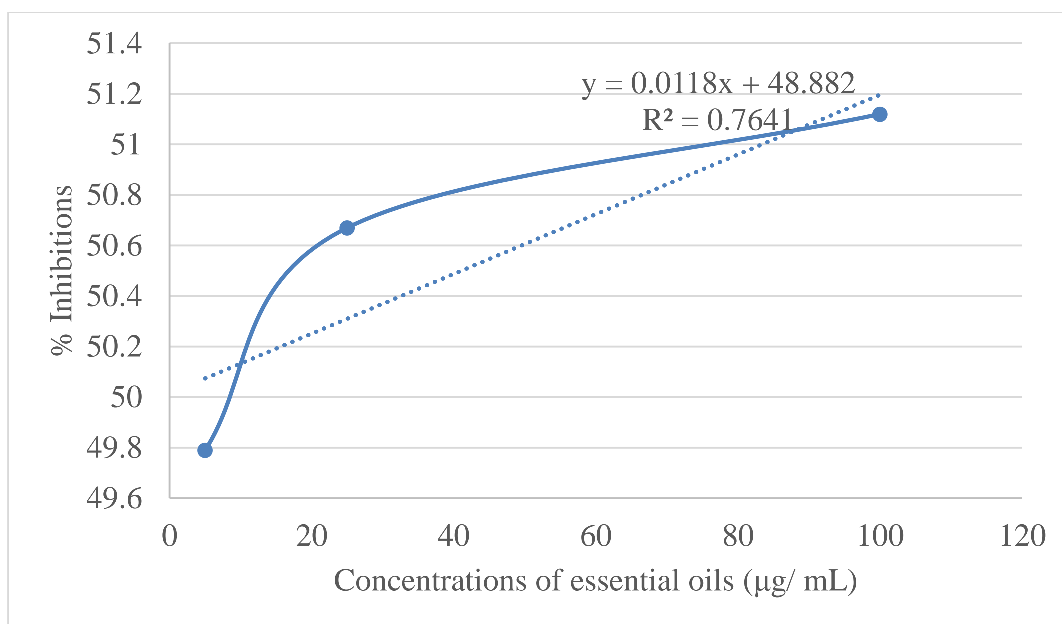
APPENDIX 51: Antioxidant activity of *Annona muricata* leaf essential oil



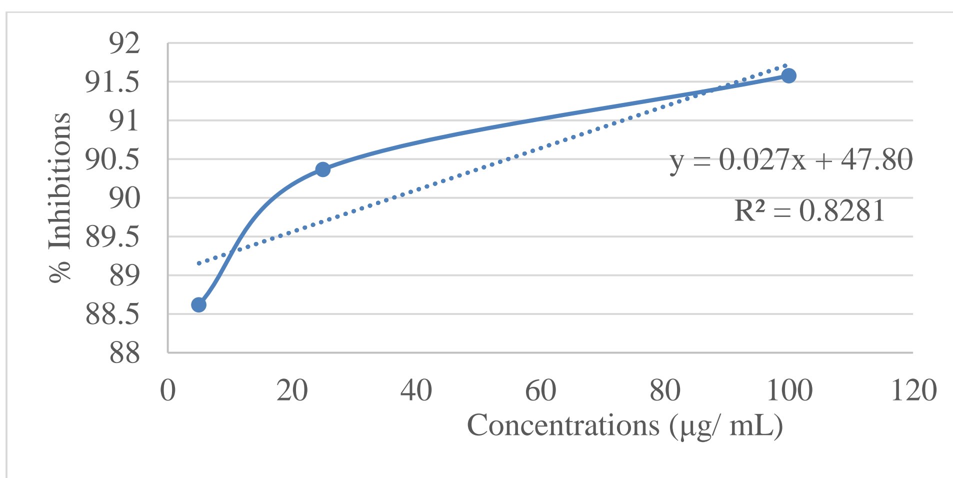
APPENDIX 52: Antioxidant activity of *Annona muricata* branches essential oil



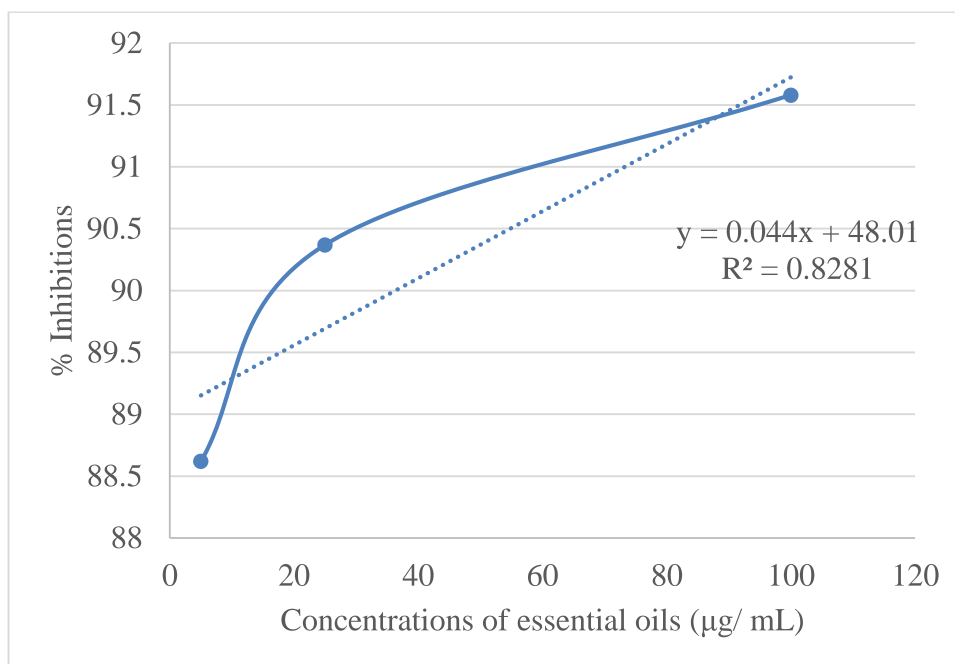
APPENDIX 53: Antioxidant activity of *Annona muricata* stem bark essential oil



APPENDIX 54: Antioxidant activity of α-Tocopherol



APPENDIX 55: Antioxidant activity of Butylatedhydroxyanisole (BHA)



APPENDIX 56: Antimicrobial activities of *Tecoma stans* essential oils

2way ANOVA

Tabular results

1	Table Analyzed	Data 1			
2					
3	Two-way ANOVA				
4					
5	Source of Variation	% of total variation	P value		
6	Interaction	30.26	< 0.0001		
7	Leaf, root, flower, stem	43.44	< 0.0001		
8	organism	13.75	< 0.0001		
9					
10	Source of Variation	P value summary	Significant?		
11	Interaction	***	Yes		
12	Leaf, root, flower, stem	***	Yes		
13	organism	***	Yes		
14					
15	Source of Variation	Df	Sum-of-squares	Mean square	F
16	Interaction	16	341.1	21.32	7.539
17	Leaf, root, flower, stem	4	489.8	122.4	43.29
18	organism	4	155.0	38.76	13.70
19	Residual	50	141.4	2.828	
20					
21	Number of missing values	0			
22					
23	Bonferroni posttests				
24					

25 **Leaf oil vs Stem oil**

26	organism	Leaf oil	Stem oil	Difference	95% CI of diff.
27	S. aureus	0.4333	0.0000	-0.4333	-4.805 to 3.939
28	E. coli	0.5667	0.0000	-0.5667	-4.939 to 3.805
29	C. albicans	3.367	2.767	-0.6000	-4.972 to 3.772
30	F. solani	2.067	1.833	-0.2333	-4.605 to 4.139
31	A. niger	2.733	1.933	-0.8000	-5.172 to 3.572

32

33	organism	Difference	t	P value	Summary
34	S. aureus	-0.4333	0.3156	P > 0.05	ns
35	E. coli	-0.5667	0.4127	P > 0.05	ns
36	C. albicans	-0.6000	0.4370	P > 0.05	ns
37	F. solani	-0.2333	0.1699	P > 0.05	ns
38	A. niger	-0.8000	0.5826	P > 0.05	ns

39

40 **Leaf oil vs seed oil**

41	organism	Leaf oil	seed oil	Difference	95% CI of diff.
42	S. aureus	0.4333	0.0000	-0.4333	-4.805 to 3.939
43	E. coli	0.5667	0.0000	-0.5667	-4.939 to 3.805
44	C. albicans	3.367	4.100	0.7333	-3.639 to 5.105
	F. solani	2.067	4.000	1.933	-2.439 to 6.305
	A. niger	2.733	0.4667	-2.267	-6.639 to 2.105

organism	Difference	t	P value	Summary
S. aureus	-0.4333	0.3156	P > 0.05	ns
E. coli	-0.5667	0.4127	P > 0.05	ns
C. albicans	0.7333	0.5341	P > 0.05	ns
F. solani	1.933	1.408	P > 0.05	ns
A. niger	-2.267	1.651	P > 0.05	ns

Leaf oil vs Flower oil

organism	Leaf oil	Flower oil	Difference	95% CI of diff.
S. aureus	0.4333	0.0000	-0.4333	-4.805 to 3.939
E. coli	0.5667	0.0000	-0.5667	-4.939 to 3.805
C. albicans	3.367	6.000	2.633	-1.739 to 7.005
F. solani	2.067	3.967	1.900	-2.472 to 6.272
A. niger	2.733	6.000	3.267	-1.105 to 7.639

organism	Difference	t	P value	Summary
S. aureus	-0.4333	0.3156	P > 0.05	ns
E. coli	-0.5667	0.4127	P > 0.05	ns
C. albicans	2.633	1.918	P > 0.05	ns
F. solani	1.900	1.384	P > 0.05	ns
A. niger	3.267	2.379	P > 0.05	ns

Leaf oil vs Gentamycin

organism	Leaf oil	Gentamycin	Difference	95% CI of diff.
S. aureus	0.4333	11.50	11.07	6.695 to 15.44
E. coli	0.5667	9.000	8.433	4.061 to 12.81
C. albicans	3.367	12.50	9.133	4.761 to 13.51
F. solani	2.067	8.000	5.933	1.561 to 10.31
A. niger	2.733	0.0000	-2.733	-7.105 to 1.639

organism	Difference	t	P value	Summary
S. aureus	11.07	8.060	P<0.001	***
E. coli	8.433	6.142	P<0.001	***
C. albicans	9.133	6.652	P<0.001	***
F. solani	5.933	4.321	P<0.001	***
A. niger	-2.733	1.991	P > 0.05	ns

APPENDIX 57: Antibacterial activities of *P. acuminata* essential

2way ANOVA

Tabular results

1	Table Analyzed	Data 1			
2					
3	Two-way RM ANOVA	Matching by rows			
4					
5	Source of Variation	% of total variation	P value		
6	Interaction	62.17	< 0.0001		
7	Row Factor	8.18	0.2691		
8	Time	8.89	< 0.0001		
9	Subjects (matching)	13.2918	< 0.0001		
10					
11	Source of Variation	P value summary	Significant?		
12	Interaction	***	Yes		
13	Row Factor	ns	No		
14	Time	***	Yes		
15	Subjects (matching)	***	Yes		
16					
17	Source of Variation	Df	Sum-of-squares	Mean square	F
18	Interaction	18	762.7	42.37	19.44
19	Row Factor	6	100.4	16.73	1.437
20	Time	3	109.0	36.34	16.67
21	Subjects (matching)	14	163.0	11.65	5.343
22	Residual	42	91.54	2.180	
23					
24	Number of missing values	0			

25					
26	**Bonferroni posttests**				
27					
28	**leaves oil vs root oil**				
29	**Row Factor**	**leaves oil**	**root oil**	**Difference**	**95% CI of diff.**
30	**0.0000**	**3.933**	**6.933**	**3.000**	**-1.192 to 7.192**
31	**0.0000**	**3.400**	**0.0000**	**-3.400**	**-7.592 to 0.7920**
32	**0.0000**	**6.000**	**3.800**	**-2.200**	**-6.392 to 1.992**
33	**0.0000**	**6.700**	**3.933**	**-2.767**	**-6.959 to 1.425**
34	**0.0000**	**8.867**	**6.000**	**-2.867**	**-7.059 to 1.325**
35	**0.0000**	**7.400**	**2.000**	**-5.400**	**-9.592 to -1.208**
36	**0.0000**	**4.033**	**0.0000**	**-4.033**	**-8.225 to 0.1587**
37					
38	**Row Factor**	**Difference**	**t**	**P value**	**Summary**
39	**0.0000**	**3.000**	**2.489**	**P > 0.05**	**ns**
40	**0.0000**	**-3.400**	**2.821**	**P > 0.05**	**ns**
41	**0.0000**	**-2.200**	**1.825**	**P > 0.05**	**ns**
42	**0.0000**	**-2.767**	**2.295**	**P > 0.05**	**ns**
43	**0.0000**	**-2.867**	**2.378**	**P > 0.05**	**ns**
44	**0.0000**	**-5.400**	**4.480**	**P<0.001**	*******

45	**Row Factor**	**Leaf oil**	**flower oil**	**Difference**	**95% CI of diff.**
46	**E. coli**	**3.933**	**1.933**	**-2.000**	**-8.015 to 4.015**
47	**Sal 2**	**2.067**	**2.000**	**-0.06667**	**-6.081 to 5.948**
48	**S. aureus**	**6.000**	**1.833**	**-4.167**	**-10.18 to 1.848**
49	**L. adecarb**	**6.700**	**3.867**	**-2.833**	**-8.848 to 3.181**
50	**M. morganti**	**8.867**	**5.867**	**-3.000**	**-9.015 to 3.015**
51	**C. freundii**	**7.400**	**6.000**	**-1.400**	**-7.415 to 4.615**
52	**K. pneumo**	**4.033**	**6.033**	**2.000**	**-4.015 to 8.015**
53					

54	Row Factor	Difference	t	P value	Summary
55	E. coli	-2.000	1.136	P > 0.05	ns
56	Sal 2	-0.06667	0.03786	P > 0.05	ns
57	S. aureus	-4.167	2.366	P > 0.05	ns
58	L. adecarb	-2.833	1.609	P > 0.05	ns
59	M. morganti	-3.000	1.704	P > 0.05	ns
60	C. freundii	-1.400	0.7951	P > 0.05	ns
61	K. pneumo	2.000	1.136	P > 0.05	ns
62					
63	Leaf oil vs Gentamycin				
64	Row Factor	Leaf oil	Gentamycin	Difference	95% CI of diff.
65	E. coli	3.933	9.000	5.067	-0.9479 to 11.08
66	Sal 2	2.067	12.50	10.43	4.419 to 16.45
67	S. aureus	6.000	11.50	5.500	-0.5145 to 11.51
68	L. adecarb	6.700	8.000	1.300	-4.715 to 7.315
69	M. morganti	8.867	0.0000	-8.867	-14.88 to -2.852
70	C. freundii	7.400	0.0000	-7.400	-13.41 to -1.385
71	K. pneumo	4.033	0.0000	-4.033	-10.05 to 1.981
72					
73	Row Factor	Difference	t	P value	Summary
74	E. coli	5.067	2.877	P < 0.05	*
75	Sal 2	10.43	5.925	P<0.001	***
76	S. aureus	5.500	3.123	P < 0.05	*
77	L. adecarb	1.300	0.7383	P > 0.05	ns
78	M. morganti	-8.867	5.035	P<0.001	***
79	C. freundii	-7.400	4.202	P<0.001	***
80	K. pneumo	-4.033	2.291	P > 0.05	ns
81					
82	Root oil vs flower oil				
83	Row Factor	Root oil	flower oil	Difference	95% CI of diff.

84	E. coli	6.933	1.933	-5.000	-11.01 to 1.015
85	Sal 2	0.0000	2.000	2.000	-4.015 to 8.015
86	S. aureus	3.800	1.833	-1.967	-7.981 to 4.048
87	L. adecarb	3.933	3.867	-0.06667	-6.081 to 5.948
88	M. morganti	6.000	5.867	-0.1333	-6.148 to 5.881
89	C. freundii	2.000	6.000	4.000	-2.015 to 10.01
90	K. pneumo	0.0000	6.033	6.033	0.01879 to 12.05
91					
92	Row Factor	Difference	t	P value	Summary
93	E. coli	-5.000	2.840	P < 0.05	*
94	Sal 2	2.000	1.136	P > 0.05	ns
95	S. aureus	-1.967	1.117	P > 0.05	ns
96	L. adecarb	-0.06667	0.03786	P > 0.05	ns
97	M. morganti	-0.1333	0.07572	P > 0.05	ns
98	C. freundii	4.000	2.272	P > 0.05	ns
99	K. pneumo	6.033	3.426	P<0.01	**
100					
101	Root oil vs Gentamycin				
102	Row Factor	Root oil	Gentamycin	Difference	95% CI of diff.
103	E. coli	6.933	9.000	2.067	-3.948 to 8.081
104	Sal 2	0.0000	12.50	12.50	6.485 to 18.51
105	S. aureus	3.800	11.50	7.700	1.685 to 13.71
106	L. adecarb	3.933	8.000	4.067	-1.948 to 10.08
107	M. morganti	6.000	0.0000	-6.000	-12.01 to 0.01454
108	C. freundii	2.000	0.0000	-2.000	-8.015 to 4.015
109	K. pneumo	0.0000	0.0000	0.0000	-6.015 to 6.015
110					
111	Row Factor	Difference	t	P value	Summary
112	E. coli	2.067	1.174	P > 0.05	ns
113	Sal 2	12.50	7.099	P<0.001	***

114	S. aureus	7.700	4.373	P<0.001	***
115	L. adecarb	4.067	2.309	P > 0.05	ns
116	M. morganti	-6.000	3.407	P<0.01	**
117	C. freundii	-2.000	1.136	P > 0.05	ns
118	K. pneumo	0.0000	0.0000	P > 0.05	ns

119

120 flower oil vs Gentamycin

121	Row Factor	flower oil	Gentamycin	Difference	95% CI of diff.
122	E. coli	1.933	9.000	7.067	1.052 to 13.08
123	Sal 2	2.000	12.50	10.50	4.485 to 16.51
124	S. aureus	1.833	11.50	9.667	3.652 to 15.68
125	L. adecarb	3.867	8.000	4.133	-1.881 to 10.15
126	M. morganti	5.867	0.0000	-5.867	-11.88 to 0.1479
127	C. freundii	6.000	0.0000	-6.000	-12.01 to 0.01454
128	K. pneumo	6.033	0.0000	-6.033	-12.05 to -0.01879

129

130	Row Factor	Difference	t	P value	Summary
131	E. coli	7.067	4.013	P<0.01	**
132	Sal 2	10.50	5.963	P<0.001	***
133	S. aureus	9.667	5.490	P<0.001	***
134	L. adecarb	4.133	2.347	P > 0.05	ns
135	M. morganti	-5.867	3.332	P < 0.05	*
136	C. freundii	-6.000	3.407	P<0.01	**
137	K. pneumo	-6.033	3.426	P<0.01	**

APPENDIX 58: Antifungal activities of *Plumeria acuminata* essential oils

	2way ANOVA Tabular results				
1	Table Analyzed	Data 1			
2					
3	Two-way ANOVA				
4					
5	Source of Variation	% of total variation	P value		
6	Interaction	17.67	< 0.0001		
7	Column Factor	50.27	< 0.0001		
8	Row Factor	33.95	< 0.0001		
9					
10	Source of Variation	P value summary	Significant?		
11	Interaction	***	Yes		
12	Column Factor	***	Yes		
13	Row Factor	***	Yes		
14					
15	Source of Variation	Df	Sum-of-squares	Mean square	F
16	Interaction	6	309.1	51.52	21.73
17	Column Factor	3	879.2	293.1	123.6
18	Row Factor	2	593.8	296.9	125.2
19	Residual	21	49.79	2.371	
20					
21	Number of missing values	3			
22					
23	Bonferroni posttests				
24					
25	Leaf oil vs Root oil				
26	Row Factor	Leaf oil	Root oil	Difference	95% CI of diff.

27	C. albicans	20.10	0.0000	-20.10	-24.86 to -15.34
28	A. niger	0.0000	0.0000	0.0000	-4.762 to 4.762
29	F. solani	6.000	1.933	-4.067	-8.829 to 0.6952
30					
31	Row Factor	Difference	t	P value	Summary
32	C. albicans	-20.10	14.30	P<0.001	***
33	A. niger	0.0000	0.0000	P > 0.05	ns
34	F. solani	-4.067	2.893	P < 0.05	*
35					
36	Leaf oil vs Flower oil				
37	Row Factor	Leaf oil	Flower oil	Difference	95% CI of diff.
38	C. albicans	20.10	10.03	-10.07	-14.83 to -5.305
39	A. niger	0.0000	0.0000	0.0000	-4.762 to 4.762
40	F. solani	6.000	2.700	-3.300	-8.062 to 1.462
41					
42	Row Factor	Difference	t	P value	Summary
43	C. albicans	-10.07	7.161	P<0.001	***
44	A. niger	0.0000	0.0000	P > 0.05	ns
45	F. solani	-3.300	2.348	P > 0.05	ns
46					
47	Leaf oil vs Ketoconazole				
48	Row Factor	Leaf oil	Ketoconazole	Difference	95% CI of diff.
49	C. albicans	20.10	21.00	0.9000	-3.862 to 5.662
50	A. niger	0.0000	10.50	10.50	5.738 to 15.26
51	F. solani	6.000	10.30	4.300	-0.4619 to 9.062
52					
53	Row Factor	Difference	t	P value	Summary
54	C. albicans	0.9000	0.6403	P > 0.05	ns
55	A. niger	10.50	7.470	P<0.001	***
56	F. solani	4.300	3.059	P < 0.05	*

57

58 Root oil vs Flower oil

59 Row Factor	Root oil	Flower oil	Difference	95% CI of diff.
60 C. albicans	0.0000	10.03	10.03	5.774 to 14.29
61 A. niger	0.0000	0.0000	0.0000	-4.259 to 4.259
62 F. solani	1.933	2.700	0.7667	-3.492 to 5.026

63

64 Row Factor	Difference	t	P value	Summary
65 C. albicans	10.03	7.980	P<0.001	***
66 A. niger	0.0000	0.0000	P > 0.05	ns
67 F. solani	0.7667	0.6098	P > 0.05	ns

68

69 Root oil vs Ketoconazole

70 Row Factor	Root oil	Ketoconazole	Difference	95% CI of diff.
71 C. albicans	0.0000	21.00	21.00	16.74 to 25.26
72 A. niger	0.0000	10.50	10.50	6.241 to 14.76
73 F. solani	1.933	10.30	8.367	4.108 to 12.63

74

75 Row Factor	Difference	t	P value	Summary
76 C. albicans	21.00	16.70	P<0.001	***
77 A. niger	10.50	8.351	P<0.001	***
78 F. solani	8.367	6.655	P<0.001	***

79

80 Flower oil vs Ketoconazole

81 Row Factor	Flower oil	Ketoconazole	Difference	95% CI of diff.
82 C. albicans	10.03	21.00	10.97	6.708 to 15.23
83 A. niger	0.0000	10.50	10.50	6.241 to 14.76
84 F. solani	2.700	10.30	7.600	3.341 to 11.86

85

86 Row Factor	Difference	t	P value	Summary
87 C. albicans	10.97	8.723	P<0.001	***
88 A. niger	10.50	8.351	P<0.001	***
89 F. solani	7.600	6.045	P<0.001	***

APPENDIX 59: Antimicrobial activities of *Plumeria rubra* essential oils

2way ANOVA

Tabular results

1	Table Analyzed	Data 1			
2					
3	Two-way ANOVA				
4					
5	Source of Variation	% of total variation	P value		
6	Interaction	14.12	< 0.0001		
7	Column Factor	67.40	< 0.0001		
8	Row Factor	12.70	< 0.0001		
9					
10	Source of Variation	P value summary	Significant?		
11	Interaction	***	Yes		
12	Column Factor	***	Yes		
13	Row Factor	***	Yes		
14					
15	Source of Variation	Df	Sum-of-squares	Mean square	F
16	Interaction	16	283.8	17.74	7.628
17	Column Factor	4	1355	338.8	145.7
18	Row Factor	4	255.4	63.84	27.45
19	Residual	50	116.3	2.326	
20					
21	Number of missing values	0			
22					
23	Bonferroni posttests				
24					

	Leaf oil vs Stem oil				
25	Leaf oil vs Stem oil				
26	Row Factor	Leaf oil	Stem oil	Difference	95% CI of diff.
27	S. aureus	0.6667	0.0000	-0.6667	-5.020 to 3.687
28	E. coli	0.0000	0.0000	0.0000	-4.353 to 4.353
29	C. albicans	2.667	2.100	-0.5667	-4.920 to 3.787
30	F. solani	6.033	2.000	-4.033	-8.387 to 0.3198
31	A. niger	6.000	0.6667	-5.333	-9.687 to -0.9802
32					
33	Row Factor	Difference	t	P value	Summary
34	S. aureus	-0.6667	0.5354	P > 0.05	ns
35	E. coli	0.0000	0.0000	P > 0.05	ns
36	C. albicans	-0.5667	0.4551	P > 0.05	ns
37	F. solani	-4.033	3.239	P < 0.05	*
38	A. niger	-5.333	4.283	P<0.001	***
39					
40	Leaf oil vs Flower oil				
41	Row Factor	Leaf oil	Flower oil	Difference	95% CI of diff.
42	S. aureus	0.6667	0.6333	-0.03333	-4.387 to 4.320
43	E. coli	0.0000	0.0000	0.0000	-4.353 to 4.353
44	C. albicans	2.667	5.933	3.267	-1.087 to 7.620
45	F. solani	6.033	0.0000	-6.033	-10.39 to -1.680
46	A. niger	6.000	5.933	-0.06667	-4.420 to 4.287
47					
48	Row Factor	Difference	t	P value	Summary
49	S. aureus	-0.03333	0.02677	P > 0.05	ns
50	E. coli	0.0000	0.0000	P > 0.05	ns
51	C. albicans	3.267	2.623	P > 0.05	ns
52	F. solani	-6.033	4.845	P<0.001	***
53	A. niger	-0.06667	0.05354	P > 0.05	ns

54

55 Leaf oil vs root oil

	Row Factor	Leaf oil	root oil	Difference	95% CI of diff.
56	Row Factor	Leaf oil	root oil	Difference	95% CI of diff.
57	S. aureus	0.6667	0.6000	-0.06667	-4.420 to 4.287
58	E. coli	0.0000	0.0000	0.0000	-4.353 to 4.353
59	C. albicans	2.667	3.367	0.7000	-3.653 to 5.053
60	F. solani	6.033	0.6333	-5.400	-9.753 to -1.047
61	A. niger	6.000	2.733	-3.267	-7.620 to 1.087

62

	Row Factor	Difference	t	P value	Summary
63	Row Factor	Difference	t	P value	Summary
64	S. aureus	-0.06667	0.05354	P > 0.05	ns
65	E. coli	0.0000	0.0000	P > 0.05	ns
66	C. albicans	0.7000	0.5622	P > 0.05	ns
67	F. solani	-5.400	4.337	P<0.001	***
68	A. niger	-3.267	2.623	P > 0.05	ns

69

70 Leaf oil vs Postive Control

	Row Factor	Leaf oil	Postive Control	Difference	95% CI of diff.
71	Row Factor	Leaf oil	Postive Control	Difference	95% CI of diff.
72	S. aureus	0.6667	11.50	10.83	6.480 to 15.19
73	E. coli	0.0000	9.000	9.000	4.647 to 13.35
74	C. albicans	2.667	21.00	18.33	13.98 to 22.69
75	F. solani	6.033	10.30	4.267	-0.08651 to 8.620
76	A. niger	6.000	10.50	4.500	0.1468 to 8.853

77

	Row Factor	Difference	t	P value	Summary
78	Row Factor	Difference	t	P value	Summary
79	S. aureus	10.83	8.700	P<0.001	***
80	E. coli	9.000	7.228	P<0.001	***
81	C. albicans	18.33	14.72	P<0.001	***
82	F. solani	4.267	3.427	P<0.01	**
83	A. niger	4.500	3.614	P<0.01	**

84

85 Stem oil vs Flower oil

86	Row Factor	Stem oil	Flower oil	Difference	95% CI of diff.
87	S. aureus	0.0000	0.6333	0.6333	-3.720 to 4.987
88	E. coli	0.0000	0.0000	0.0000	-4.353 to 4.353
89	C. albicans	2.100	5.933	3.833	-0.5198 to 8.187
90	F. solani	2.000	0.0000	-2.000	-6.353 to 2.353
91	A. niger	0.6667	5.933	5.267	0.9135 to 9.620

92

93	Row Factor	Difference	t	P value	Summary
94	S. aureus	0.6333	0.5086	P > 0.05	ns
95	E. coli	0.0000	0.0000	P > 0.05	ns
96	C. albicans	3.833	3.079	P < 0.05	*
97	F. solani	-2.000	1.606	P > 0.05	ns
98	A. niger	5.267	4.230	P<0.001	***

99

100 Stem oil vs root oil

101	Row Factor	Stem oil	root oil	Difference	95% CI of diff.
102	S. aureus	0.0000	0.6000	0.6000	-3.753 to 4.953
103	E. coli	0.0000	0.0000	0.0000	-4.353 to 4.353
104	C. albicans	2.100	3.367	1.267	-3.087 to 5.620
105	F. solani	2.000	0.6333	-1.367	-5.720 to 2.987
106	A. niger	0.6667	2.733	2.067	-2.287 to 6.420

107

108	Row Factor	Difference	t	P value	Summary
109	S. aureus	0.6000	0.4819	P > 0.05	ns
110	E. coli	0.0000	0.0000	P > 0.05	ns
111	C. albicans	1.267	1.017	P > 0.05	ns
112	F. solani	-1.367	1.098	P > 0.05	ns
113	A. niger	2.067	1.660	P > 0.05	ns

114

115 Stem oil vs Postive Control

	Row Factor	Stem oil	Postive Control	Difference	95% CI of diff.
116	Row Factor	Stem oil	Postive Control	Difference	95% CI of diff.
117	S. aureus	0.0000	11.50	11.50	7.147 to 15.85
118	E. coli	0.0000	9.000	9.000	4.647 to 13.35
119	C. albicans	2.100	21.00	18.90	14.55 to 23.25
120	F. solani	2.000	10.30	8.300	3.947 to 12.65
121	A. niger	0.6667	10.50	9.833	5.480 to 14.19

122

	Row Factor	Difference	t	P value	Summary
123	Row Factor	Difference	t	P value	Summary
124	S. aureus	11.50	9.236	P<0.001	***
125	E. coli	9.000	7.228	P<0.001	***
126	C. albicans	18.90	15.18	P<0.001	***
127	F. solani	8.300	6.666	P<0.001	***
128	A. niger	9.833	7.897	P<0.001	***

129

130 Flower oil vs root oil

	Row Factor	Flower oil	root oil	Difference	95% CI of diff.
131	Row Factor	Flower oil	root oil	Difference	95% CI of diff.
132	S. aureus	0.6333	0.6000	-0.03333	-4.387 to 4.320
133	E. coli	0.0000	0.0000	0.0000	-4.353 to 4.353
134	C. albicans	5.933	3.367	-2.567	-6.920 to 1.787
135	F. solani	0.0000	0.6333	0.6333	-3.720 to 4.987
136	A. niger	5.933	2.733	-3.200	-7.553 to 1.153

137

	Row Factor	Difference	t	P value	Summary
138	Row Factor	Difference	t	P value	Summary
139	S. aureus	-0.03333	0.02677	P > 0.05	ns
140	E. coli	0.0000	0.0000	P > 0.05	ns
141	C. albicans	-2.567	2.061	P > 0.05	ns
142	F. solani	0.6333	0.5086	P > 0.05	ns
143	A. niger	-3.200	2.570	P > 0.05	ns

144

145 Flower oil vs + Control

146 Row Factor	Flower oil	Postive Control	Difference	95% CI of diff.
147 S. aureus	0.6333	11.50	10.87	6.513 to 15.22
148 E. coli	0.0000	9.000	9.000	4.647 to 13.35
149 C. albicans	5.933	21.00	15.07	10.71 to 19.42
150 F. solani	0.0000	10.30	10.30	5.947 to 14.65
151 A. niger	5.933	10.50	4.567	0.2135 to 8.920

152

153 Row Factor	Difference	t	P value	Summary
154 S. aureus	10.87	8.727	P<0.001	***
155 E. coli	9.000	7.228	P<0.001	***
156 C. albicans	15.07	12.10	P<0.001	***
157 F. solani	10.30	8.272	P<0.001	***
158 A. niger	4.567	3.667	P<0.01	**

159

160 root oil vs Postive Control

161 Row Factor	root oil	Postive Control	Difference	95% CI of diff.
162 S. aureus	0.6000	11.50	10.90	6.547 to 15.25
163 E. coli	0.0000	9.000	9.000	4.647 to 13.35
164 C. albicans	3.367	21.00	17.63	13.28 to 21.99
165 F. solani	0.6333	10.30	9.667	5.313 to 14.02
166 A. niger	2.733	10.50	7.767	3.413 to 12.12

167 Row Factor	Difference	t	P value	Summary
168 S. aureus	10.90	8.754	P<0.001	***
169 E. coli	9.000	7.228	P<0.001	***
170 C. albicans	17.63	14.16	P<0.001	***
171 F. solani	9.667	7.763	P<0.001	***
172 A. niger	7.767	6.237	P<0.001	***

APPENDIX 60: Antimicrobial activities of *Delonix regia* essential oils

2way ANOVA

Tabular results

1	Table Analyzed	Data 1			
2					
3	Two-way ANOVA				
4					
5	Source of Variation	% of total variation	P value		
6	Interaction	10.38	< 0.0001		
7	Column Factor	76.64	< 0.0001		
8	Row Factor	8.50	< 0.0001		
9					
10	Source of Variation	P value summary	Significant?		
11	Interaction	***	Yes		
12	Column Factor	***	Yes		
13	Row Factor	***	Yes		
14					
15	Source of Variation	Df	Sum-of-squares	Mean square	F
16	Interaction	12	189.9	15.83	7.722
17	Column Factor	3	1403	467.6	228.2
18	Row Factor	4	155.7	38.91	18.99
19	Residual	40	81.98	2.050	
20					
21	Number of missing values	0			
22					
23	Bonferroni posttests				
24					

25 Twig oil vs Flower oil

Line	Row Factor	Twig oil	Flower oil	Difference	95% CI of diff.
27	S. aureus	0.6667	0.0000	-0.6667	-4.608 to 3.275
28	E. coli	0.0000	0.0000	0.0000	-3.941 to 3.941
29	C. albicans	2.167	2.067	-0.1000	-4.041 to 3.841
30	F. solani	2.067	1.300	-0.7667	-4.708 to 3.175
31	A. niger	2.000	1.967	-0.03333	-3.975 to 3.908

32

Line	Row Factor	Difference	t	P value	Summary
34	S. aureus	-0.6667	0.5703	P > 0.05	ns
35	E. coli	0.0000	0.0000	P > 0.05	ns
36	C. albicans	-0.1000	0.08555	P > 0.05	ns
37	F. solani	-0.7667	0.6559	P > 0.05	ns
38	A. niger	-0.03333	0.02852	P > 0.05	ns

39

40 Twig oil vs root oil

Line	Row Factor	Twig oil	root oil	Difference	95% CI of diff.
42	S. aureus	0.6667	0.0000	-0.6667	-4.608 to 3.275
43	E. coli	0.0000	0.0000	0.0000	-3.941 to 3.941
44	C. albicans	2.167	2.633	0.4667	-3.475 to 4.408
45	F. solani	2.067	4.000	1.933	-2.008 to 5.875
46	A. niger	2.000	0.6000	-1.400	-5.341 to 2.541

47

Line	Row Factor	Difference	t	P value	Summary
49	S. aureus	-0.6667	0.5703	P > 0.05	ns
50	E. coli	0.0000	0.0000	P > 0.05	ns
51	C. albicans	0.4667	0.3992	P > 0.05	ns
52	F. solani	1.933	1.654	P > 0.05	ns
53	A. niger	-1.400	1.198	P > 0.05	ns

54

55 Twig oil vs Postive Control

56 Row Factor	Twig oil	Postive Control	Difference	95% CI of diff.
57 S. aureus	0.6667	11.50	10.83	6.892 to 14.77
58 E. coli	0.0000	9.000	9.000	5.059 to 12.94
59 C. albicans	2.167	21.00	18.83	14.89 to 22.77
60 F. solani	2.067	10.30	8.233	4.292 to 12.17
61 A. niger	2.000	10.50	8.500	4.559 to 12.44

62

63 Row Factor	Difference	t	P value	Summary
64 S. aureus	10.83	9.268	P<0.001	***
65 E. coli	9.000	7.700	P<0.001	***
66 C. albicans	18.83	16.11	P<0.001	***
67 F. solani	8.233	7.044	P<0.001	***
68 A. niger	8.500	7.272	P<0.001	***

69

70 Flower oil vs root oil

71 Row Factor	Flower oil	root oil	Difference	95% CI of diff.
72 S. aureus	0.0000	0.0000	0.0000	-3.941 to 3.941
73 E. coli	0.0000	0.0000	0.0000	-3.941 to 3.941
74 C. albicans	2.067	2.633	0.5667	-3.375 to 4.508
75 F. solani	1.300	4.000	2.700	-1.241 to 6.641
76 A. niger	1.967	0.6000	-1.367	-5.308 to 2.575

77

78 Row Factor	Difference	t	P value	Summary
79 S. aureus	0.0000	0.0000	P > 0.05	ns
80 E. coli	0.0000	0.0000	P > 0.05	ns
81 C. albicans	0.5667	0.4848	P > 0.05	ns
82 F. solani	2.700	2.310	P > 0.05	ns
83 A. niger	-1.367	1.169	P > 0.05	ns

84

85 Flower oil vs Postive Control

86 Row Factor	Flower oil	Postive Control	Difference	95% CI of diff.
87 S. aureus	0.0000	11.50	11.50	7.559 to 15.44
88 E. coli	0.0000	9.000	9.000	5.059 to 12.94
89 C. albicans	2.067	21.00	18.93	14.99 to 22.87
90 F. solani	1.300	10.30	9.000	5.059 to 12.94
91 A. niger	1.967	10.50	8.533	4.592 to 12.47

92

93 Row Factor	Difference	t	P value	Summary
94 S. aureus	11.50	9.838	P<0.001	***
95 E. coli	9.000	7.700	P<0.001	***
96 C. albicans	18.93	16.20	P<0.001	***
97 F. solani	9.000	7.700	P<0.001	***
98 A. niger	8.533	7.300	P<0.001	***

99

100 root oil vs Postive Control

101 Row Factor	root oil	Postive Control	Difference	95% CI of diff.
102 S. aureus	0.0000	11.50	11.50	7.559 to 15.44
103 E. coli	0.0000	9.000	9.000	5.059 to 12.94
104 C. albicans	2.633	21.00	18.37	14.43 to 22.31
105 F. solani	4.000	10.30	6.300	2.359 to 10.24
106 A. niger	0.6000	10.50	9.900	5.959 to 13.84

107 Row Factor	Difference	t	P value	Summary
108 S. aureus	11.50	9.838	P<0.001	***
109 E. coli	9.000	7.700	P<0.001	***
110 C. albicans	18.37	15.71	P<0.001	***
111 F. solani	6.300	5.390	P<0.001	***
112 A. niger	9.900	8.469	P<0.001	***

APPENDIX 61: Antimicrobial activities of *Duranta repens* essential oils

2way ANOVA

Tabular results

1	Table Analyzed	Data 1			
2					
3	Two-way ANOVA				
4					
5	Source of Variation	% of total variation	P value		
6	Interaction	10.42	< 0.0001		
7	Column Factor	73.12	< 0.0001		
8	Row Factor	13.88	< 0.0001		
9					
10	Source of Variation	P value summary	Significant?		
11	Interaction	***	Yes		
12	Column Factor	***	Yes		
13	Row Factor	***	Yes		
14					
15	Source of Variation	Df	Sum-of-squares	Mean square	F
16	Interaction	16	219.0	13.69	12.65
17	Column Factor	4	1536	384.1	355.0
18	Row Factor	4	291.7	72.92	67.40
19	Residual	50	54.09	1.082	
20					
21	Number of missing values	0			
22					
23	Bonferroni posttests				
24					

25 **Leaf oil vs Stem oil**

	Row Factor	Leaf oil	Stem oil	Difference	95% CI of diff.
27	S. aureus	0.6000	0.0000	-0.6000	-3.569 to 2.369
28	E. coli	0.0000	0.0000	0.0000	-2.969 to 2.969
29	C. albicans	5.633	4.033	-1.600	-4.569 to 1.369
30	F. solani	0.0000	0.6667	0.6667	-2.302 to 3.636
31	A. niger	0.7000	0.0000	-0.7000	-3.669 to 2.269

32

33	Row Factor	Difference	t	P value	Summary
34	S. aureus	-0.6000	0.7065	P > 0.05	ns
35	E. coli	0.0000	0.0000	P > 0.05	ns
36	C. albicans	-1.600	1.884	P > 0.05	ns
37	F. solani	0.6667	0.7850	P > 0.05	ns
38	A. niger	-0.7000	0.8242	P > 0.05	ns

39

40 **Leaf oil vs Fruit**

41	Row Factor	Leaf oil	Fruit	Difference	95% CI of diff.
42	S. aureus	0.6000	0.6667	0.06667	-2.902 to 3.036
43	E. coli	0.0000	0.0000	0.0000	-2.969 to 2.969
44	C. albicans	5.633	6.133	0.5000	-2.469 to 3.469
45	F. solani	0.0000	6.033	6.033	3.064 to 9.002
46	A. niger	0.7000	6.167	5.467	2.498 to 8.436

47

48	Row Factor	Difference	t	P value	Summary
49	S. aureus	0.06667	0.07850	P > 0.05	ns
50	E. coli	0.0000	0.0000	P > 0.05	ns
51	C. albicans	0.5000	0.5887	P > 0.05	ns
52	F. solani	6.033	7.104	P<0.001	***
53	A. niger	5.467	6.437	P<0.001	***

54

55 Leaf oil vs root

56 Row Factor	Leaf oil	root	Difference	95% CI of diff.
57 S. aureus	0.6000	0.0000	-0.6000	-3.569 to 2.369
58 E. coli	0.0000	0.0000	0.0000	-2.969 to 2.969
59 C. albicans	5.633	0.6667	-4.967	-7.936 to -1.998
60 F. solani	0.0000	0.0000	0.0000	-2.969 to 2.969
61 A. niger	0.7000	0.0000	-0.7000	-3.669 to 2.269

62

63 Row Factor	Difference	t	P value	Summary
64 S. aureus	-0.6000	0.7065	P > 0.05	ns
65 E. coli	0.0000	0.0000	P > 0.05	ns
66 C. albicans	-4.967	5.848	P<0.001	***
67 F. solani	0.0000	0.0000	P > 0.05	ns
68 A. niger	-0.7000	0.8242	P > 0.05	ns

69

70 Leaf oil vs Positive control

71 Row Factor	Leaf oil	Positive control	Difference	95% CI of diff.
72 S. aureus	0.6000	11.50	10.90	7.931 to 13.87
73 E. coli	0.0000	9.000	9.000	6.031 to 11.97
74 C. albicans	5.633	21.00	15.37	12.40 to 18.34
75 F. solani	0.0000	10.30	10.30	7.331 to 13.27
76 A. niger	0.7000	10.50	9.800	6.831 to 12.77

77

78 Row Factor	Difference	t	P value	Summary
79 S. aureus	10.90	12.83	P<0.001	***
80 E. coli	9.000	10.60	P<0.001	***
81 C. albicans	15.37	18.09	P<0.001	***
82 F. solani	10.30	12.13	P<0.001	***
83 A. niger	9.800	11.54	P<0.001	***

84

85 Stem oil vs Fruit

86	Row Factor	Stem oil	Fruit	Difference	95% CI of diff.
87	S. aureus	0.0000	0.6667	0.6667	-2.302 to 3.636
88	E. coli	0.0000	0.0000	0.0000	-2.969 to 2.969
89	C. albicans	4.033	6.133	2.100	-0.8690 to 5.069
90	F. solani	0.6667	6.033	5.367	2.398 to 8.336
91	A. niger	0.0000	6.167	6.167	3.198 to 9.136

92

93	Row Factor	Difference	t	P value	Summary
94	S. aureus	0.6667	0.7850	P > 0.05	ns
95	E. coli	0.0000	0.0000	P > 0.05	ns
96	C. albicans	2.100	2.473	P > 0.05	ns
97	F. solani	5.367	6.319	P<0.001	***
98	A. niger	6.167	7.261	P<0.001	***

99

100 Stem oil vs root

101	Row Factor	Stem oil	root	Difference	95% CI of diff.
102	S. aureus	0.0000	0.0000	0.0000	-2.969 to 2.969
103	E. coli	0.0000	0.0000	0.0000	-2.969 to 2.969
104	C. albicans	4.033	0.6667	-3.367	-6.336 to -0.3976
105	F. solani	0.6667	0.0000	-0.6667	-3.636 to 2.302
106	A. niger	0.0000	0.0000	0.0000	-2.969 to 2.969

107

108	Row Factor	Difference	t	P value	Summary
109	S. aureus	0.0000	0.0000	P > 0.05	ns
110	E. coli	0.0000	0.0000	P > 0.05	ns
111	C. albicans	-3.367	3.964	P<0.01	**
112	F. solani	-0.6667	0.7850	P > 0.05	ns
113	A. niger	0.0000	0.0000	P > 0.05	ns

114

115 Stem oil vs Positive control

116	Row Factor	Stem oil	Positive control	Difference	95% CI of diff.
117	S. aureus	0.0000	11.50	11.50	8.531 to 14.47
118	E. coli	0.0000	9.000	9.000	6.031 to 11.97
119	C. albicans	4.033	21.00	16.97	14.00 to 19.94
120	F. solani	0.6667	10.30	9.633	6.664 to 12.60
121	A. niger	0.0000	10.50	10.50	7.531 to 13.47

122

123	Row Factor	Difference	t	P value	Summary
124	S. aureus	11.50	13.54	P<0.001	***
125	E. coli	9.000	10.60	P<0.001	***
126	C. albicans	16.97	19.98	P<0.001	***
127	F. solani	9.633	11.34	P<0.001	***
128	A. niger	10.50	12.36	P<0.001	***

129

130 Fruit vs root

131	Row Factor	Fruit	root	Difference	95% CI of diff.
132	S. aureus	0.6667	0.0000	-0.6667	-3.636 to 2.302
133	E. coli	0.0000	0.0000	0.0000	-2.969 to 2.969
134	C. albicans	6.133	0.6667	-5.467	-8.436 to -2.498
135	F. solani	6.033	0.0000	-6.033	-9.002 to -3.064
136	A. niger	6.167	0.0000	-6.167	-9.136 to -3.198

137

138	Row Factor	Difference	t	P value	Summary
139	S. aureus	-0.6667	0.7850	P > 0.05	ns
140	E. coli	0.0000	0.0000	P > 0.05	ns
141	C. albicans	-5.467	6.437	P<0.001	***
142	F. solani	-6.033	7.104	P<0.001	***
143	A. niger	-6.167	7.261	P<0.001	***

144

145 Fruit vs Positive control

146 Row Factor	Fruit	Positive control	Difference	95% CI of diff.
147 S. aureus	0.6667	11.50	10.83	7.864 to 13.80
148 E. coli	0.0000	9.000	9.000	6.031 to 11.97
149 C. albicans	6.133	21.00	14.87	11.90 to 17.84
150 F. solani	6.033	10.30	4.267	1.298 to 7.236
151 A. niger	6.167	10.50	4.333	1.364 to 7.302

152

153 Row Factor	Difference	t	P value	Summary
154 S. aureus	10.83	12.76	P<0.001	***
155 E. coli	9.000	10.60	P<0.001	***
156 C. albicans	14.87	17.51	P<0.001	***
157 F. solani	4.267	5.024	P<0.001	***
158 A. niger	4.333	5.102	P<0.001	***

159

160 root vs Positive control

161 Row Factor	root	Positive control	Difference	95% CI of diff.
162 S. aureus	0.0000	11.50	11.50	8.531 to 14.47
163 E. coli	0.0000	9.000	9.000	6.031 to 11.97
164 C. albicans	0.6667	21.00	20.33	17.36 to 23.30
165 F. solani	0.0000	10.30	10.30	7.331 to 13.27
166 A. niger	0.0000	10.50	10.50	7.531 to 13.47

167 Row Factor	Difference	t	P value	Summary
168 S. aureus	11.50	13.54	P<0.001	***
169 E. coli	9.000	10.60	P<0.001	***
170 C. albicans	20.33	23.94	P<0.001	***
171 F. solani	10.30	12.13	P<0.001	***
172 A. niger	10.50	12.36	P<0.001	***

APPENDIX 62: Antimicrobial activities of *Zanthoxylum zanthoxyloides* essential oils

2way ANOVA
Tabular results

1	Table Analyzed	Data 1			
2					
3	Two-way ANOVA				
4					
5	Source of Variation	% of total variation	P value		
6	Interaction	13.31	< 0.0001		
7	Column Factor	67.98	< 0.0001		
8	Row Factor	14.83	< 0.0001		
9					
10	Source of Variation	P value summary	Significant?		
11	Interaction	***	Yes		
12	Column Factor	***	Yes		
13	Row Factor	***	Yes		
14					
15	Source of Variation	Df	Sum-of-squares	Mean square	F
16	Interaction	8	205.8	25.72	12.87
17	Column Factor	2	1051	525.4	262.8
18	Row Factor	4	229.2	57.29	28.66
19	Residual	30	59.98	1.999	
20					
21	Number of missing values	0			
22					
23	Bonferroni posttests				
24					
25	Leaf oil vs Stem bark				

26	Row Factor	Leaf oil	Stem bark	Difference	95% CI of diff.
27	S. aureus	0.7667	0.6333	-0.1333	-3.815 to 3.548
28	E. coli	0.0000	0.0000	0.0000	-3.682 to 3.682
29	C. albicans	2.100	6.067	3.967	0.2852 to 7.648
30	F. solani	2.733	3.933	1.200	-2.482 to 4.882
31	A. niger	5.867	0.0000	-5.867	-9.548 to -2.185

32

33	Row Factor	Difference	t	P value	Summary
34	S. aureus	-0.1333	0.1155	P > 0.05	ns
35	E. coli	0.0000	0.0000	P > 0.05	ns
36	C. albicans	3.967	3.436	P<0.01	**
37	F. solani	1.200	1.039	P > 0.05	ns
38	A. niger	-5.867	5.082	P<0.001	***

39

40 Leaf oil vs positive control

41	Row Factor	Leaf oil	positive control	Difference	95% CI of diff.
42	S. aureus	0.7667	11.50	10.73	7.052 to 14.41
43	E. coli	0.0000	9.000	9.000	5.318 to 12.68
44	C. albicans	2.100	21.00	18.90	15.22 to 22.58
45	F. solani	2.733	10.30	7.567	3.885 to 11.25
46	A. niger	5.867	10.50	4.633	0.9518 to 8.315

47

48	Row Factor	Difference	t	P value	Summary
49	S. aureus	10.73	9.297	P<0.001	***
50	E. coli	9.000	7.796	P<0.001	***
51	C. albicans	18.90	16.37	P<0.001	***
52	F. solani	7.567	6.554	P<0.001	***
53	A. niger	4.633	4.013	P<0.01	**

54

55 Stem bark vs positive control

56 Row Factor	Stem bark	positive control	Difference	95% CI of diff.
57 S. aureus	0.6333	11.50	10.87	7.185 to 14.55
58 E. coli	0.0000	9.000	9.000	5.318 to 12.68
59 C. albicans	6.067	21.00	14.93	11.25 to 18.61
60 F. solani	3.933	10.30	6.367	2.685 to 10.05
61 A. niger	0.0000	10.50	10.50	6.818 to 14.18

62

63 Row Factor	Difference	t	P value	Summary
64 S. aureus	10.87	9.412	P<0.001	***
65 E. coli	9.000	7.796	P<0.001	***
66 C. albicans	14.93	12.93	P<0.001	***
67 F. solani	6.367	5.515	P<0.001	***
68 A. niger	10.50	9.095	P<0.001	***

APPENDIX 63: Antimicrobial activities of *Ceiba pentandra* essential oils

2way ANOVA

Tabular results

1	Table Analyzed	Data 1			
2					
3	Two-way ANOVA				
4					
5	Source of Variation	% of total variation	P value		
6	Interaction	9.59	< 0.0001		
7	Column Factor	78.57	< 0.0001		
8	Row Factor	9.15	< 0.0001		
9					
10	Source of Variation	P value summary	Significant?		
11	Interaction	***	Yes		
12	Column Factor	***	Yes		
13	Row Factor	***	Yes		
14					
15	Source of Variation	Df	Sum-of-squares	Mean square	F
16	Interaction	8	158.1	19.76	13.35
17	Column Factor	2	1296	647.9	437.7
18	Row Factor	4	150.9	37.72	25.49
19	Residual	30	44.41	1.480	
20					
21	Number of missing values	0			
22					
23	Bonferroni posttests				
24					

25 Stembark vs Heartwood

	Row Factor	Stembark	Heartwood	Difference	95% CI of diff.
27	S. aureus	0.6667	0.0000	-0.6667	-3.834 to 2.501
28	E. coli	0.0000	0.0000	0.0000	-3.168 to 3.168
29	C. albicans	2.000	2.067	0.06667	-3.101 to 3.234
30	F. solani	2.067	1.967	-0.1000	-3.268 to 3.068
31	A. niger	2.100	0.0000	-2.100	-5.268 to 1.068

32

	Row Factor	Difference	t	P value	Summary
34	S. aureus	-0.6667	0.6711	P > 0.05	ns
35	E. coli	0.0000	0.0000	P > 0.05	ns
36	C. albicans	0.06667	0.06711	P > 0.05	ns
37	F. solani	-0.1000	0.1007	P > 0.05	ns
38	A. niger	-2.100	2.114	P > 0.05	ns

39

40 Stembark vs positive control

	Row Factor	Stembark	positive control	Difference	95% CI of diff.
42	S. aureus	0.6667	11.50	10.83	7.666 to 14.00
43	E. coli	0.0000	9.000	9.000	5.832 to 12.17
44	C. albicans	2.000	21.00	19.00	15.83 to 22.17
45	F. solani	2.067	10.30	8.233	5.066 to 11.40
46	A. niger	2.100	10.50	8.400	5.232 to 11.57

47

	Row Factor	Difference	t	P value	Summary
49	S. aureus	10.83	10.91	P<0.001	***
50	E. coli	9.000	9.060	P<0.001	***
51	C. albicans	19.00	19.13	P<0.001	***
52	F. solani	8.233	8.288	P<0.001	***
53	A. niger	8.400	8.456	P<0.001	***

54

55 **Heartwood vs positive control**

	Row Factor	Heartwood	positive control	Difference	95% CI of diff.
56					
57	S. aureus	0.0000	11.50	11.50	8.332 to 14.67
58	E. coli	0.0000	9.000	9.000	5.832 to 12.17
59	C. albicans	2.067	21.00	18.93	15.77 to 22.10
60	F. solani	1.967	10.30	8.333	5.166 to 11.50
61	A. niger	0.0000	10.50	10.50	7.332 to 13.67

62

	Row Factor	Difference	t	P value	Summary
63					
64	S. aureus	11.50	11.58	P<0.001	***
65	E. coli	9.000	9.060	P<0.001	***
66	C. albicans	18.93	19.06	P<0.001	***
67	F. solani	8.333	8.389	P<0.001	***
68	A. niger	10.50	10.57	P<0.001	***

APPENDIX 64: Antimicrobial activities of *Gliricidia sepium* essential oils

	2way ANOVA				
	Tabular results				
1	Table Analyzed	Data 1			
2					
3	Two-way ANOVA				
4					
5	Source of Variation	% of total variation	P value		
6	Interaction	11.58	< 0.0001		
7	Column Factor	74.45	< 0.0001		
8	Row Factor	10.97	< 0.0001		
9					
10	Source of Variation	P value summary	Significant?		
11	Interaction	***	Yes		
12	Column Factor	***	Yes		
13	Row Factor	***	Yes		
14					
15	Source of Variation	Df	Sum-of-squares	Mean square	F
16	Interaction	8	193.4	24.17	14.51
17	Column Factor	2	1243	621.7	373.1
18	Row Factor	4	183.2	45.81	27.50
19	Residual	30	49.98	1.666	
20					
21	Number of missing values	0			
22					
23	Bonferroni posttests				
24					

25	Leaf oil vs Stem				
26	Row Factor	Leaf oil	Stem	Difference	95% CI of diff.
27	S. aureus	0.6000	0.0000	-0.6000	-3.961 to 2.761
28	E. coli	0.0000	0.0000	0.0000	-3.361 to 3.361
29	C. albicans	0.0000	4.833	4.833	1.473 to 8.194
30	F. solani	3.967	3.300	-0.6667	-4.027 to 2.694
31	A. niger	0.0000	0.6333	0.6333	-2.727 to 3.994
32					

33	Row Factor	Difference	t	P value	Summary
34	S. aureus	-0.6000	0.5693	P > 0.05	ns
35	E. coli	0.0000	0.0000	P > 0.05	ns
36	C. albicans	4.833	4.586	P<0.001	***
37	F. solani	-0.6667	0.6326	P > 0.05	ns
38	A. niger	0.6333	0.6010	P > 0.05	ns
39					

40	Leaf oil vs positive control				
41	Row Factor	Leaf oil	positive control	Difference	95% CI of diff.
42	S. aureus	0.6000	11.50	10.90	7.539 to 14.26
43	E. coli	0.0000	9.000	9.000	5.639 to 12.36
44	C. albicans	0.0000	21.00	21.00	17.64 to 24.36
45	F. solani	3.967	10.30	6.333	2.973 to 9.694
46	A. niger	0.0000	10.50	10.50	7.139 to 13.86
47					

48	Row Factor	Difference	t	P value	Summary
49	S. aureus	10.90	10.34	P<0.001	***
50	E. coli	9.000	8.540	P<0.001	***
51	C. albicans	21.00	19.93	P<0.001	***
52	F. solani	6.333	6.010	P<0.001	***
53	A. niger	10.50	9.963	P<0.001	***

54

55 Stem vs positive control

56 Row Factor	Stem	positive control	Difference	95% CI of diff.
57 S. aureus	0.0000	11.50	11.50	8.139 to 14.86
58 E. coli	0.0000	9.000	9.000	5.639 to 12.36
59 C. albicans	4.833	21.00	16.17	12.81 to 19.53
60 F. solani	3.300	10.30	7.000	3.639 to 10.36
61 A. niger	0.6333	10.50	9.867	6.506 to 13.23

62

63 Row Factor	Difference	t	P value	Summary
64 S. aureus	11.50	10.91	P<0.001	***
65 E. coli	9.000	8.540	P<0.001	***
66 C. albicans	16.17	15.34	P<0.001	***
67 F. solani	7.000	6.642	P<0.001	***
68 A. niger	9.867	9.362	P<0.001	***

APPENDIX 65: Antimicrobial activities of *Annona muricata* essential oils

2way ANOVA

Tabular results

1	Table Analyzed	Data 1			
2					
3	Two-way ANOVA				
4					
5	Source of Variation	% of total variation	P value		
6	Interaction	8.66	< 0.0001		
7	Column Factor	76.22	< 0.0001		
8	Row Factor	11.37	< 0.0001		
9					
10	Source of Variation	P value summary	Significant?		
11	Interaction	***	Yes		
12	Column Factor	***	Yes		
13	Row Factor	***	Yes		
14					
15	Source of Variation	Df	Sum-of-squares	Mean square	F
16	Interaction	12	162.1	13.51	7.717
17	Column Factor	3	1426	475.4	271.5
18	Row Factor	4	212.7	53.18	30.37
19	Residual	40	70.03	1.751	
20					
21	Number of missing values	0			
22					
23	Bonferroni posttests				
24					

25	leaf vs branches				
26	Row Factor	leaf	branches	Difference	95% CI of diff.
27	S. aureus	0.6667	0.0000	-0.6667	-4.039 to 2.706
28	E. coli	0.0000	0.0000	0.0000	-3.373 to 3.373
29	C. albicans	2.000	2.067	0.06667	-3.306 to 3.439
30	F. solani	2.067	1.967	-0.1000	-3.473 to 3.273
31	A. niger	2.100	0.0000	-2.100	-5.473 to 1.273
32					
33	Row Factor	Difference	t	P value	Summary
34	S. aureus	-0.6667	0.6171	P > 0.05	ns
35	E. coli	0.0000	0.0000	P > 0.05	ns
36	C. albicans	0.06667	0.06171	P > 0.05	ns
37	F. solani	-0.1000	0.09256	P > 0.05	ns
38	A. niger	-2.100	1.944	P > 0.05	ns
39					
40	leaf vs stem bark				
41	Row Factor	leaf	stem bark	Difference	95% CI of diff.
42	S. aureus	0.6667	0.0000	-0.6667	-4.039 to 2.706
43	E. coli	0.0000	0.0000	0.0000	-3.373 to 3.373
44	C. albicans	2.000	5.367	3.367	-0.006061 to 6.739
45	F. solani	2.067	2.000	-0.06667	-3.439 to 3.306
46	A. niger	2.100	0.0000	-2.100	-5.473 to 1.273
47					
48	Row Factor	Difference	t	P value	Summary
49	S. aureus	-0.6667	0.6171	P > 0.05	ns
50	E. coli	0.0000	0.0000	P > 0.05	ns
51	C. albicans	3.367	3.116	P < 0.05	*
52	F. solani	-0.06667	0.06171	P > 0.05	ns
53	A. niger	-2.100	1.944	P > 0.05	ns

54

55 leaf vs positive control

	Row Factor	leaf	positive control	Difference	95% CI of diff.
56	Row Factor	leaf	positive control	Difference	95% CI of diff.
57	S. aureus	0.6667	11.50	10.83	7.461 to 14.21
58	E. coli	0.0000	9.000	9.000	5.627 to 12.37
59	C. albicans	2.000	21.00	19.00	15.63 to 22.37
60	F. solani	2.067	10.30	8.233	4.861 to 11.61
61	A. niger	2.100	10.50	8.400	5.027 to 11.77

62

	Row Factor	Difference	t	P value	Summary
63	Row Factor	Difference	t	P value	Summary
64	S. aureus	10.83	10.03	P<0.001	***
65	E. coli	9.000	8.330	P<0.001	***
66	C. albicans	19.00	17.59	P<0.001	***
67	F. solani	8.233	7.621	P<0.001	***
68	A. niger	8.400	7.775	P<0.001	***