Phytochemical and Biological Investigation of *Jatropha pelargoniifolia* Roots Native to the Kingdom of Saudi Arabia

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The research described in this thesis was conducted at the Department of Pharmacognosy (Pharmacy College, King Saud University, Kingdom Saudi Arabia) according to the requirements of the External joint supervision program for PhD degree (King Saud University) and at the Department of Biochemical and Chemical Engineering, Chair of Technical Biochemistry, TU Dortmund University.

Dedication

I wish to dedicate this thesis to

My wonderful parents (Yahya & Fatimah)

For their endless love, continued support and encouragement.

My husband (Hamad)

who has been a constant source of support and encouragement during the challenges of life.

My lovely children

They are five flowers in my life (Shahad, Abdulmalik, Fares, Taleen & Zeyad)

I do not forget to send a bunch of thanks and gratitude

For my love friend (Jwaher Al-Qahtani)

For my sisters (Amal & Amoon)

For my brothers (Sultan, Salman & Abdulrahman)

Acknowledgements

In the name of Allah, most gracious, most merciful. All praise and glory to Allah the almighty who alone made this objective to be accomplished. My deep appreciation goes to my thesis supervisors, Dr. Oliver Kayser, Professor of the Chair Technical Biochemistry, TU Dortmund University, Germany and Dr. Ali A. El-Gamal, Professor of Pharmacognosy, College of Pharmacy, King Saud University whose gave me a lot of their expertise and research insight. They gave me a lot of their time and helped me go through the hard way with constant encouragement and influential discussions. I am deeply grateful to their valuable guidance and advices. It would have never been possible for me to complete this work without their incredible support and encouragement.

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Last but not the least, I wish to express my heartfelt gratitude to my parents for their continuous support. They taught me how to be patient, strong and sincere in my work. No words can express my appreciation to my dear husband Hamad Aati and my children (Shahad, Abdulmalik, Fares, Taleen & Zeyad) for their love that give me strength to move forward and make our dream come true. Furthermore, I wish to express my thanks to my brothers (Sultan, Salman and Abdulrahman), sisters (Amal and Amoon), for their support and constant love. Finally, this dissertation is really dedicated to my family.

Table of contents

Abstract

Reviewing the current literature for the phytochemical importance of the plants belonging to genus *Jatropha* revealed broad range of secondary metabolites isolated such as, diterpenoids, triterpenoids, non-conventional coumarino-lignoids, alkaloids, coumarins, flavonoids, cyclic peptides and steroids. This is indeed a reflection on the versatility of the enzymatic system present in the plants belonging to the family Euphorbiaceae but nothing was reported regarding *pelargoniifolia* species. Thus, it was of interest to explore the active constituents and biological importance for the titled plant which is used traditionally to cure ulcers and wounds in Ethiopia and Saudi Arabia.

In vivo and *in vitro* studies were carried out to evaluate the potential hepatoprotective, antinociceptive, anti-inflammatory and antidiabetic/hypoglycemic effects of the total alcoholic extracts of aerial parts and roots of *J. pelargoniifolia* and *J. glauca* species in comparison to those of standard clinical drugs. As the result, the roots of *J. glauca* showed higher hepatoprotective and antinociceptive (63.85%) activities in comparison to indomethacin (69.87%). In addition, the root extracts from *J. pelargoniifolia* exhibited greater anti-inflammatory (50.63%) and hypoglycemic (37.98%) activities when compared with phenylbutazone (64.63%) and glibenclamide (51.57%), respectively. Finally, extracts from the aerial parts of *J. glauca* showed significantly higher anti-diabetic activity in mice with alloxan-induced diabetes (39.93%) when compared with glibenclamide (47.13%).

Moreover, the chemical composition of the essential oil separated from *J. pelargoniifolia* roots was determined via GC-FID, resulting in isolation of 80 compounds representing 99.99% of the total oil constituents. Among these, 77.31% were sesquiterpenes, 14.62% were fatty acids, 7.21% were other components (i.e., phenolics, hydrocarbons, etc.), and 0.85% were monoterpenes. Then, the essential oil was evaluated for its potency as an anti-inflammatory, antioxidant, antipyretic, and antinociceptive agent by *in vivo* and *in vitro* models. The obtained results demonstrated that the investigated essential oil of *J. pelargoniifolia* roots could be used as a natural remedy for their anti-inflammatory, antinociceptive, antipyretic, and antioxidant effects.

Additionally, the chemical composition of the essential oil isolated from roots of *J. pelargoniifolia* was compared with the essential oils isolated from aerial parts of the same plant as well with different *Jatropha* species, *J. glauca* aerial parts and roots. Oxygenated sesquiterpenes and fatty acids were predominant chemical classes in oil isolated from roots of both species. On the other hand, oils from aerial parts were high in oxygenated diterpenes (35.30% in *J. glauca* and 32.23% in *J. pelargoniifolia*). Oils from *J. pelargoniifolia* aerial parts were high in oxygenated sesquiterpenes followed by fatty acids, while those from *J. glauca* were rich in fatty acids and other phenolics (hydrocarbons/cyclic). *J. glauca* and *J. pelargoniifolia* have similar morphology, except for leaf surface (hairy in *J. pelargoniifolia* and smooth in *J. glauca*) and fruit color (darker in *J. pelargoniifolia*), and these species are therefore often confused by native people in southern Saudi Arabia. This study clarified chemotaxonomic characters of *J. glauca* and *J. pelargoniifolia* via chemical composition analysis of essential oils of roots and aerial parts.

Finally, extensive phytochemical study for different fractions of *J. pelargoniifolia*, resulted in the isolation and identification of a total twenty-two compounds belonging to different chemical classes of secondary metabolites by using different chromatographic techniques. Two new compounds belong to coumarin glucoside and alkaloid; 6-hydroxy-8-methoxycoumarin-7-O-*β*-D-glycopyranoside and 2-hydroxymethyl-*N*-methyltryptamine, respectively were isolated and identified for the first time from a natural source. The anti-inflammatory, antinociceptive, antipyretic and antioxidant activities were evaluated for some isolated compounds which are available in good yields. Compound 2-hydroxymethyl-*N*-methyltryptamine can be used as starting material for semi synthesis of related analogous aiming to produce new drug leads used for treatment of contemporary disease such as; depression, anxiety, pain, obesity and many more. The roots of *J. pelargoniifolia* showed also significant antinociceptive, anti-inflammatory, antipyretic and free radical scavenging activities may be due to its content of many bioactive constituents belong to various chemical classes and those results were support the efficient use of this plant in Saudi Traditional Medicine as remedy for pain relieve and curing many inflammatory conditions.

Chapter 1

Introduction and scope of thesis

Hanan Yahya Aati (H.A), Ali A. El-Gamal (A.A.G) and Oliver Kayser (O.K) jointly planned and designed the chapter; H.A performed the literature review; H.A wrote the manuscript with inputs from all coauthors; A.A.G and O.K oversaw the entire project as supervisors of H.A.

The data for this chapter are unpuplished

1.1. Background

Since ancient times, nature has been an important source of medicinal agents. This fact is illustrated by the large number of natural products currently used medically. The value of natural products in this regard can be assessed using three criteria: first, the rate of introduction of new chemical entities of wide structural diversity including natural products serving as templates for semi-synthetic and total synthetic analogues. Secondly, the large number of diseases treated or prevented by these natural substances. Thirdly, their frequent use in treatment of diseases. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. This interest in drugs of plant origin is due to several reasons, namely, the frequent inefficiency of conventional medicine, possible development of side effects of synthetic drugs, and that a large percentage of the world's poor population doesn't have access to conventional medical treatment. In addition, the long history of use of folk medicine suggests that "natural" products are usually harmless. The plant kingdom offers a unique and renewable resource for the discovery of potential new drugs and important leads against various medical targets including pain, cancer, HIV/AIDS, SARS, Alzheimer's and malaria [1].

The Arabian Peninsula is recognized as an arid area dominated by deserts and poor biodiversity. However, the Kingdom of Saudi Arabia (henceforth abbreviated into KSA) has a wide range of flora, consisting of different species of trees, herbs, and shrubs and containing numerous edible and medicinal plants. The KSA is characterized by its vast area of diverse geographical landscapes and climates. Consequently, there is enormous variation in the distribution of plants across the Kingdom. The traditional use of ethnomedical plants in the KSA represents a strong interconnection among familiar remedies, health, diet, and traditional healing practices characterized by specific cultures [2].

1.2. Scope and objective of thesis

Reviewing the current literature for the importance of genus *Jatropha* growing worldwide and comprising about 200 species, revealed many biological interests such as, treatments of skin inflammation, eye infection, chest pain, stomach pain, itching and as a vermifuge or as ornamental plants and energy crops [3]. *J. glauca, J. curcas, J. spinose* and *J. pelargoniifolia* are the only four *Jatropha* species distributed in Saudi Arabia and employed as traditional herbal medicines due to their anti-inflammatory, antioxidant, antiseptic and analgesic activities [4, 5].

Interestingly, nothing was reported concerning the study of *Jatropha pelargoniifolia* growing in Saudi Arabia. Thus, it was of interest to explore the active constituents and the biological activities for this plant. *Jatropha pelargoniifolia* used in traditional medicine such as, sap of the petiole is applied to ulcers and wound healing as well treatment for various type of skin inflammation. It is widely distributed in East tropical Africa (Sudan, Eritrea, Ethiopia, Somalia and Kenya) and Arabian Peninsula (Yemen, Oman and Saudi Arabia) [6].

The aim of this thesis is to give an overview of the values of *J. pelargoniifolia* root which could be serve as one of the most promising candidate groups of natural compounds for the development of safer therapeutic agents used for treatment of many contemporary diseases.

The objectives of this cumulative thesis are worked out as individual chapters including the following contents:

- **Chapter 1:** Included scope of presented thesis which inclusive the main aim for conducting this work. As well a brief discussion of the taxonomy of the family Euphorbiaceae and the genus *Jatropha* and biological activities of *Jatropha* extracts and their constituents. In addition, the biologically-active compounds isolated from genus *Jatropha* were discussed with emphasis on diterpenes, flavonoids, coumarins and alkaloids.

- **Chapter 2:** Covered the biological activity of the ethanolic extracts of *J. pelargoniifolia* (root and aerial parts) and compared with other *Jatroph* species growing in Saudi Arabia (*J. glauca*). The folkloric use of *J. pelargoniifolia* and *J. glauca* in the management of pain and inflammatory conditions were guide us to select some

biological activities to be tested. The selected biological activities were an analgesic, anti-inflammatory and hepatoprotective activities as well hypoglycemic activity.

- Chapter 3: It was revealed to the chemical compositions and the biological potentialities of the essential oil of Saudi plant, *J. pelargoniifolia* roots. The promising biological activities to be test were anti-inflammatory, antinociceptive, antipyretic and antioxidant.

- Chapter 4: It was indicated the chemical compositions of essential oils of two Saudi plants, *J. pelargoniifolia* and *J. glauca* roots and aerial parts. This study has a significant value from taxonimical point of view. Whereas, the discernibly different chemical composition of the essential oils of the two *Jatropha* species might be useful as an aid in solving problems of scientific plant classification of *Jatropha* species.

- Chapter 5: This is representing the first report on phytochemical investigation of *Jatropha pelargoniifolia* roots led to isolate many biologically-active compounds some of them were new and isolated here for the first time from a natural source also some of them have been isolated and identified for the first time in family Euphorbiaceae. The anti-inflammatory, antinociceptive, antipyretic and antioxidant activities were evaluated for some isolated compounds which are available in good yields. This work has enhanced understanding about the phytoconstituents of the selected plant and gave evidence about the efficient use of this plant traditionally and this is referred to the presence of these active compounds in high yield.

- Chapter 6: A review of ethnomedicinal native plants used traditionally in Saudi Arabia.

- Chapter 7: Summary and outlook.

- Chapter 8: Appendices.

1.3. Family Euphorbiaceae

It is considered as one of the sixth largest plant families in the the plant kingdom. It covers about 300 genera distributed in approximately 7,800 species and five subfamilies worldwide. These genera occur in tropical and subtropical reigons [7, 8].

In Saudi Arabia, family Euphorbiaceae is represented by 15 genera (*Andrachne* L., *Flueggea* Willd, *Phyllanthus* L., *Clutia* L., *Chrozophora* Neck. ex Juss., *Ricinus* L., *Mercurialis* L., *Erythrococca* L., *Micrococca* Benth., *Acalypha* L., *Tragia* L., *Dalechampia* L., *Jatropha* L., *Croton* L. and *Euphorbia* L.) [9].

1.4. Genus *Jatropha*

The genus *Jatropha* belongs to the family Euphorbiaceae and comprises about 200 species which are distributed mainly in the tropical and subtropical regions of Americas and Africa [10]. It is represented in Saudi Arabia by four species *glauca, curcas, spinosa* and *pelargoniifolia* [11].

1.5. *Jatropha pelargoniifolia* Courb.

It is a frutescent plant about 30 cm. high. Leaves bluish-green small, petioled, 3-5 digitately lobed with serrate margin and covered with smooth silky hairs. Female flowers larger and with larger perianth than the male. Capsule pale, straw-colored, scaly, 1 cm. long with smooth black seeds. The stem and roots are excreted red latex [12].

In Saudi Arabia, *J. pelargoniifolia* Courb. grows wildly in the Southern region (Asir) and is known locally as "Obab" **(عبـــب)** [12] (Figures. 1, 2 and 3).

1.6. Taxonomic classification of *J. pelargoniifolia*

The taxonomists classify the plant as follows:

Kingdom: Plantae, family: Euphorbiaceae, genus: *Jatropha*, and species: *pelargoniifolia.*

Figure 1: *Jatropha pelargoniifolia* Courb.

Figure 2: *Jatropha pelargoniifolia* Courb. (leaves and fruits)

Figure 3: *Jatropha pelargoniifolia* Courb. red latex

1.7. Some reported biological activities of various *Jatropha* species

Jatropha species are spread worldwide and there are some remarkable similarities in their medicinal uses. This might well indicate the presence of the same or similar compounds with a particular pharmacological action. Sufficient knowledge of the biological activity of *Jatropha* extracts and constituents is now known to account for the major traditional uses of this genus.

1.7.1. Anticancer and antileukaemic activity

Although there are many kinds of compounds found in *Jatropha* plants, most attention was paid to diterpenes because of their antitumor activity. Kupchan *et al.* isolated jatrophone from *J. gossypiifolia* which exhibited antileukaemia and anti-nasopharyngeal carcinoma activity [13].

1.7.2. Anti-inflammatory activity

Anti-inflammatory activity of topical application of *J. multifida* root powder in paste form for ear inflammation was confirmed in albino mice. Its anti-inflammatory activity might be due to effects on several mediators and arachidonic acid metabolism involving cyclo-oxygenase pathway resulting in prostaglandin formation anti-proliferative activity leading to reduction in granular tissue formation and leukocyte migration from the vessels [14].

1.7.3. Anticoagulant and coagulant activities

Jatropha curcas, a medicinal plant, which is commonly grown in the tropics, is traditionally used as a haemostatic. Investigation of the coagulant activity of the latex of *J. curcas* showed that the whole latex significantly reduced the clotting time of human blood. Diluted latex, however, prolonged the clotting time, at high dilutions; the blood did not clot at all. This indicates that *J. curcas* latex possesses both procoagulant and anticoagulant activities, the former being evident at high concentrations of the latex, while the latter is exhibited at low concentrations. This designates that the *J. curcas* latex anticoagulant inhibits a factor or factors in the intrinsic pathway of blood coagulation. At this stage, there is no indication of how the procoagulant in the latex acts since it affected the prothrombin time (PT) and activated partial thromboplastin time (APTT) in a similar manner, causing instant coagulation in both cases. The

observation that the latex actually changed from being a procoagulant to an anticoagulant upon dilution raises a few possibilities. One possibility is that the two activities are exhibited by different factors, each of which functions optimally at different concentrations. It is also possible that the same factor acts as a procoagulant and as an anticoagulant under different conditions. For example, it has been shown that thrombin acts as a procoagulant when it cleaves fibrinogen and promotes the formation of a fibrin clot but functions as an anticoagulant when it activates protein C in the presence of the cofactor thrombomodulin [15].

1.7.4. Antibacterial Activity

Extracts of *J. multifida* roots effectively inhibited the growth of *Bacillus subtilis* and *Staphylococcus aureus* at a concentration of 200 mg/disk. Japodagrin and Jatrogrossidentadione two macrocylic diterpenoids possessing lathyrane and jatrophane skeletons, respectively, isolated from the root of *J. podagrica* displayed antibacterial activity against some *Gram*-positive bacteria [10].

1.7.5. Antidiarrheal activity

The MeOH extract of *J. curcas* root showed antidiarrheal activity against castor oil-induced diarrhea and intraluminal accumulation of fluid. It also reduced gastrointestinal motility after charcoal meal administration in albino mice. These results indicated that action of *J. curcas* root MeOH extract could be through a combination of inhibition of elevated prostaglandin biosynthesis and reduced propulsive movement of the small intestine [10].

1.7.6. Antiviral properties

It is reported that the aqueous extract of the branches of *J. curcas* inhibited strongly the HIV-induced cytopathic effects with low cytotoxicity [16].

1.7.7. Pregnancy-terminating effect

The fertility regulatory effect of the fruit of *J. curcas* was investigated by oral administration of different extracts to pregnant rats for varying periods of time. Fetal resorption was observed with different extracts, indicating the abortifacient properties of this fruit [10].

1.7.8. Other Activities

There are also other activities of *Jatropha* plants. Hypotensive and vasorelaxant effects of alcoholic extract from *J. gossypiifolia* L. in rats were reported in 2003 [17].

It has been found that a formulation from *J. curcas*, can eliminate malaria parasites (*Plasmodium falciparum* and *P. malarie*) from the peripheral blood of patients with malaria. Additionally, it did not show any undesired effects in the patients as well as in laboratory rats [18].

Furthermore, *Jatropha* oil was used as biodiesel preparation by lipase-catalyzed transesterification [19]. *J. curcas* seed oil is regarded as a potential alternative to diesel fuel, and vegetable oils have numerous advantages in this respect because they are safe to store, handle and of their high flash points. The fact that *jatropha* oil cannot be used for nutritional purposes without detoxification makes its use as an energy source for fuel production, very attractive [20].

1.8. Phytochemical investigation of different *Jatropha* species

Previous phytochemical investigation of *Jatropha* species revealed the presence of variety of constituents. These compounds include diterpenes, triterpenes, lignanes, coumarins, flavonoids, alkaloids, phytosterols, and some others Figure 4. Some selected structures are shown below, and their names and the corresponding plant sources are collected in the Table 1. As shown in the Table, diterpenes are the predominant constituents within the genus of *Jatropha*.

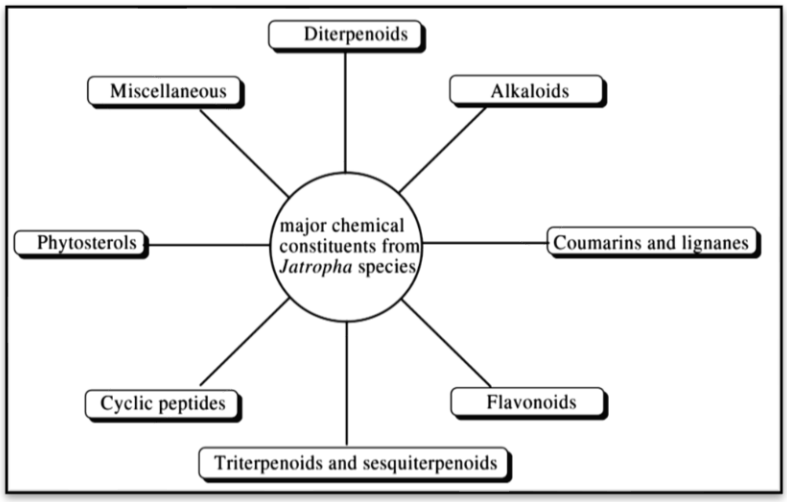
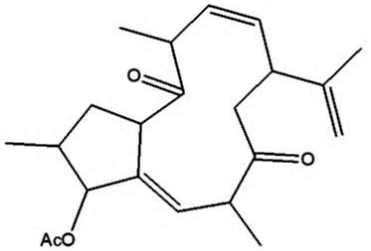
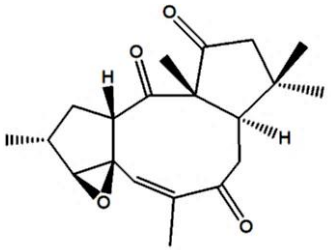


Figure 4: Major chemical constituents reported in genus *Jatroph*

Table 1: Representative examples for chemical constituents isolated from genus *Jatropha.*

<table>
<tr><th>Compound names</th><th>Class</th><th>Source</th><th>Structure</th><th>Reference</th></tr>
<tr><td>Integerrimene</td><td>Diterpene
(Rhamnofolane)</td><td>J. integerrima</td><td>  </td><td>[21]</td></tr>
<tr><td>Citlalitrione</td><td>Diterpene
(Lathyrane)</td><td>J. gossypiifolia</td><td>  </td><td>[22]</td></tr>
<tr><td>Spruceanol

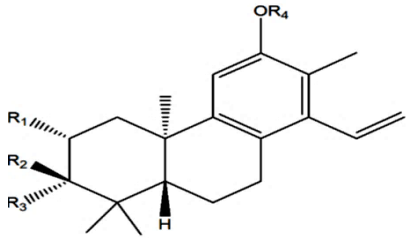
Cleistanthol</td><td>Diterpene
(Pimarane)</td><td>J. divaricata</td><td> 
<table>
<tr><th>R1</th><th>R2</th><th>R3</th><th>R4</th><th>Name</th></tr>
<tr><td>H</td><td>H</td><td>OH</td><td>H</td><td>Spruceanol</td></tr>
<tr><td>OH</td><td>H</td><td>OH</td><td>H</td><td>Cleistanthol</td></tr>
</table></td><td>[23]</td></tr>
</table>

Table 1: Cont.

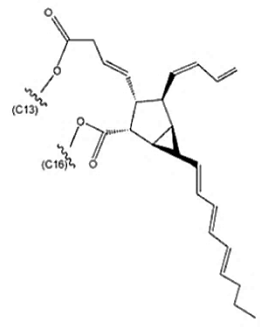
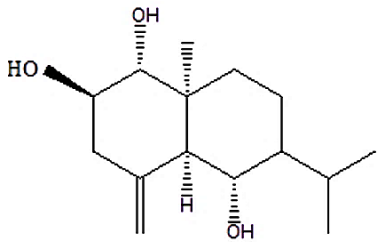
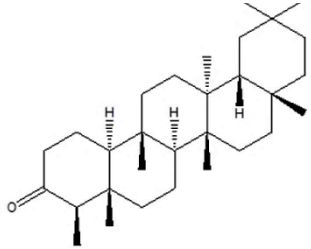
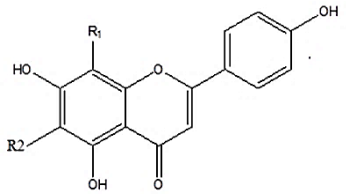
Compound name	Class	Source	Structure	Reference
Jatropha factor C2 (Phorobol ester)	Diterpene (Tigliane)	*J. curcas*		[24]
5-Epieudesmene-1,2,6-triol	Sesquiterpenoid	*J. neopauciflora*		[25]
Friedelin	Triterpene	*J. curcas*		[26]

Table 1: Cont.

<table>
<tr><th>Compound names</th><th>Class</th><th>Source</th><th>Structure</th><th>Reference</th></tr>
<tr><td>Vitexin
Isovitexin
Apigenin</td><td>Flavonoids</td><td>J. gossypiifolia</td><td> 

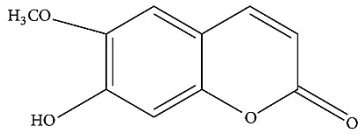
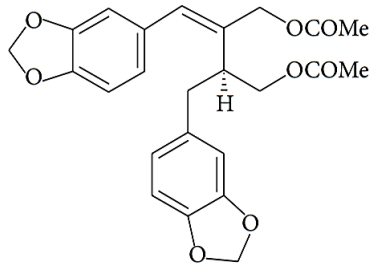
<table>
<tr><th>R1</th><th>R2</th><th>Name</th></tr>
<tr><td>β-D-Glu.</td><td>H</td><td>Vitexin</td></tr>
<tr><td>H</td><td>β-D-Glu.</td><td>Isovitexin</td></tr>
<tr><td>H</td><td>H</td><td>Apigenin</td></tr>
</table></td><td>[27]</td></tr>
<tr><td>Scopoletin</td><td>Coumarin</td><td>J. gossypiifolia</td><td> 
</td><td>[10]</td></tr>
<tr><td>Gossypiline</td><td>Lignane</td><td>J. gossypiifolia</td><td> 
</td><td>[10]</td></tr>
</table>

Table 1: Cont.

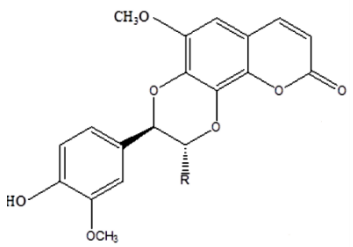
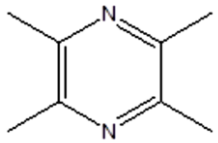
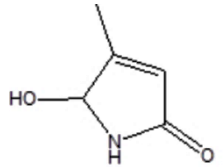
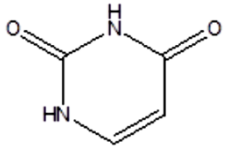
Compound names	Class	Source	Structure	Reference
Propacin Cleomiscosin A	Coumarino-lignan	*J. gossypiifolia*	 **R** / **Name**: CH_3 — Propacin; CH_2OH — Cleomiscosin A	[10]
Tetramethylpyrazine	Alkaloid	*J. podagrica*		[10]
Jatropham	Alkaloid	*J. macrorhiza*		[10]
Pyrimidine-2,4-dione	Alkaloid	*J. curcas*		[28]

Table 1: Cont.

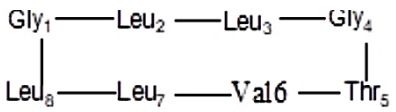
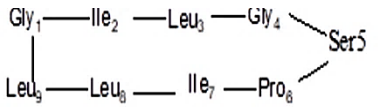
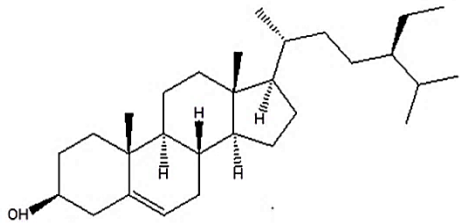
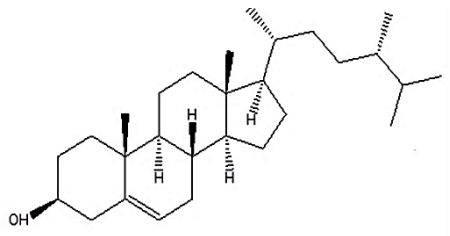
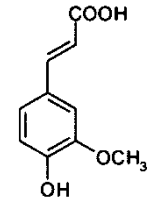
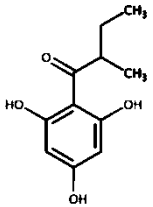
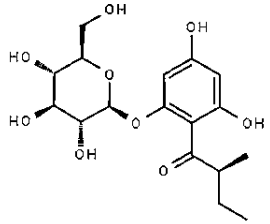
Compound names	Class	Source	Structure	Reference
Curacycline A	Cyclic peptide	*J. curcas*		[29]
Curacycline B	Cyclic peptide	*J. curcas*		[29]
β-Sitosterol	Phytosterol	*J. maheshwarii*		[10]
Campesterol	Phytosterol	*J. unicostata*		[30]

Table 1: Cont.

Compound names	Class	Source	Structure	Reference
Ferulic acid	Miscellaneous	*J. gossypiifolia*		[10]
Multifidol	Miscellaneous	*J. multifida*		[10]
Multifidol glucoside	Miscellaneous	*J. multifida*		[10]

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Chapter 2

Comparative Study of the Biological Activities of *Jatropha pelargoniifolia* and *Jatropha glauca* Native to Saudi Arabia

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Hanan Yahya Aati (H.A), Ali A. El-Gamal (A.A.G) and Oliver Kayser (O.K) conceived and designed the experiments; H.A prepared different tested extracts; H.A., A.A.G analyzed the data; H.A wrote the paper; A.E.G helped to draft the manuscript; O.K participated in the experiment design and supervised.

Submitted to Phytomedicine

2.1. Abstract

***Background*:** Traditionally, in Saudi Arabia as well as Ethiopia, *Jatropha glauca*, are mashed in water and the resultant liquid is ingested to treat constipation and used as ear drops to treat ear pain. Furthermore, the sap is used as an astringent to treat chronic skin diseases. The sap from the petiole of *Jatropha pelargoniifolia* has been used to cure ulcers and wounds.

Objective: The present study aimed to evaluate the biological activities of the total alcoholic extracts obtained from the roots and aerial parts of *J. pelargoniifolia* and *J. glauca*, which belong to the Euphorbiaceae family and are native to Saudi Arabia.

Materials and methods: *In vivo* and *in vitro* studies were carried out to evaluate the potential hepatoprotective, antinociceptive, anti-inflammatory, and anti-diabetic/hypoglycemic effects of the extracts in comparison to those of standard clinical drugs. Their hepatoprotective effect was assessed by examining the changes in levels of serum biochemical markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), and total bilirubin. The presence of tissue markers such as malonaldehyde (MDA), non-protein sulfhydryl groups (NP-SH), and total protein (TP) content was evaluated. Antinociceptive activity was explored by the hot plate and writhing methods and the anti-inflammatory effect was evaluated by carrageenan-induced paw edema. Finally, hypoglycemic and anti-diabetic activities of the extracts from both *Jatropha* plants were evaluated in alloxan-induced diabetic mice.

Results: The roots of *J. glauca* showed higher hepatoprotective and antinociceptive (63.85%) activities in comparison to indomethacin (69.87%). In addition, the root extracts from *J. pelargoniifolia* exhibited greater anti-inflammatory (50.63%) and hypoglycemic (37.98%) activities when compared with phenylbutazone (64.63%) and glibenclamide (51.57%), respectively. Finally, extracts from the aerial parts of *J. glauca* showed significantly higher anti-diabetic activity in mice with alloxan-induced diabetes (39.93%) when compared with glibenclamide (47.13%).

Conclusion: Ethanolic extracts from *J. glauca* and *J. pelargoniifolia* (root and aerial parts) induced analgesic and anti-inflammatory effects; these results justify the use of these plants in folk medicine as analgesics and for the treatment of inflammatory conditions. In addition, the extracts also showed significant hepatoprotective and hypoglycemic/anti-diabetic activities.

2.2. Introduction

Medicinal plants have been used by various cultures globally for many generations; they are still used as a primary form of treatment in many parts of the world [1]. However, among the 220,000 known plant species, only a small percentage are used as dietary supplements or herbal medicinal products by people and for animals [2]. Many other plants might also have the potential to be employed as medicinal plants. About 40% of the drugs used today in modern society are derived from natural products, which is why major pharmaceutical industries are interested in drug discovery from plant sources. In low-income countries, about 80% of the population relies on folk medicine for their primary health care needs, and about 85% of these traditional medicines include the use of plant extracts [3, 4].

Jatropha species belonging to the Euphorbiaceae family are used in folk medicine to cure several illnesses in Africa, Asia, and Latin America [5, 6]. Important medicinal species, including *J. gossypifolia, J. curcas, J. glandulifera, J. grossidentata, J. macrorhiza, J. podagrica, J. multifida, and J. ciliata,* have sparked interest in recent years because they exhibit significant pharmacological activities, including cytotoxic, anti-inflammatory, molluscicidal, insecticidal, fungicidal, antioxidant, antiplatelet-aggregative, and diuretic activities [7].

The only medicinal species growing in Saudi Arabia are *J. glauca, J. curcas, J. spinose* and *J. pelargoniifolia,* which are known locally as Obeeb, Shareb, Atheb, and Obab, respectively. They are used as traditional herbal medicines due to their anti-inflammatory, antioxidant, and antinociceptive activities [8].

Traditionally, in Saudi Arabia as well as Ethiopia, all parts of the *J. glauca* plant, including the roots, are mashed in water and the resultant liquid is ingested to treat constipation, and used as ear drops to treat ear pain. Furthermore, the sap is used as an astringent to treat chronic skin diseases [9]. The sap from the petiole of *J. pelargoniifolia* has been used to cure ulcers and wounds in Ethiopia and Saudi Arabia [10].

According to Al Zanbagi *et al*. (2000), only alcoholic and chloroform extracts of *J. glauca* leaves exhibited molluscicidal activity. However, to our knowledge, *J. glauca* and *J. pelargoniifolia* species have not been studied [11]. The main purpose of

our study was to explore the biological activities of both these species in detail in order to gain a broader perspective regarding their ethnomedicinal use. Further, we aimed to prove the ethnopharmacological claims regarding these species by rational studies. For these purposes, ethanolic extracts of the root and aerial parts were obtained and tested for their ability to counter pain, inflammation, hepatic problems, and diabetes.

2.3. Materials and methods

2.3.1. Chemicals

Analytical grade solvents (Sigma-Aldrich, St Louis, MO, USA) were used for the extraction process. Silymarin was procured from Sigma Aldrich Chemical Company, St Louis, MO, USA. Carbon tetrachloride (CCl_4) was purchased from E. Merck, Germany. The kits for *in vitro* enzymatic diagnosis were obtained from Roche Diagnostics Corporation, UK. Alloxan was purchased from Sigma (USA), while Daonil® (glibenclamide), indomethacin, and phenylbutazone were purchased from Spimaco (Saudi Arabia).

2.3.2. Plant material

J. glauca and *J. pelargoniifolia* were collected in September 2015 from Wadi Shogare and Wadi Mojasas, respectively, in Jazan City, located in the southern region of the Kingdom of Saudi Arabia. The identity of the plants was verified by Dr. Jacob Thomas, a taxonomist from the Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia. Voucher specimens (#23063 and 23064, respectively) for *J. glauca* and *J. pelargoniifolia* were kept at the herbarium of the College of Science, King Saud University.

2.3.3. Preparation of organic extracts

J. glauca and *J. pelargoniifolia* plants (500 g each) were collected locally and shade-dried; their roots and aerial parts were separated from each other and ground. Powdered roots and aerial parts were subjected to ethanol extraction by cold maceration and treatment with 80% EtOH (3 × 700 mL each). The ethanolic extracts were concentrated using a rotary evaporator at 40°C, to obtain a dark green residue from the aerial parts (75 g and 69 g for *J. glauca* and *J. pelargoniifolia*, respectively). The root parts yielded

a dark brown residue (36 g and 45 g of residue were obtained for *J. glauca* and *J. pelargoniifolia*, respectively).

2.3.4. Animals

Male Wistar rats and white male Swiss albino mice with approximate body weights of 200 g and 20–25 g, respectively, were divided into groups of six. The animals were obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University. After a 7-day period in animal accommodation, they were divided into groups and maintained at 12 h:12 h light-dark conditions at 55% humidity. Purina chow rat diet (UAR-Panlab, Barcelona, Spain) and drinking water were supplied to the animal *ad libitum*. The protocols for the present study were based on the recommendations of the Ethical Committee of King Saud University's Experimental Animal Care Center (approval number CPR-7569).

2.3.5. Acute toxicity assay

The acute toxicity of the alcoholic extracts was examined in rats by adapting the procedures of the Organization for Economic Co-operation and Development (OECD) (guideline No. 423) [12]. The animals fasted overnight, after which they were administered *J. glauca* and *J. pelargoniifolia* (from root and aerial parts) extracts suspended in 3% gum acacia in distilled water at fixed oral doses of 50–2000 mg/kg. The animals were then assessed every 30 min for 3 h to understand their general behavioral, neurological, and autonomic profiles, and every 24 h for a 2-week period to determine survival. The extracts did not cause any significant behavioral changes and no mortality was observed.

2.3.6. Study of Biological activities

2.3.6.1. Anti-inflammatory activity

2.3.6.1.1. Carrageenan-induced paw edema in rats

Male Wistar rats weighing around 200 g each were divided into 10 groups (six rats in each group). The first group (Group I) received only carrageenan as a control. Groups II to IX also received total alcoholic extracts from *J. glauca* and *J. pelargoniifolia* (from root and aerial parts) at concentrations of 200 and 400 mg/kg, respectively. Group X

was administered phenylbutazone (100 mg/kg) as a positive control. Rat paw-edema was induced using carrageenan to obtain a model for acute inflammation. A dose of 0.2 mL of 1% carrageenan was injected into the paw of each rat in the 10 groups. The animals were administered suspensions of different extracts an hour before carrageenan was injected via the intraperitoneal route.

The volume of rat paws was measured before receiving carrageenan injection and then 3 h after the injection using a Hydro-Plethysmograph (Model 7150, Basile, USA). The rats were held firmly, and the right paw was immersed in a pool of mercury up to the tibiotarsal joints. The increase in pressure resulting from the slight rise in the mercury level was transmitted to the pressure transducer. After amplification, the level of transduced signals registered as a deflection in the pen on the chart. These results are represented as the percentage (%) inhibition of edema (protection against inflammation) in the drug-treated groups 3 h after carrageenan injection as compared to the % inhibition observed in the control group [13]. The % inhibition was calculated using the following equation:

$$I = 1 – (dt/dc) \times 100\ \%$$

Here, “dt” is the change in paw volumes recorded for drug-treated groups, “dc” is the change in paw volumes recorded for reference groups, and “I” denotes inhibition.

2.3.6.2. Antinociceptive (analgesic) activity

Pain is frequently associated with inflammation; it is the final product of a complex information-processing network involving the central and peripheral neuronal pathways. Drugs with an anti-inflammatory effect very often possess analgesic properties. The analgesic activities of total alcoholic extracts from the root and aerial parts of *J. glauca* and *J. pelargoniifolia* were evaluated against the acetic acid-induced writhing response, which is a standard method for measuring peripheral analgesic activity. The central analgesic effect was measured using the hot plate method.

2.3.6.2.1. Acetic acid-induced writhing response (chemical stimuli)

The test was carried out using a method adapted from the procedure put forward by Amresha *et al*. (2007) [14]. Mice were divided into 10 groups of six animals each. Group I was intraperitoneally injected solely with 0.6% acetic acid solution. Group II to IX orally received 200 and 400 mg/kg doses each of extracts from the root and aerial parts of *J. glauca and J. pelargoniifolia*. Group X received indomethacin [4 mg/kg, per os (p.o.)] as a positive control after an overnight fast. One hour after treatment, the mice from groups II to X were injected intraperitoneally with a 0.6% acetic acid solution to induce the characteristic writhing response. The number of writhings observed in the 5 to 20 min period following acetic acid administration was counted, recorded, and compared to the number recorded for the indomethacin group.

2.3.6.2.2. Hot plate test method (thermal stimuli)

The test was performed using a slightly modified version of the protocol described previously by Asongalem *et al.* (2004) [15]. The mice were placed in a 24-cm diameter glass cylinder on the heated surface of a hot plate at a constant temperature of 56 ± 1°C. Animals were divided into nine groups of six animals each. Group I to VIII orally received 200 and 400 mg/kg doses each of extracts from *J. glauca* and *J. pelargoniifolia* (root and aerial parts), respectively. Group IX was treated with indomethacin (4 mg/kg, p.o.) as a positive control. Mice were chosen 24 h before the experiment based on their reactivity to the test. The animals were tested at 0, 60, 90, and 120 min after treatments were administered. The time interval from the moment that the animal reached the hot plate until it licked its forefeet or jumped off was considered as the reaction time. The stop time of 20 seconds was set to avoid heat damage to the paws of animals.

2.3.6.3. Hypoglycemic and anti-diabetic activities

Male Swiss albino mice with body weights ranging between 20–25 g were divided into 10 groups (each group consisted of four mice). Extracts from *J. glauca* and *J. Pelargoniifolia* (root and aerial parts) at doses of 200 and 400 mg/kg of body weight, respectively, were administered to each animal. Tween 80 solvent (1 mL) was added to each extract. The experiment was carried out using the procedure described by El Tahir (2007) [16]. The first group (control) was given a normal saline solution with tween 80; the second group was administered a reference oral anti-diabetic drug, Daonil®

(glibenclamide) at a dose of 1 mg/kg of body weight, while the remaining animal groups were given plant extracts. The extracts and reference drugs were orally administered to animals after 24 h of fasting. Blood samples were taken before administering the drugs, at time 0 and 2 h after administration. Blood glucose levels were measured using the Reflotron® instrument (ROCH).

For anti-diabetic screening, alloxan (125 mg/kg) was intraperitoneally injected for the induction of diabetes in the overnight fasted animals; 72 h after administering the injection, the animals became diabetic and the experiment was performed using the protocol described for the hypoglycemia-testing experiment. The significance of the differences between the treatments and control values were determined using ANOVA.

2.3.6.4. Hepatoprotective activity

Male Wistar rats were divided into 11 groups of six animals each. Group I received normal saline solution and was used as the control group. Group II received only CCl_4 (1.25 mL/kg of body weight) treatment, while group III was administered silymarin (Sil) at a dose of 10 mg/kg p.o. (20.7 μmol/kg). Groups IV–XI were treated with 200 and 400 mg/kg of the total alcoholic extracts of both plants. Treatment started 6 days prior to CCl_4 administration and continued until day 7. On day 7, 24 h after CCl_4 administration, ether was used as a general anesthetic to immobilize the animals. The heart puncture method was used to collect blood samples, after which the serum was separated from plasma for the assessment of biochemical parameters.

2.3.6.4.1. Determination of biochemical parameters

The levels of biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), and total bilirubin were determined according to the method described by Edwards and Bouchier (1991) [17]. The enzymatic activities were evaluated using Reflotron® (ROCH) analytical strips and visualized on a Reflotron® Plus instrument (ROCH).

2.3.6.4.2. Determination of malonaldehyde and non-protein sulfhydryl groups, and total protein content

A beaker containing isolated liver samples was cooled by immersion in an ice bath. The tissues were homogenized in 0.02 M ethylenediaminetetraacetic acid (EDTA) in a Potter–Elvehjem type C homogenizer. Homogenate equivalents of 100 mg of tissues were used for the measurements.

For quantifying the level of MDA, aliquots of the homogenate (1 mL) were incubated at 37°C for 3 h in a metabolic shaker. One milliliter of 10% aqueous trichloroacetic acid (TCA) was added. The mixture thus obtained was centrifuged at 800 rpm for 10 min. One milliliter of the supernatant was separated and mixed with 1 mL of 0.67% w-thiobarbituric acid in water and placed in a water bath containing hot water for 10 min. The mixture was cooled and diluted with 1 mL of distilled water. The absorbance of the solution was measured at 535 nm. The MDA content (nmol/g wet tissue) (index of the magnitude of lipid peroxidation) was then calculated with reference to a standard curve for MDA solution [18].

Non-protein sulfhydryl groups (NP-SH) were quantified by mixing the homogenate with 4 mL of distilled water and 1 mL of 50% TCA in 15 mL test tubes. The tubes were shaken intermittently for 10–15 min and then centrifuged for 15 min at approximately 3000 rpm to precipitate the protein. Two milliliters of the supernatant were mixed with 4 mL of 0.4 M Tris buffer (pH 8.9) and 0.1 mL of 0.01 M DTNB [5,50-dithio-bis-(2-nitrobenzoic acid)]; the tube was shaken to mix the contents. Absorbance was measured spectrophotometrically within 5 min after the addition of DTNB at 412 nm against a reagent blank with no homogenate [19].

For TP evaluation, parts of the homogenate (1mL) were treated with 0.7 mL of Lowry's solution, mixed, and incubated for 20 min in dark at room temperature. Diluted Folin's reagent (1 mL) was then added, and samples were incubated at room temperature in dark for 30 min. The absorbances of the resultant solutions were then measured at 750 nm [20].

2.4. Statistical analysis

Data are expressed as mean ± standard error (SE). The results were first checked for normality by Kolmogorov and Smirnov test and homogeneity by Bartlett's test, and then analyzed using the Student's t test and analysis of variance (ANOVA) test. Dunnett posttest was used to determine which groups significantly differed from the control group. The statistical analysis was performed using GraphPad Prism version 3. Results were considered significantly different if the $p < 0.05$ [21].

2.5. Results

The total alcoholic extracts from the root and aerial parts of *J. glauca* and *J. pelargoniifolia* were tested for anti-inflammatory activities; the corresponding results are presented in Table 1. Furthermore, all extracts were evaluated for analgesic activity using chemical (writhing method) and thermal stimuli (hot plate method). All results are presented in Tables 2 and 3. The results obtained after the assessment of hypoglycemic and anti-diabetic activities are summarized in Table 4. Finally, the hepatoprotective activity of the different alcoholic extracts was tested against CCl_4-induced toxicity. All results are presented in Tables 5 and 6.

The effect of crude alcoholic extracts from the roots and aerial parts of *J. glauca* and *J. pelargoniifolia* on the carrageenan-induced acute inflammation model was evaluated at concentrations of 200 mg/kg and 400 mg/kg. The tested extracts reduced edema in a dose and time-dependent manner (Table 1). In carrageenan-administered animals (Group I), severe swelling was observed within 3 h (paw volume was 1.617 ± 0.030 mL), and the swelling reduced to 0.655 ± 0.015 mL in the next 180 min. The groups treated with extracts exhibited decreased paw edema and showed improvement throughout the period of study. Swelling was significantly reduced during 3 h in *J. pelargoniifolia* root extract-treated rats and was decreased by 50.63% and 39.94% at doses of 400 mg/kg and 200 mg/kg, respectively, as compared to the 64.63% reduction observed in phenylbutazone-treated rats. *J. glauca* root extracts also caused a reduction in edema at a dose of 400 mg/kg (44.52%). Extracts from the aerial parts of *J. glauca* and *J. pelargoniifolia* showed better anti-inflammatory activities at the higher dose of 400 mg/kg, while at the lower dose of 200 mg/kg, they showed moderate to low activity

(0.522 ± 0.022 mL and 0.547 ± 0.037 mL, respectively) as compared to the activity in the control group (0.232 ± 0.018 mL).

Table 1. Effects of *Jatropha pelargoniifolia* (J.P.) and *J. glauca* (J.G.) extracts (root and aerial parts) on carrageenan-induced hind paw edema in rats

Group (n=6)	**Dose (mg/kg)**	**Paw volume before carrageenan treatment (mL) (Mean ± SE)**	**Paw volume 3 h after treatment (mL) (Mean ± SE)**	**Increase observed 3 h after treatment (mL) (Mean ± SE)**	**% Inhibition**
Carrageenan	0.2 mL	0.962±0.028	1.617±0.030$^{\bullet\bullet\bullet}$	0.655±0.015	—
J.P. aerial part	200	0.908±0.038	1.455±0.025$^{\bullet\bullet\bullet}$	0.547±0.037*	16.53
J.P. aerial part	400	0.953±0.029	1.362±0.022$^{\bullet\bullet\bullet}$	0.408±0.037**	37.65
J.P. root	200	0.930±0.027	1.323±0.024$^{\bullet\bullet\bullet}$	0.393±0.034**	39.94
J.P. root	400	0.947±0.033	1.270±0.012$^{\bullet\bullet\bullet}$	0.323±0.032**	50.63
J.G. aerial part	200	0.943±0.033	1.467±0.020$^{\bullet\bullet\bullet}$	0.522±0.022**	20.35
J.G. aerial part	400	0.915±0.031	1.323±0.018$^{\bullet\bullet\bullet}$	0.408±0.023**	37.65
J.G. root	200	0.978±0.026	1.498±0.016$^{\bullet\bullet\bullet}$	0.520±0.015**	20.61
J.G. root	400	0.958±0.031	1.322±0.028$^{\bullet\bullet\bullet}$	0.363±0.015**	44.52
Phenylbutazone	100	0.943±0.032	1.175±0.019$^{\bullet\bullet\bullet}$	0.232±0.018$^{\#\#\#}$	64.63

All values represent mean ± SE. *$p < 0.05$; **$p < 0.01$ *vesus* carrageenan group (one-way ANOVA, followed by Dunnett's test), $^{\#\#\#}p < 0.001$ *versus* carrageenan group (unpaired *t*-test), $^{\bullet\bullet\bullet}p < 0.001$ *versus* the corresponding pre-carrageenan treatment group (paired t-test).

Table 2 shows the analgesic effect of indomethacin and the extracts tested by using the writhing method in mice. The average number of writhings over a period of 20 min after acetic acid was injected in the control group mice was 27.667 ± 0.955. The standard drug indomethacin (at a dose of 4 mg/kg) resulted in a 69.87% reduction in the number of writhings (8.333 ± 0.558). The total alcoholic extracts (200 and 400 mg/kg) from the *J. glauca* root resulted in a significant reduction in the number of writhings in mice (51.20% and 63.85%, respectively) as compared to that caused by the analgesic drug. Meanwhile, the total alcoholic extracts (400 mg/kg) from the *J. pelargoniifolia* (root) and *J. glauca* (aerial parts) resulted in a moderate reduction in the writhing response, i.e. 14.166 ± 0.477 (48.76%) and 17.000±0.577 (38.55%), respectively, as opposed to the reduction in writhings in control animals. The aerial parts of *J. pelargoniifolia* showed the least effect on writhing in mice at 200 mg/kg. However, all results revealed that significant analgesic effects ($p < 0.01$) resulted from the administration of all the treated doses.

Table 2. Determination of the analgesic effects of *Jatropha pelargoniifolia* (J.P.) and *J. glauca* (J.G.) extracts (root and aerial parts) using the acetic acid-induced writhing method in mice

Group (n=6)	Dose mg/kg	Number of writhings in 20 min	% Inhibition
Acetic acid	0.2 mL	27.667±0.955	—
J.P. aerial part	200	25.833±0.946	6.62
J.P. aerial part	400	22.500±0.428**	18.67
J.P. root	200	18.333±0.422**	33.73
J.P. root	400	14.166±0.477**	48.79
J.G. aerial part	200	20.500±1.310**	25.90
J.G. aerial part	400	17.000±0.577**	38.55
J.G. root	200	13.500±0.428**	51.20
J.G. root	400	10.000±0.577**	63.85
Indomethacin	4	8.333±0.558###	69.87

All values represent mean ± SE.; **$p < 0.01$ *versus* acetic acid group (one-way ANOVA, followed by Dunnett's test), ###$p < 0.001$ *versus* acetic acid group (unpaired t-test).

In addition, indomethacin showed 116.66% analgesia after 120 min of treatment, the extracts from the root and aerial parts of *J. glauca* resulted in the highest percentage inhibition of pain (73.77% and 72.13%, respectively) as compared to inhibition with the control drug. Furthermore, *J. pelargoniifolia* root extracts showed moderate activity (50.79%), and the activity was lower in extracts obtained from the aerial parts (42.42%), as documented in Table 3.

Table 3. Determination of the analgesic effects of *Jatropha pelargoniifolia* (J.P.) and *J. glauca* (J.G.) (root and aerial parts) extracts using the hot plate latency method in mice

Group (n=6)	Dose mg/kg	Before treatment	After treatment					
		0 min	60 min		90 min		120 min	
		Mean ± SEM	*Mean ± SE*	*% Change*	*Mean ± SE*	*% Change*	*Mean ± SE*	*% Change*
J.P. aerial part	200	10.000±0.258	11.333±0.333	13.33	12.666±0.494**	26.66	13.667±0.422**	36.66
J.P. aerial part	400	11.000±0.365	12.167±0.307	10.60	14.667±0.333**	33.33	15.667±0.422**	42.42
J.P. root	200	10.833±0.307	11.167±0.307	3.07	12.167±0.307**	12.30	12.500±0.428**	15.38
J.P. root	400	10.500±0.342	12.833±0.477**	22.22	14.833±0.307**	41.26	15.833±0.477**	50.79
J.G. aerial part	200	11.167±0.307	13.000±0.365**	16.41	13.000±0.365**	16.41	13.333±0.333**	19.40
J.G. aerial part	400	10.167±0.307	13.833±0.478**	36.06	15.833±0.477**	55.73	17.500±0.224**	72.13
J.G. root	200	9.833±0.307	11.167±0.307	13.55	12.833±0.401**	30.50	13.500±0.563**	37.28
J.G. root	400	10.167±0.307	14.000±0.447**	37.70	17.000±0.258**	67.21	17.667±0.422**	73.77
Indomethacin	4	10.000±0.258	15.833±0.601**	58.33	19.667±0.615**	96.66	21.667±0.422**	116.66

All values represent mean ± SE. **$p < 0.01$ *versus* the corresponding group before treatment (Repeted measures ANOVA, followed by Dunnett's test).

Furthermore, the total alcoholic extracts from *J. glauca* and *J. pelargoniifolia* (root and aerial parts) were examined for hypoglycemic and anti-diabetic activity. The blood glucose level of both normal and diabetic mice were significantly decreased ($p < 0.001$) after administration with the extracts a dose of 400 mg/kg of body weight, when compared to glibenclamide. Changes in blood glucose levels of diabetic mice after treatment with extracts at a dose of 400 mg/kg of body weight are illustrated in Table 4. The blood glucose levels of the animals treated with extracts from *J. glauca* aerial parts showed a significant decrease of 39.93%. However, the blood glucose level was lower after treatment with *J. pelargoniifolia* (26.68%), and the least percentage reduction in the glucose levels were achieved by extracts of *J. glauca* root (13.77%) and *J. pelargoniifolia* aerial parts (9.53%).

Table 4. Effects of *Jatropha pelargoniifolia* (J.P.) and *J. glauca* (J.G.) extracts (root and aerial parts) on the glucose levels in normal and diabetic mice

Groups (n=6)	**Dose mg/kg (p.o)**	**Glucose levels in normal mice (mg/dL)**			**Glucose levels in diabetic mice (mg/dL)**		
		Before treatments	**After treatments (2 h)**	**% decrease**	**Before treatments**	**After treatments (2 h)**	**% decrease**
Normal	–	102.517±3.169	102.567±5.543	–	–	–	–
Alloxanized	125 (i.p)	–	–	–	-	309.667±3.084	-
Glibenclamide	1	110.167±2.535	53.350±1.881***, [a]	51.57	299.167±6.306	158.167±3.497***, [b]	47.13
J.P. aerial part	200	101.850±3.187	90.567±3.312	11.07	299.833±8.499	285.500±4.617	4.78
J.P. aerial part	400	100.533±3.706	88.300±3.215	12.16	304.000±5.744	275.000±4.008	9.53
J.P. root	200	113.000±2.646	79.750±1.546***, [a]	29.42	309.167±8.056	279.167±7.245	9.70
J.P. root	400	110.667±2.290	68.633±1.351***, [a]	37.98	303.833±5.474	216.667±8.762***, [b]	26.68
J.G. aerial part	200	104.850±3.352	82.133±2.702	21.66	297.833±5.375	255.000±5.079	14.38
J.G. aerial part	400	109.750±2.988	71.267±2.919***, [a]	35.06	315.50±6.212	189.500±5.078***, [b]	39.93
J.G. root	200	112.000±2.898	88.117±2.866	21.32	309.000±5.398	283.000±5.132	8.41
J.G. root	400	114.833±3.081	81.617±2.777	28.92	304.833±4.799	262.833±3.962***, [b]	13.77

All values represent mean ± SE. ***$p < 0.001$ (paired t-test), i.p= intraperitoneal.[a] *versus* normal group before treatment, [b] *versus* alloxanized group.

Treatment with *J. glauca* and *J. pelargoniifolia* (root and aerial part) total extracts showed a dose-dependent reduction in the levels of all the measured markers. AST, ALT, ALP, GGT, and bilirubin levels decreased significantly ($p < 0.01$) to 31.77%, 37.22%, 13.21%, 31.38%, and 30.13% in animals treated with *J. glauca* root extracts at a dose of 200 mg/kg; while the corresponding levels in animals treated with a dose of 400 mg/kg were 38.24%, 55.60%, 18.59%, 45.48%, and 47.15%, respectively, indicating that *J. glauca* root extract was more protective against CCl4-induced liver damage as compared to silymarin. Moreover, treatment with alcoholic extracts obtained from the aerial parts of *J. glauca* at doses of 200 and 400 mg/kg resulted in a lesser improvement in these parameters. However, all results were highly significant ($p < 0.01$), except for the reduction in ALT (7.34%) ($p < 0.05$) at a dose of 200 mg/kg. On the other hand, animals treated with *J. pelargoniifolia* extracts (from root and aerial parts) showed the least improvement as compared to animals treated with *J. glauca* (root and aerial parts), especially at a lower dose of 200 mg/kg. However, the results were highly significant, $p < 0.05$ and 0.01 (Table 5).

Table 5. Effects of *Jatropha pelargoniifolia* (J.P.) and *J. glauca* (J.G.) extracts (root and aerial parts) on serum biochemical parameter

Treatments (n=6)	**Dose mg/kg**	**AST (U/L)**		**ALT (U/L)**		**ALP (U/L)**		**GGT (U/L)**		**Bilirubin (mg/dL)**	
		Mean ± SE	***% decrease***	***Mean ± SE***	***% decrease***	***Mean ± SE***	***% decrease***	***Mean ± SE***	***% decrease***	***Mean ± SE***	***% decrease***
Normal	–	100.383±4.857	–	26.983±1.732	–	319.500±7.584	–	5.783±0.206	–	0.550±0.011	–
CCl_4	1.25mL/kg	320.333±7.706$^{###, a}$	–	292.833±5.991$^{###, a}$	–	587.833±7.157$^{###, a}$	–	19.017±0.968$^{###, a}$	–	3.078±0.059$^{###, a}$	–
Silymarin + CCl_4	10	178.500±6.536$^{###, b}$	44.27	86.100±7.039$^{###, b}$	70.59	377.167±11.794$^{###, b}$	35.83	8.000±0.392$^{###, b}$	57.93	1.165±0.063$^{###, b}$	62.15
J.P. aerial part+ CCl_4	200	284.666±5.731$^{**, b}$	11.13	263.500±5.390$^{**, b}$	10.01	557.000±5.882$^{*, b}$	5.24	17.033±0.444$^{, b}$	10.42	2.953±0.055$^{, b}$	4.06
J.P. aerial part+ CCl_4	400	255.000±3.715$^{**, b}$	20.39	224.500±4.288$^{**, b}$	23.33	527.333±7.297$^{*, b}$	10.29	13.617±0.224$^{**, b}$	28.39	2.483±0.064$^{**, b}$	19.32
J.P. root+ CCl_4	200	308.333±4.787$^{, b}$	3.74	276.833±4.700$^{, b}$	5.46	576.833±7.855$^{, b}$	1.87	16.067±0.682$^{*, b}$	15.51	2.863±0.058$^{*, b}$	6.98
J.P. root+ CCl_4	400	284.333±5.289$^{**, b}$	11.23	281.166±3.894$^{, b}$	3.98	568.167±4.415$^{, b}$	3.34	13.917±0.348$^{**, b}$	26.81	2.647±0.066$^{**, b}$	14.02
J.G. aerial part+ CCl_4	200	272.500±6.260$^{**, b}$	14.93	271.333±6.960$^{*, b}$	7.34	544.833±5.474$^{**, b}$	7.31	14.867±0.351$^{**, b}$	21.82	2.883±0.071$^{, b}$	6.33
J.G. aerial part+ CCl_4	400	253.833±4.969$^{**, b}$	20.76	248.833±5.243$^{**, b}$	15.00	536.333±7.873$^{**, b}$	8.76	13.600±0.440$^{**, b}$	28.48	2.290±0.039$^{**, b}$	25.60
J.G. root+ CCl_4	200	218.500±6.244$^{**, b}$	31.77	183.833±5.576$^{**, b}$	37.22	510.167±6.625$^{**, b}$	13.21	12.967±0.463$^{**, b}$	31.38	2.151±0.044$^{**, b}$	30.13
J.G. root+ CCl_4	400	197.833±3.449$^{**, b}$	38.24	130.000±3.120$^{**, b}$	55.60	478.050±6.054$^{**, b}$	18.59	10.367±0.233$^{**, b}$	45.48	1.627±0.059$^{**, b}$	47.15

All values represent mean ± SE. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ (one-way ANOVA, followed by Dunnett's test), $^{###}p < 0.001$ (unpaired t-test), ^{a} *versus* normal control group, ^{b} *versus* CCl_4 group.

Moreover, the normal control group showed MDA levels of 0.465 ± 0.018 nmol/g in their healthy liver tissues. The levels of MDA increased to 5.270 ± 0.220 nmol/g in liver tissues following CCl_4 treatment. The standard drug silymarin was effective in reducing these elevated levels to 1.262 ± 0.038 nmol/g. The total extracts induced a dose-dependent reduction in the MDA levels. Treatment of animals with extracts from *J. glauca* root, *J. pelargoniifolia* root, *J. pelargoniifolia* aerial parts, and *J. glauca* aerial parts at a dose of 400 mg/kg of body weight decreased the level of MDA to 1.970 ± 0.058, 2.203 ± 0.069, 3.273 ± 0.095, and 4.342 ± 0.136 nmol/g, respectively (Table 6). All treatments with extracts at a dose of 200 mg/kg resulted in a significant ($p < 0.05$, 0.01) reduction in MDA levels in the liver tissues.

In addition, treatment of animals with CCl_4 reduced the hepatic NP-SH level from 8.083 ± 0.198 to 4.028 ± 0.214 nmol/g of wet weight tissue (Table 6). Pretreatment of animals with silymarin at a dose of 10 mg/kg significantly suppressed the reduction in NP-SH content by increasing it to 7.492 ± 0.353 nmol/g ($p < 0.001$) of wet weight in hepatic tissues. Animals that received the total extracts derived from the *J. pelargoniifolia* root showed a significant recovery of the NP-SH content ($p < 0.001$) with doses of 200 and 400 mg/kg as well. However, the NP-SH level after administration of 400 mg/kg of extracts was 7.892 ± 0.263 nmol/g, which was better than the levels observed with silymarin. Pretreatment with total alcoholic extracts from aerial parts of *J. glauca* at doses of 200 and 400 mg/kg resulted in a significant improvement in NP-SH levels to 5.407 ± 0.164 and 6.431 ± 0.163 nmol/g wet weight tissues, respectively ($p < 0.01$). Treatments with extracts from the *J. glauca* root and aerial parts of *J. pelargoniifolia* (200 and 400 mg/kg) were less effective, but the results were statistically significant ($p < 0.01$) for the results obtained after *J. glauca* root extracts administration.

It is well-known that carbon tetrachloride (CCl_4)-induced acute liver injury model shares a similar molecular mechanism with acute chemical liver injury in humans [22]. Especially, oxidative stress, inflammation response, and apoptosis have been recognized as the most important pathomechanisms during the progress of CCl_4-induced acute liver injury. Within the liver, CCl_4 as a hepatotoxicant is metabolized by the cytochrome P450 enzyme to a highly reactive trichloromethyl free radical and/or trichloromethyl peroxyl radical, which sequentially attacks hepatic tissue, leading to

liver lipid peroxidation and oxidative damage. In addition, CCl_4 exposure could trigger the production of inflammatory cytokines and chemokines, stimulating the recruitment of inflammatory cells. Moreover, as another crucial event involved in the acute liver injury induced by CCl_4, apoptosis is a programmed cell death leading to morphological changes and death in the hepatocytes [22].

The treatment of rats with CCl_4 resulted in a reduction of more than 50% in liver tissue protein content, which eventually reached a value of 54.291 ± 1.336 g/L (Table 6). Treatment with silymarin increased the levels of TP in liver tissues to 94.211 ± 2.811 g/L in control mice. The treatment of rats with total extracts from the root of *J. glauca* and *J. pelargoniifolia* roots and aerial parts (400 mg/kg) increased the TP levels in liver tissues to 85.828 ± 1.439, 75.050 ± 1.712, and 68.263 ± 1.829 g/L, respectively. Treatment with a dose of 200 mg/kg body weight of total extracts from roots of *J. glauca* and *J. pelargoniifolia* increased the protein level significantly to 70.658 ± 1.931 and 65.070 ± 2.265 g/L ($p < 0.01$) in liver tissues, respectively. When 200 mg/kg doses of extracts from aerial parts of both *J. glauca* and *J. pelargoniifolia* were administered, they caused lesser increase in the TP levels to 59.880 ± 2.051 and 59.880 ± 3.330 g/L, respectively, in liver tissues.

Table 6. Effects of *Jatropha pelargoniifolia* (J.P.) and *J. glauca* (J.G.) (root and aerial parts) extracts on MDA, NP-SH, and TP in rat liver

Treatments (n=6)	**Dose mg/kg**	**MDA (nmol/g)**	**NP-SH (nmol/g)**	**Total protein (g/l)**
		Mean ± SM	***Mean ± SE***	***Mean ± SE***
Normal	–	0.465±0.018	8.083±0.198	110.578±1.143
CCl_4	1.25 mL/kg	5.270±0.220$^{###, a}$	4.028±0.214$^{###, a}$	54.291±1.336$^{###, a}$
Silymarin + CCl_4	10	1.262±0.038$^{###, b}$	7.492±0.353$^{###, b}$	94.211±2.811$^{###, b}$
J.P. aerial part+ CCl_4	200	4.541±0.401$^{, b}$	5.073±0.534$^{, b}$	59.880±3.330$^{, b}$
J.P. aerial part+ CCl_4	400	3.273±0.095$^{**, b}$	5.599±0.379$^{, b}$	68.263±1.829$^{*, b}$
J.P. root+ CCl_4	200	3.319±0.147$^{**, b}$	6.757±0.304$^{**, b}$	65.070±2.265$^{**, b}$
J.P. root+ CCl_4	400	2.203±0.069$^{**, b}$	7.892±0.263$^{***, b}$	75.050±1.712$^{**, b}$
J.G. aerial part+ CCl_4	200	4.673±0.126$^{*, b}$	5.407±0.164$^{**, b}$	58.283±1.181$^{, b}$
J.G. aerial part+ CCl_4	400	4.342±0.136$^{**, b}$	6.431±0.163$^{**, b}$	59.880±2.051$^{*, b}$
J.G. root+ CCl_4	200	3.070±0.106$^{**, b}$	5.755±0.276$^{**, b}$	70.658±1.931$^{**, b}$
J.G. root+ CCl_4	400	1.970±0.058$^{**, b}$	6.378±0.230$^{**, b}$	85.828±1.439$^{**, b}$

All values represent mean ± SE. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ (one-way ANOVA, followed by Dunnett's test), $^{###}p < 0.001$ (unpaired t-test), ^{a} *versus* normal control group, ^{b} *versus* CCl_4 group.

2.6. Discussion

Our results revealed that all tested extracts protected the rats from carrageenan-induced inflammation and showed significant anti-inflammatory activity (decrease in paw swelling) against the control group ($p < 0.05$ and $p < 0.01$) at both doses (Table 1). Generally, carrageenan is used in experimental pharmacology to induce acute arthritis [23]. The intraperitoneal administration of the total extracts of *J. glauca* and *J. pelargoniifolia* caused a pronounced ($p < 0.05$ and $p < 0.01$) inhibition of late phase edema, induced by the sub-plantar injection of carrageenan; it caused no inhibitory effect in the initial phase of the experiment. The second phase (late phase) of carrageenan-induced edema was susceptible to anti-inflammatory drugs that have been commonly used clinically to evaluate the antiphlogistic effects of natural products [24]. *Jatropha* species might exhibit anti-inflammatory activity due to its flavonoid content, which might lead to a reduction in the total leukocyte and monocyte percentage and/or number of circulating phagocytes [25].

The antinociceptive activity of the alcoholic extracts from *J. glauca* and *J. pelargoniifolia* (root and aerial parts) were evaluated by using the acetic acid writhing and the hot plate methods. The results obtained for the writhing test were very similar to those obtained for the induction of edema using carrageenan.

The analgesic effect of *J. glauca* and *J. pelargoniifolia* has not been previously reported; thus, the mechanism by which it occurs is not fully understood. The administration of acetic acid causes increased membrane phospholipase activity and greater synthesis of pain and inflammation mediators, such as cytokines, eicosanoids, leukotrienes, and prostaglandins; therefore, protection against acetic acid-induced writhing is indicative of the inhibition of prostaglandin-like mediators [26].

Hot plate paw licking is used to study the neuronal mechanisms of opioids and stimulation-induced antinociceptive effects. Thermal stimuli were used in the test; however, the hot plate paw latency response is a supraspinally-integrated response [27]. In conclusion, all extract-treated groups showed a significant analgesic response ($p < 0.05$ and $p < 0.01$) as compared to the control group. Further studies are needed to understand if a significant level of analgesic activity occurs via the effect on the central nervous system due to the presence of significant amounts of cyclopeptides and

alkaloids in the studied *Jatropha* species [28]. The docking study confirmed that an interaction occurs between the cyclopeptide alkaloids and μ-opioid receptor [29].

The current study demonstrates the efficacy of the extracts from *J. glauca* and *J. pelargoniifolia* as analgesic and anti-inflammatory agents, and scientifically justifies the use of these plants as non-specific therapeutic agents in Arabian traditional medicine [10, 11]. Therefore, these two species can serve as unique natural sources of secondary metabolites for use as antinociceptive agents.

Alloxan and streptozotocin are the most popular diabetogenic agents used for assessing the antidiabetic or hypoglycemic capacity of test compounds/extracts. The diabetogenicity of alloxan is under lined by its selective cellular up take by beta cells of the pancreas and consequent accumulation in these cells. Notably, alloxan is far less expensive and more readily available than streptozotocin. On this ground, one will logically expect a preference for use of alloxan in experimental diabetes studies [30]. Although, streptozotocin is actually preferred chemical agent to stimulate experimental diabetes because it has longe half-life (15 min), sustained hyperglycemia with regard to longer duration and also the development associated with well characterized diabetic problems with less incidences associated with ketosis in addition to mortality [31]. Alloxan not just has poisonous effects upon islets associated with Langerhans, but additionally affect additional body internal organs. It generally produces serious diabetes. Streptozotocin is actually more particular to beta cells instead of alloxan. Streptozotocin is much better especially whenever inducing DM type-2. Alloxan altered the standard pathways associated with cellular metabolic process, including the actual inactivation associated with certain nutrients, which resulted in liver harm and passing away [31].

The decrease in glucose level caused by the extracts from roots of *J. pelargoniifolia* at 400 mg/kg was found to be 37.98%, which was a significant reduction in glucose level, as compared to glibenclamide. On the other hand, aerial parts and roots of *J. glauca* resulted in moderate reductions of 35.06% and 28.92% in glucose levels, respectively. The aerial parts of *J. pelargoniifolia* caused the least reduction in the glucose level as compared to the control group (Table 4).

The significant lowering of blood glucose level in both normal and diabetic experimental animals, particularly because of the alcoholic extracts of *J. glauca* aerial parts and the roots of *J. pelargoniifolia*, ranged from 39.93% to 37.98%, which highlighted the hypoglycemic activity of the plant constituents. A number of mechanisms of action for these extracts have been suggested. Some hypotheses state that the possible effects on the activity of pancreatic β-cells are attributable to the fact that the extracts might increase the inhibitory action against insulinase enzyme and increase insulin sensitivity or insulin-like activity. Other mechanisms have also been suggested, such as an increase in the peripheral utilization of glucose, increase in the synthesis of hepatic glycogen, or decrease in glycogenolysis, inhibition of intestinal glucose absorption, reduction in glycemic index of carbohydrate, and a reduction in the action of glutathione [32]. All are equally important because they could potentially be used for the treatment of different aspects of diabetes mellitus. Therefore, plant extracts are a rich source of new hypoglycemic agents [33].

A previous phytochemical study on *Jatropha* species revealed that they contain numerous classes of secondary metabolites, such as terpenoids, steroids, flavonoids, alkaloids, and coumarinolignans [7]. The hypoglycemic and anti-diabetic activity of *J. glauca* and *J. pelargoniifolia* may be attributable to the presence of different classes of active ingredients that can act synergistically or independently under the conditions of type II diabetes as shown in our experiments.

Furthermore, the CCl_4-induced hepatic toxicity is commonly used as an experimental model for screening the hepatoprotective activity for convenient drugs or natural products [34]. Increasing the level of the liver enzymes indicating liver injury beside histopathological imaging [17]. Induction of liver injury by CCI_4 lead to significant elevation of liver enzymes and total bilirubin levels beside increase the levels of MDA and reduction of both total protein and NP-SH levels, (Tables 5 & 6) and indicates also injury caused to the hepatocytes. Here, ALT and AST were observed to be the most sensitive markers of hepatocellular injury [35]. Release of enzymes into the circulation is mainly due to a disturbance effect in the transport function of liver cells due to change in permeability of hepatocytes membrane which lead to elevation the level of biomarker enzymes, particularly AST and ALT [36]. The elevated levels of AST, ALT, ALP, GGT, and bilirubin caused by the CCl_4 injury (44.27, 70.59, 20.56,

57.93, and 62.15%, respectively), were effectively suppressed after the pretreatment of rats with silymarin. The oral administration of *J. glauca* and *J. pelargoniifolia* extracts after CCl_4 treatment also significantly protected against the elevation of transaminase levels, indicating an improvement in cellular integrity and status of hepatic cells. Increased levels of GGT and ALP are a significant sign of liver damage [37]. Improvement in the GGT and ALP levels after treatment with plant extracts is indicative of their membrane-stabilizing effect. Perhaps, the increase in total serum bilirubin concentration following CCl_4 treatment is attributable to the failure in normal uptake, conjugation, and excretion by the damaged hepatic parenchyma. The increased total bilirubin content due to CCl_4 intoxication was reduced after treatment with total extracts from *J. glauca* and *J. pelargoniifolia*, which is also indicative of their ability to enable normal uptake, conjugation, and excretion [38].

Attack of polyunsaturated fatty acid peroxidation by free radical lead to the production of malonaldehyde as end product. It is a reactive aldehyde and is one of the many reactive electrophilic species that cause toxic stress in cells and form covalent protein complex [39]. The level of oxidative stress is measured by this biomarker aldehyde in an organism [40]. The treatment of the animals with roots and aerial parts of *J. glauca* and *J. pelargoniifolia* decreased the level of MDA, reflecting the significant level of protection that the extracts could provide (Table 6).

Pretreatment of the animals with silymarin significantly increased the NP-SH content that had been reduced in hepatic tissues. Animals that received the total *J. pelargoniifolia* root extract showed a significant recovery of the NP-SH content at doses of 200 and 400 mg/kg. However, the NP-SH levels in such animals were better than the levels in animals given silymarin at a dose of 10 mg/kg. Pretreatment with total alcoholic extracts from aerial parts of *J. glauca* at doses of 200 and 400 mg/kg also resulted in a significant improvement in NP-SH levels, while treatment with the root of *J. glauca* and aerial parts of *J. pelargoniifolia* were less effective but statistically significant (Table 6). The NP-SHs are known to be involved in several defense processes against oxidative damage; they protect cells against free radical peroxides and poisonous substances [41].

Protein synthesis by the liver is considered a vital function necessary for having a healthy liver. Liver injury leads to the interruption and disassociation of

polyribosomes on the endoplasmic reticulum, because of which the total protein biosynthesis is reduced. The total protein level in the recovered and healthy liver would return to normal [42]. Treatment with the total extracts from the *J. glauca* root and *J. pelargoniifolia* root caused a significant increase in the TP levels in liver tissues. These results indicated that the extracts had a protective effect that was almost similar to the effect of silymarin ($p < 0.01$) at dose 400 mg/kg, while treatment with the extracts from aerial parts of *J. glauca* and *J. pelargoniifolia* resulted in a lesser, but significant improvement in the liver TP levels ($p < 0.05$). Treatment with total extracts from the roots of *J. glauca* and *J. pelargoniifolia* even at low dose were significantly increased the level of proteins in liver tissues ($p < 0.01$).

Finally, a chemical investigation of the *Jatropha* genus revealed the presence of terpenes, alkaloids, coumarinolignans, flavonoids, and steroids. Hence, the prominent hepatoprotective activity observed could be attributable to the high percentages of phenolics and flavonoids in *J. glauca* and *J. pelargoniifolia* [43].

2.7. Conclusions

In conclusion, the ethanolic extracts from *J. glauca* and *J. pelargoniifolia* (root and aerial parts) induced analgesic and anti-inflammatory effects; these results justify the traditional use of *J. pelargoniifolia* and *J. glauca* as analgesics for the treatment of inflammatory conditions. The changes in liver parameters induced by CCl_4 intoxication were reversed by the alcoholic extracts of *J. pelargoniifolia* and *J. glauca*. The NP-SH assay indicated that the antioxidant nature of the extracts could be responsible for the hepatoprotective activity of both the *Jatropha* species. Finally, hypoglycemic and anti-diabetic effects of the extracts were significant, especially for extracts obtained from *J. glauca* aerial parts and *J. pelargoniifolia* roots. All results presented in this study need to be supported with further experiments to clarify the exact mechanism of action before the plants and their extracts can be clinically recommended.

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Chapter 3

Chemical Composition and Biological Activity of the Essential Oil from the Roots of *Jatropha pelargoniifolia* Courb. Native to Saudi Arabia

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3.1. Abstract

The chemical composition of the essential oil from *Jatropha pelargoniifolia* roots was determined via GC-FID. There were 80 compounds, representing 99.99% of the total oil constituents. Among these, 77.31% were sesquiterpenes, 14.62% were fatty acids, 7.21% were other components (i.e., phenolics, hydrocarbons, etc.), and 0.85% were monoterpenes. The major compounds in the oil were γ-eudesmol (35.31%), 5-guaien-11-ol (14.43%), epi-cedrol (8.19%), oleic acid (5.23%), bulnesol (4.45%), α-linoleic acid (4.20%), 3,4-dimethoxycinnamic acid (3.83%), palmitic acid (2.69%), isolongifolanone (2.68%), eicosane (1.41%), and cedrol (1.14%). Oxygenated sesquiterpenes were found to represent more than 50% percent of the total oil content. Moreover, the essential oil was evaluated for anti-inflammatory, antioxidant, antipyretic, and antinociceptive activities using *in vivo* and *in vitro* models. Additionally, the antioxidant potential of the oil was evaluated using various *in vitro* antioxidant tests, including $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and FRAP. At a dose of 240 µl/kg, the oil showed anti-inflammatory (55.64%), antipyretic (37.033±0.120), and antinociceptive (47.58%) activities and showed significant ($p < 0.01$) effect as compared to a standard drug (phenylbutazone and indomethacin). These findings demonstrated that the essential oil of *J. pelargoniifolia* root could be used as a natural source for their anti-inflammatory, antinociceptive, antipyretic, and antioxidant effects.

3.2. Introduction

Jatropha pelargoniifolia is a member of the Euphorbiaceae family. It is a frutescent plant of about 30 cm in height with bluish-green small petioles; there are usually 3–5 digitately-lobed ones with serrate margins covered with smooth silky hairs. Female flowers are larger in size with a larger perianth than the male flowers. Capsule is pale, straw-colored, scaly, and about 1 cm long with smooth black seeds. This plant is known locally in Saudi Arabia as "Obab" [1].

Species of *Jatropha* became popular over the years to treat various diseases. The roots of some species of *Jatropha* (*J. glandulifera, J. gossypiifolia, J. multifida*) were used to cure individuals suffering from leprosy and gonorrhea [2]. Traditionally, the sap of *J. pelargoniifolia* petiole is applied to cure ulcers and wounds in Ethiopia and Saudi Arabia [3].

Essential oils are complex mixtures of various chemical classes derived from secondary plant metabolism. Mostly, essential oils contain terpenes, including monoterpenes and sesquiterpenes, as well phenolics and some lipophilic constituents. These oils are extracted from specific plant organs (e.g., leaves, seeds, and peels) and also from the roots and rhizome. Generally, the synergistic biological activity of essential oils attributed to the presence of active constituents [4]. In fact, the essential oils were used in human being and animals and exhibited many promising biological activities such as; antimicrobial, antioxidant, anti-inflammatory, antispasmodic, and muscle relaxing properties [5]. Biologically active essential oils represent a potential source for alternative medicine [4]. As a consequence, essential oils are one of the promising candidates amongst natural compounds for the development of safe therapeutic agents.

Much phytochemical and biological work has been carried out on essential oils isolated from the genus *Jatropha*, such as *J. curcas, J. ribifolia, J. gossypifolia*, and *J. mutabilis* [6, 7, 8, 9]. It is worthy to note that this is the first study describing the chemical constituents and biological activities of the essential oil isolated from *J. pelargoniifolia* roots. The motivation of this study was to shed light on the composition of *J. pelargoniifolia* essential oil and to prove if some of the ethnomedical claims about the species are linked to the essential oil and its related constituents.

3.3. Materials and methods

3.3.1. Chemicals

ABTS [2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)], trichloroacetic acid (TCA), and DPPH (1,1-diphenyl-2-picrylhydrazyl) were procured from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Potassium Ferricyanide from Loba Chemie Pvt. Ltd. (Mumbai, India) and ascorbic acid was obtained from SD Fine Chem. Ltd. (Biosar, India) while indomethacin and phenylbutazone were purchased from Spimaco (Saudi Arabia). The rest of chemicals and solvents used were of analytical grade and purchased from Sigma-Aldrich (St Louis, MO, USA).

3.3.2. Plant material

The roots of *J. pelargoniifolia* were collected (1 kg) in September 2015 from Wadi Mojasas, in Jazan area in the south of Kingdom of Saudi. It was identified by Jacob Thomas, taxonomist of the Botany and Microbiology Department, King Saud University, College of Science. A voucher sample (number- 23064) has been placed at the Herbarium Center College of Science, King Saud University.

3.3.3. Essential oil prepared

The freshly cut roots (1 kg) were exposed to hydrodistillation for 6 hr. Clevenger type apparatus was used following the technique prescribed in the European Pharmacopoeia [10]. Anhydrous sodium sulfate powder was used for drying the obtained oil. The dried oil was kept on and stored in air-tight, amber colored glass vials at 4°C for further study.

3.3.4. GC-MS analysis

Sample components analysis was carried out by using Gas Chromatograph System Series (Agilent 5975MS /6890) combined with a flame ionization type of detector (FID) with fused silica capillary column HP-5 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm inner diameter, 0.25 μm film thickness). Oven temperature was gradually increased from 40 to 280°C at a rate of 3 °C min^{-1}. The temperature of the injection port was kept at 230°C while the detector temperature kept constantly at 280°C. The split ratio was (1:20). The injection volume was 1.0 μL. A C7-C21 *n*-alkanes mixture was mixed with n-hexane and used for determination of the temperature programmed

retention indices. The sample was analyzed in n-hexane solution. An internal standard (*n*-alkanes) was then mixed with the sample to help in the standardization of retention times, and the sample was reanalyzed. Retention indices (RI) for all components were verified. The identity of essential oil components was achieved by comparing their retention indices (RI) and mass spectra with those reported from authentic samples and/ or the Wiley and NBS/NIST libraries and those published by Adams, 2001 [11]. The quantitative data regarding the essential constituents were obtained by peak-area normalization using the chromatographic technique with flame ionization detection (GC-FID) operated under similar conditions to the GC-MS. Compounds with concentrations equal or greater than 0.001% were considered for quantification. The false-positive results were prevented via avoiding coelution phenomena through the identification and deconvolution of coeluting peaks are done with Agilent Chemstation in combination with AMDIS as deconvolution software. For the identification we are using comprehensive internal MS-libraries as well as commercial libraries like NIST. Additionally, a retention index calibration is done to have RI as second factor for a correct identification.

3.3.5. Animal testing

The experiments were performed on male Wistar rats (with six in each group (I-IV) at 180-200 g b.w., 8/10 weeks old) and Swiss albino mice (with six in each group at 25±5 g b.w., 8/9 weeks old) of either sex. The required animals were provided from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Those animals were housed in large polypropylene cages in 22±2°C and gave standard pelleted nourishment and drinking water *ad libitum*. The current study was accepted by the Institutional Animal Ethical Committee of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia (approval number CPR-7569).

3.3.6. Determination of median lethal dose (LD_{50})

The LD_{50} value was evaluated adapting the procedure described by Karber, 1931 [12]. The minimal dose that completely killed 100% of the animal (LD_{100}) was carried out while the highest dose that failed to kill any animal was also calculated. Many doses at equal logarithmic intervals were selected in between these two doses; each dose was

administrated orally in a group of six rats. The rats were then evaluated for 24 h and recorded any behavioral, neurological symptoms as well as any sign of toxicity and mortality in each group.

3.3.7. Determination of anti-inflammatory activity

3.3.7.1. Carrageenan-induced paw edema in rats

The inflammation in rat's paw was induced following the procedure described by Winter *et al.*, 1962 [13]. Animals were divided into four groups of six animals each as follows: Group I was given an injection of 0.05 ml of 1% carrageenan solution in the right hind paw of each rat under the plantar aponeurosis. Group II and III rats were treated orally with 120 and 240 µl/kg b.w. of the oil suspended in distilled water 1 h before the carrageenan injection. Group IV (control rats) was administered standard anti-inflammatory drug (phenylbutazone, 100 mg/kg b.w., orally) 1 h before the carrageenan administration. The measurements of foot volume were carried by the displacement technique using a plethysmometer (Apelex, France) immediately 3 h after the injection of carrageenan. The inhibitory effect percentage was measured following the equation shown below:

$$\% \text{ Inhibition} = (1- a\text{-}x/b\text{-}y) \text{ X } 100$$

Where ‘b’ is the mean paw volume of control rats after carrageenan injection and ‘y’ before the injection; whereas ‘x’ is the mean paw volume of treated rats before injection and ‘a’ is the mean paw volume after carrageenan injection.

3.3.7.2. Cotton pellet granuloma test in rats

Goldstein *et al*. 1967 method was applied with minor modification [14]. 30 mg of sterilized cotton pellet was entered subcutaneous in the groin region of rats. Animals were divided into four groups of six animals each. Animals in the control group administered normal saline. Group I and II were administered the oil orally, in a dose of 120 and 240 µl/kg b.w. once daily for four consecutive days. Phenylbutazone 100 mg/kg b.w. was given to positive control group. On the fifth day, the animals were sacrificed using ether. The removed dried cotton pellets from extraneous tissue were weight after 24 h.

3.3.8. Determination of antipyretic activity in mice (Brewer's yeast-induced pyrexia method)

Fever was induced in mice by s.c. injection of 20% aqueous suspension of Brewer's yeast in the back, below the nape of the neck (20 ml/kg b.w.) [15]. Animals were divided into three groups of six animals each. The animals were then fasted for the duration of the experiment (approximately 1 day), with free water supply. Regular temperature measurements were performed 24 h after the yeast administration to determine the pyretic response. The oil suspension (120 and 240 µl/kg b.w.) was given 24 h after the yeast injection and the temperatures were recorded at 30, 60, and 120 minutes after its administration. Indomethacin (4 mg/kg b.w., administered orally) was gave to positive control group.

3.3.9. Antinociceptive activity

The analgesic activity was measured against chemical and thermal stimuli.

3.3.9.1. Chemical method

3.3.9.1.1. Inhibition of acetic acid-induced writhing in mice

The experiment was done according to the method approved by Siegmund *et al.*, 1957 with minor changes by Koster *et al*., 1959 [16, 17]. Animals were divided into four groups of six Swiss albino mice each. Group I was injected i.p with 0.2 ml of 3% acetic acid solution only. Group II and III were treated with the suspension of the oil mixture in doses of 120 and 240 µl/kg b.w. orally, and group IV was administered indomethacin (4 mg/kg b.w., p.o.), as a positive control, after an overnight fast. One hour after the treatment, the mice from groups II, III, and IV were injected i.p. with acetic acid solution to stimulate the distinctive writhings. The writhing numbers that occurred between 5 and 15 minutes after the acetic acid injection was calculated.

3.3.9.2. Thermal methods

3.3.9.2.1. Hot-plate test

The experiment was carried out in order to assess the potential of the response as designated by Eddy and Leimback, 1953 with a few modifications [18]. Hot plate

temperature was kept at 56±1°C. The mice were placed in a 24-cm diameter glass cylinder on the heated surface. The time between placement and licking of the paws or jumping was recorded as response latency. Animals were divided into three groups of six animals each. Distilled water was administered orally for the control group, and indomethacin was used as positive control (4 mg/kg b.w., administered orally). The choice of mice was achieved one day before the test based on their reactivity to the experiment. The animals were evaluated at 30, 60, and 120 minutes after oral administration of the oil suspension (120 and 240 µl/kg b.w.) and indomethacin administration. The cut off time was 30 seconds.

3.3.9.2.2. Tail-flick test

Acute nociception was induced using tail flick apparatus using (Tail flick Apparatus Harvard), according to the method described by D'amour and Smith, 1941 [19]. Briefly, each mouse was placed in a restrainer (three groups of six animals each) for 2 minutes before treatment; baseline reaction time was determined by focusing on an intensity-controlled beam of light on the distal one-third portion of the animal tail. The essential oil doses of 120 µl/kg and 240 µl/kg were administered intraperitonially. The post-drug reaction time was measured after 30, 60, and 120 minutes. Indomethacin was used as positive control (4 mg/kg b.w., administered orally).

3.3.10. Estimating antioxidant activity

3.3.10.1. DPPH(2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The antioxidant effect of the oil, based on the scavenging potency of the stable 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical, was measured using the technique designed by Braca *et al*., 2001 [20]. Different concentrations of oil were mixed with 3 mL of a 0.004% ethanol solution of DPPH. One ml methanol instead of oil was used to prepare control. The absorbance of color intensity was measured at 520 nm after 30 minutes and the percentage inhibition of antioxidant effect was calculated using the below formula:

$$[(A0-A1)/ A0] \times 100$$

Where A0 is the absorbance of the control (DPPH solution) and A1 is the absorbance of the oil/standard.

3.3.10.2. ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assay

The radical scavenging potency of the oil against ABTS radical cation was evaluated using the technique described by Re *et al*., 1999 [21]. The ABTS solution was prepared in water with a concentration of 7 mmol/L; an aqueous solution of potassium persulphate was also prepared with a concentration of 2.45 mmol/L. The two solutions were added in equal volume (1: 1) and stored in dark for 6 hr. at room temperature. During that period, ABTS radical was produced. The ABTS stock solution was diluted with ethanol to an absorbance of 0.70±0.02 at 734 nm and equilibrated at 30°C. An aliquot of oil was mixed with 2.9 ml of diluted ABTS radical cation solution. After the reaction was incubated at 30°C for 20 minutes, absorbance was measured at 734 nm. The ability of the oil to quench ABTS free radical was calculated according to the formula:

$$\text{Scavenging (\%)} = [\ (Ac - Aa / Ac)] \times 100$$

Where Ac= absorbance of control and Aa= absorbance of the oil.

3.3.10.3. Ferric reducing antioxidant power (FRAP) assay

The ferric free radical scavenging power was measured based on the technique recommended by Oyaizu, 1986 [22]. The reduction of ferric ion to ferrous ion is confirmed by formation of Perl's Prussian blue color where its intensity is related to the antioxidant activity. Serial dilution of the oil (20-100 µg/ml) in 1 mL of distilled water were added to 0.2 M phosphate buffer (2.5 mL, pH 6.6) and 1 % potassium ferricyanide (2.5 mL). The mixture was incubated at 50°C for 20 minutes. 2.5 ml of 10 % trichloroacetic acid was added to the mixture, followed by centrifugation at 3000 rpm for 10 minutes. 2.5 ml of distilled water was added to equal amount of the supernatant followed by addition of 0.5 ml of 0.1 % $FeCl_3$, the absorbance was recorded at 700 nm.

2.11. Statistical analysis

Data are expressed as mean ± standard error (SE). The results were first checked for normality by Kolmogorov and Smirnov test and homogeneity by Bartlett's test, and then analyzed using the Student's t test and analysis of variance (ANOVA) test. Dunnett posttest was used to determine which groups significantly differed from the control

group. The statistical analysis was performed using GraphPad Prism version 3. Results were considered significantly different if the $p < 0.05$ [23].

3.4. Results and discussion

Hydrodistillation of *J. pelargoniifolia* roots provided a yellowish essential oil with a yield of 0.52% (v/w), depend on fresh weight of the plant. Analysis and identification by mass fragmentation and retention index revealed the presence of 80 compounds, representing 99.99% of the total oil with 77.31% of these compounds being sesquiterpenes, 14.62% fatty acids, 7.21% other components (i.e., phenolics, hydrocarbons, cyclic compounds, etc.), and 0.85% were monoterpenes. The chemical composition of the essential oil and the percentage of each component determined from GC-MS analysis is shown (Table 1). The compounds are arranged in order of their elution from column together with their retention indices.

The major compounds in the essential oil were γ-eudesmol (35.31), 5-guaien-11-ol (14.43%), epi-cedrol (8.19%), oleic acid (5.23%), bulnesol (4.45%), linoleic acid (4.20%), 3,4-dimethoxycinnamic acid (3.83%), palmitic acid (2.69%), isolongifolanone (2.68%), eicosane (1.41%), and cedrol (1.40%). Oxygenated sesquiterpenes were found to be the major group of compounds that represented more than half of the oil content. It consisted almost entirely of γ-eudesmol (35.31%), 5-guaien-11-ol (14.43%), and epi-cedrol (8.19%). While oxygenated monoterpenes, fatty acids, and sesquiterpenes hydrocarbons covered a small percentage of the oil content.

A review of recent literature confirmed that γ-eudesmol is identified here for the first time in the genus *Jatropha*. Previously, secondary metabolites have been reported for many *Jatropha* essential oils, especially in the species: *J. ribifolia, J. gossypifolia*, and *J. mutabilis* [7, 8, 24, 9]. Major constituents in *J. ribifolia* aerial part essential oil were β-pinene, isoeugenol methyl ether, α-gurjunene, endo-8-hydroxy-cycloisolongifolene, α-pinene, and p-menth-1-en-8-ol, while phytol, spathulenol, epi-α-cadinol, caryophyllene oxide, germacerene D, and α-cadinol were the main components derived from *J. mutabilis* leaves essential oil. Essential oil from the leaves of *J. gossypifolia* was composed mainly of phytol, octadecanal, and viridiflorol, while major components of its stem oil were phytol, α-copaene, and limonene.

Table 1: Chemical constituents of *J. pelargoniifolia* root essential oil

#	Constituents	Rt (min.)	Conc. (%)	RI
1	Isovaleric acid	6.79	0.006	834
2	8-α-Pinenol	11.92	0.016	939
3	Phenol	12.15	0.013	965
4	Capronic acid	13.99	0.005	987
5	Eucalyptol	16.03	0.010	1033
6	*cis*-Linalool oxide	16.46	0.013	1074
7	Heptanoic acid	17.03	0.010	1083
8	Guaiacol	17.10	0.998	1086
9	*trans*-Linalool oxide	17.66	0.012	1088
10	Linalool	19.14	0.005	1098
11	2-Phenylethyl alcohol	19.54	0.004	1110
12	Fenchol	20.00	0.018	1112
13	Camphene hydrate	20.52	0.065	1148
14	1,8-Epoxy-2-*p*-Exo-Menthanol	20.53	0.004	1158
15	Borneol	20.92	0.003	1165
16	4-Terpinenol	21.06	0.527	1177
17	8-*p*-Cymenol	21.40	0.008	1183
18	Caprylic acid	21.87	0.024	1187
19	Butyl-*n*-hexanoate	22.35	0.006	1188
20	α-Terpinol	22.45	0.128	1189
21	Myrtenol	23.24	0.030	1194
22	4-Vinyl-phenol	23.53	0.108	1229
23	Bornyl acetate	25.34	0.014	1285
24	α-Cubebene	26.15	0.511	1351
25	Eugenol	28.05	0.004	1356
26	Octadecanal	28.65	0.157	1357
27	Cyclosativene	29.13	0.006	1368
28	α-Copaene	29.39	0.589	1376
29	β-Elemene	29.77	0.061	1382
30	Vanillin	30.18	0.042	1391
31	α-Cyperone	30.30	0.109	1398
32	Capric acid	30.54	0.039	1399
33	*cis*-Isoeugenol	30.77	0.104	1402
34	Caryophylladiene	31.45	0.074	1404
35	α-Gurjunene	31.88	0.030	1409
36	Caryophyllene	31.89	0.555	1418
37	α-Guaiene	32.43	0.125	1439
38	*E*-β-Farnesene	32.86	0.049	1458
39	Alloaromadendrene	33.26	0.091	1461
40	2,6-di(t-Butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	33.73	0.011	1478
41	β-Selinene	33.74	0.071	1485
42	*Epi*-Bicyclosesquiphellandrene	33.75	0.809	1490
43	Valencene	34.01	0.168	1491

Table 1: Cont.

#	Constituents	Rt (min.)	Conc. (%)	RI
44	*cis*-Methyl isoeugenyl	34.10	0.067	1492
45	α-Selinene	34.18	0.095	1494
46	β-Dihydroagarofuran	34.71	0.157	1496
47	δ-Selinene	34.72	0.028	1497
48	α-Muurolene	34.73	1.317	1499
49	δ-Guajene	34.73	1.336	1505
50	Butylated hydroxytoluene	35.50	0.032	1512
51	δ-Cadinene	35.93	0.379	1524
52	α-Calacorene	36.30	0.647	1542
53	Elemol	36.78	0.628	1549
54	Guajoxid	37.06	0.315	1595
55	Cedrol	37.48	1.140	1596
56	*Epi*-Cedrol	38.18	8.191	1611
57	*trans*-Isolongifolanone	38.29	2.679	1618
58	10-*Epi*- γ-Eudesmol	39.07	1.447	1619
59	5-Guaien-11-ol	39.91	14.429	1625
60	γ-Eudesmol	41.10	35.309	1630
61	δ-Cadinol	41.19	0.383	1645
62	4-α-Hydroxy dihydro agarofuran	41.20	0.388	1648
63	Bulnesol	41.94	4.454	1666
64	Ethyl myristate	45.40	0.065	1777
65	Cryptomeridiol	47.71	0.510	1808
66	Myristic acid	47.89	0.760	1842
67	8,11-Heptadecadienal	48.87	0.262	1855
68	Pentadecanoic acid	49.40	0.350	1902
69	Methyl hexadecanoate	50.46	0.023	1927
70	3,4-Dimethoxycinnamic acid	51.55	3.828	1949
71	Palmitic acid	54.95	2.689	1972
72	Ethyl palmitate	55.42	0.118	1993
73	Eicosane	56.26	1.411	2000
74	Geranyl linalool	56.63	0.227	2020
75	4-Vinylguaiacol	57.07	0.076	2156
76	α-Linoleic acid	57.86	4.196	2173
77	Oleic acid	58.17	5.232	2175
78	Stearic acid	59.43	0.732	2192
79	5,9-Farnesyl propanone	59.76	0.417	2377
80	Dehydrodiisoeugenol	60.06	0.041	2723
Oxygenated monoterpenes		-	0.849	-
Oxygenated sesquiterpenes		-	70.366	-
Sesquiterpene hydrocarbones		-	6.941	-
Fatty acids (saturated)		-	4.939	-
Fatty acids (unsaturated)		-	9.690	-
Other		-	7.205	-
Total percentages		-	99.99	-

*Rt: retention time, *RI: retention indices, GC-MS, gas chromatography-mass spectroscopy; component concentrations were calculated from GC-FID peak areas in the order of column elution.

The 24-hours LD_{50} was approximately more than 1.2 ml/kg b.w. for the essential oil. These results showed that the essential oil of *J. pelargoniifolia* roots is safe and non-toxic because no mice died or showed any severe side effects or intoxication.

Table 2 shows the effects of oil and phenylbutazone on carrageenan-induced rat paw edema. Injection of carrageenan into the sub-plantar tissue of the right hind paw of rats in the control group led to rapid growth of edema, which peaked (1.795±0.029 in paw volume) at 180 min post-phlogistic agent injection. The percent reduction in edema after oil administration was 31.68 and 55.64% inhibition observed at 120 µl/kg and 240 µl/kg, respectively. This effect was significantly different ($P < 0.01$) as compared to that produced by 100 mg/kg phenylbutazone (77.42% inhibition).

Carrageenan-induced paw edema is widely used experiment for screening the anti-inflammatory drugs including natural products [25]. We found that the administration of oil significantly decreased edema volume induced in animal's paw by carrageenan.

The inflammatory response by carrageenan hind paw in the rodent is including many stages. Firstly, after carrageenan injection, the paw volume increased due to release of internal chemical mediators such as, histamine and serotonin [26]. Then, the increase in vascular permeability was sustained by the release of prostaglandins and nitric oxide lead to stimulate migration of leukocytes into the inflamed site [27]. The last stage of edema is susceptible for commonly used anti-inflammatory drug. So, that oil might prevent increased in vascular permeability (edema) and leukocyte aggregation. The exact mechanism of action of *J. pelargoniifolia* root essential oil need further investigation.

Table 2**:** Effect of *J. pelargoniifolia* essential oil on carrageenan-induced paw edema in Wistar rats

Group (n=6)	Dose (µl/kg)	Before Carrageenan	3 hours after	Net	% Inhibition
Only carrageenan	0.05 ml	0.953±0.034	1.795±0.029‴	0.842±0.017	-
Essential Oil	120	0.993±0.036	1.568±0.033‴	0.575±0.022**	31.68
Essential Oil	240	0.982±0.034	1.355±0.028‴	0.373±0.015**	55.64
Phenylbutazone	100 mg/kg	1.007±0.028	1.197±0.028‴	0.190±0.005###	77.42

All values represent mean ± SE. ** $p < 0.01$ *versus* carrageenan group (one-way ANOVA, followed by Dunnett's test), ### $p < 0.001$ *versus* carrageenan group (unpaired t-test), ‴ $p < 0.001$ *versus* the corresponding group before treatment (paired t-test).

Cotton pellet-induced granuloma test was done to assess the efficacy of oil and standard anti-inflammatory drug against the proliferative phase of inflammation in which tissue degeneration and fibrosis occur .

According to these results, the anti-proliferative potency of the oil (120 µl/kg and 240 µl/kg) was calculated to be 69.667±1.787 and 51.217±1.463 ($P < 0.001$), respectively, while for phenylbutazone (100 mg/kg), was 41.700±1.065. After cotton pellet drying, the anti-proliferative effects were calculated based on the dry weights. The inhibition of inflammation by the oil and was 39.667±1.787 (23.57%) and 21.217±1.463 (59.12%) (P< 0.01), respectively, while for phenylbutazone was 11.700±1.065 (77.45%).

The cotton pellet-induced granuloma model is popular experimental technique used to evaluate the transudative, exudative, and proliferative events during chronic inflammation [28]. Perhaps, the reduction in granuloma size revealed the ability of oil to decrease the total number of fibroblasts and extending the time need for the synthesis of mucopolysaccharide and collagen which are involved in granuloma formation [29].

It is better to point out that sesquiterpenes and unsaturated fatty acids, which are the major constituents of the currently investigated oil and which play a significant role as anti-inflammatory compounds, are all derived from natural sources [30]. The essential oil of *J. pelargoniifolia* roots have been reported to have more than half of its constitutes made up of oxygenated sesquiterpenes (70.36%). It consists almost entirely of γ-eudesmol, 5-guaien-11-ol, epi-cedrol and bulnesol; these compounds have shown significant anti-inflammatory properties due to their various pharmacological activities

[31]. In addition, review of literature confirmed that both oleic acid (5.23%) and α-linoleic acid (4.19%) possess strong anti-inflammatory activities [32]. On the basis of this result, it could be proposed that the anti-inflammatory potential exerted by the essential root oil may be attributable to the synergistic action of sesquiterpenes and fatty acids since they are present in high yield.

The action of oil and indomethacin on yeast-induced fever in mice is illustrated in Table 3. The s.c. injection of an aqueous suspension of brewer's yeast has significantly elevated the rectal temperature by 3.2°C after 24 h of injection. Animals treated with the essential oil and indomethacin (positive control) showed significant reduction in animal's rectal temperature. *J. pelargoniifolia* oil showed significant suppression in rectal temperature after 30 min; the decrease observed was by 0.217°C and 0.53°C, i.e., from 38.350±0.126°C to 37.750±0.106°C and from 38.383±0.108°C to 37.850±0.112°C ($p < 0.05$), with doses of 120 µl/kg and 240 µl/kg, respectively. The antipyretic effect of oil began 30 min after its administration especially at dose 240 µl/kg ($p < 0.05$) and the decrease in rectal temperature was continued for 2 h ($p < 0.01$ and 0.05). Indomethacin also displayed a significant decrease in rectal temperature by 1.85°C, i.e., from 38.750±0.112°C to 36.900±0.086°C, and the percentage inhibition of pyrexia was significant ($p < 0.001$).

Additionally, after 120 min, there was no important variance between the antipyretic effect of oil in comparison to indomethacin. The current study displays that *J. pelargoniifolia* root oil has antipyretic effect in mice. Various reports have verified that yeast-induced temperature elevation is a pathogenic pyrexia [33]. At a higher body temperature, the thermoregulatory center in the hypothalamus start to release prostaglandins. If a central CNS effect or peripheric inhibition of cyclooxygenase which is responsible for the pyretic effect, that aspect remains to be studied but was not a part of the present study.

Table 3: Effect of *J. pelargoniifolia* essential oil on yeast-induced hyperthermia in mice

Treatment (n=6)	Dose (µl/kg)	Normal rector temperature	Rectal temperature after yeast administration (20 ml/kg of 20%)	Rectal temperature °C (Post Drug)		
				30 m	60 m	120 m
Essential Oil	120	35.150±0.076	38.350±0.126‴	38.133±0.123	37.833±0.109*	37.750±0.106*
Essential Oil	240	35.183±0.095	38.383±0.108‴	37.850±0.112*	37.183±0.128**	37.033±0.120**
Indomethacin	4 mg/kg	35.450±0.157	38.750±0.112‴	36.900±0.086**	36.583±0.140**	36.033±0.120**

All values represent mean ± SE. *$p < 0.05$; **$p < 0.01$ versus the corresponding group before drug treatment (Repeted ANOVA, followed by Dunnett's test), ‴ $p < 0.001$ (paired t-test), the hyperthermic group were compared with normal group befor the yeast administration.

As seen in Table 4, the mean writhes of the mouse decreased from 25.500±1.176 to 18.000±0.816 as the dose of the oil increased from 120 to 240 µl/kg, which compared to 4 mg/kg of indomethacin (8.500±0.428; 75.00% inhibition of writhing).

The mechanism of antinociceptive is not obvious in the current study. It is expected that prevention of prostaglandins caused reduction in mice visceral pain. Thus, it seems that the oil of *J. pelargoniifolia* prevents the second stage of inflammation and causes the inhibition of prostaglandin peripherally [34]. This comment is supported by our finding that oil effectively suppressed yeast-induced pyrexia in animal's model.

The oil at the two doses showed significant analgesic action peaking at 30, 60, and 120 minutes at doses of 120 and 240 µl/kg (i.p.) as compared with indomethacin.

The tail flick method indicated the central analgesic effect of *J. pelargoniifolia* roots oil. The response of the tail flick is assumed to be a spinally mediated reflex, which is controlled by a supraspinal inhibitory mechanism [35]. The oil was produced significant analgesic activity ($p < 0.01$) compared to the positive control group.

Moreover, in the hot plate test, treatment with oil showed significant antinociceptive effect ($p < 0.01$) as compared to that in the positive control group. The oil was produced the analgesic effect via increasing the time of response to heat sensation, from 6.333±0.211 to 8.667±0.333 at 120 minutes (36.84% analgesia) with a dose of 120 µl/kg and 6.667±0.333 to 11.667±0.333 at 120 minutes (75.00% analgesia) with a dose of 240 µl/kg. Indomethacin also significantly delayed the reaction time by 120.00% ($p < 0.001$). The analgesic effect caused by indomethacin was significantly

stronger than that induced by the oil (8.667±0.333 and 11.667±0.333 at doses of 120 and 240 µl/kg, respectively) versus 14.667±0.333 after 120 minutes ($p < 0.001$).

In numerous studies, the pharmacological action of essential oils has been referred to the synergistic effect of active components. The oil inactive principles pharmacokinetics and bioavailability could be supported by active components. Further, it is difficult to establish a relationship between oil constituents and its activity [36]. Perhaps, the analgesic activity of the essential oil derived from *J. pelargoniifolia* roots is mainly due to the combined effect of sesquiterpenes as suggested by Lee *et al*., 2002 [37], it is maybe responsible for blocking the release of endogenous substances, which excite the pain in nociceptive pathway.

Table 4: Analgesic effect of *J. pelargoniifolia* essential oil on Acetic acid –induced writhing in mice

Treatments (n=6)	**Dose (µl/kg)**	**Number of writhing in 20 min.**	**% Inhibition**
Control (Acetic acid)	0.1 ml of 20%	34.000±1.983	-
Essential Oil	120	25.500±1.176**	25.00
Essential Oil	240	18.000±0.816**	47.58
Indomethacin	4 mg/kg	8.500±0.428###	75.00

All values represent mean ± SE. **$p < 0.01$ *versus* control (one-way ANOVA, followed by Dunnett's test), ###$p < 0.001$ *versus* control (unpaired t-test).

The antioxidant activity was measured by using three methods including DPPH, ABTS and FRAP. As illustrated in Table 5, the DPPH radical scavenging assay was used to assess the antioxidant activity of the essential oil, it was displayed a concentration-dependent antioxidant effect by $DPPH^-$ ion. As expected, ascorbic acid showed a higher potency (99.69 %) of free radical scavenging at 100 µg/ml as compared to the oil at same concentrations (78.15%).

The results and mechanism of action obtained from DPPH, ABTS methods were similar to each other since their mechanism more or less similar which based on the donation of electrons or hydrogen atoms to free radical lead to its inactivation [38]. The results obtained from these two methods, regarding scavenging activity of the oil increased with the increasing concentration.

From the investigation of Table 5, we can conclude that the oil showed significant ABTS radical-scavenging property (84.10%), which was comparable to that of ascorbic acid (90.50%) at 100 µg/ml.

These results showed that the high antioxidant potential of the oil derived from *J. pelargoniifolia* roots could be attributed to the high percentage of oxygenated compounds, such as γ-eudesmol, 5-guaien-11-ol, and *epi*-cedrol [39].

The result obtained from third antioxidant experiment (FRAP) method, is also based on the reducing power of the essential oil ingredients which can reduce the ferric ions (Fe^{3+}) to ferrous ion (Fe^{2+}) by electron donation. In this experiment the intensity of Perl's Prussian blue color was measured at 700 nm. The higher increase in the Prussian blue color intensity revealed strong antioxidant activity [40].

As indicated in Table 5, the reducing capabilities of the root oil towards FRAP were compared to ascorbic acid as the reference standard. The oil exhibited moderate ferric reduction capability (1.06) in comparison to ascorbic acid (1.48) at the same concentration (100 µg/ml). As result, the oil showed good reducing activity with increasing concentrations as compared to the most famous antioxidant drug; ascorbic acid.

Table 5: Antioxidant effect of *J. pelargoniifolia* essential oil (DPPH, ABT, and FRAP assays)

Treatments	**DPPH assay**			**ABTS assay**		**FRAP assay**	
	Concentration (µg/ml)	**%Average scavenging**	**Mean ±S.D**	**%Average scavenging**	**Mean ±S.D**	**Average OD.**	**Mean ±S.D**
Essential Oil	10	13.65	13.650±12.010	13.65	13.650 ±12.090	0.47	0.470±0.090
Essential Oil	20	32.05	32.050±1.060	43.40	43.400±11.870	0.74	0.745±0.020
Essential Oil	50	57.30	57.30±2.120	57.05	57.050±13.050	0.89	0.890±0.020
Essential Oil	100	78.15	78.150±6.570	84.10	84.100±9.330	1.06	1.060±0.020
Ascorbic acid	100	99.69	99.690±0.030	90.50	90.500±7.770	1.48	1.475±0.007

OD. = Optical density

3.5. Conclusion

The essential oil constituents obtained from the roots of *J. pelargoniifolia* are reported here for the first time*;* we have also investigated selected biological activities with regard to the ethnomedicinal use of the essential oil of the roots considering that it is a native plant to Saudi Arabia [41]. It was found that the essential oil showed promising activities as anti-inflammatory, antinociceptive, antipyretic and antioxidant agent. Perhaps, these significant biological activities can be referred to the effect of its active components. The inactive components could work by influencing pharmacokinetics and bioavailability of the active compounds. Furthermore, it is difficult to create a relationship between specific oil constituent and certain biological activity due to the combined effect between its various constituents. The essential oil isolated from roots of *J. pelargoniifolia* may serve as a promising candidate for the development of safer therapeutic agents to be used for treatment of contemporary diseases, such as various skin inflammatory condition and acute arthritis.

3.6. References

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Chapter 4

Comparative Study of the Chemical Composition of Essential Oils of *Jatropha pelargoniifolia* and *Jatropha glauca* Native to Saudi Arabia

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The data for this chapter are unpuplished

4.1. Abstract

The roots and aerial parts of *Jatropha pelargoniifolia* and *Jatropha glauca*, which belong to the family Euphorbiaceae that is native to Saudi Arabia, contain essential oils. Here, we studied the essential oil components in these plant parts in a comparative study of the two species. The essential oils were obtained by hydrodistillation and were analyzed by GC-FID and GC-MS. In total, 80, 42, 45, and 68 compounds, which accounted for 99.99%, 86.92%, 92.83%, and 89.84% of the total composition of essential oils in *J. pelargoniifolia* roots, *J. pelargoniifolia* aerial parts, *J. glauca* roots, and *J. glauca* aerial parts, respectively, were identified. We conducted a comparative study on both species to reveal the chemotaxonomic relationship between *J. glauca* and *J. pelargoniifolia* based on predominant sesquiterpenoids and diterpenoids. Qualitative and quantitative similarities were observed in the oil composition of both species, mainly regarding the major components, which for the roots of *J. glauca* and *J. pelargoniifolia* were γ-eudesmol (28.82% and 35.31%) and 5-guaien-11-ol (22.86% and 14.43%), respectively, and for the aerial parts were phytol (33.05 and 24.19 %) and palmitic acid (20.43 and 11.53%), respectively. Oxygenated sesquiterpenes and fatty acids were predominant chemical classes in oil isolated from roots of both species. On the other hand, oils from aerial parts were high in oxygenated diterpenes (35.30% in *J. glauca* and 32.23% in *J. pelargoniifolia*). Oils from *J. pelargoniifolia* aerial parts were high in oxygenated sesquiterpenes followed by fatty acids, while those from *J. glauca* were rich in fatty acids and other phenolics (hydrocarbons/cyclic). *J. glauca* and *J. pelargoniifolia* have similar morphology, except for leaf surface (hairy in *J. pelargoniifolia* and smooth in *J. glauca*) and fruit color (darker in *J. pelargoniifolia*), and these species are therefore often confused by native people in southern Saudi Arabia. This study clarified chemotaxonomic characters of *J. glauca* and *J. pelargoniifolia* via chemical composition analysis of essential oils of roots and aerial parts.

4.2. Introduction

Medicinal plants play a vital role in healthcare provision in numerous countries, and they have been used as drugs for the treatment of major human diseases [1, 2]. Herbs have been classified by the World Health Organization (WHO) as a superior source for drug discovery [3]. Plants belonging to the genus *Jatropha* are popularly used to treat various diseases [4]. Traditionally, *J. pelargoniifolia* sap of the petiole was applied as a healing agent for ulcers and wounds in Ethiopia and Saudi Arabia, while the whole plant of *J. glauca*, including the root, is mashed in water and the liquid is taken to treat constipation and as ear drops to treat earache, and the leaf sap is taken as an astringent [5].

The Euphorbiaceae family, which is considered one of the largest families of angiosperms worldwide, covers about 7,800 species distributed in approximately 300 genera and five subfamilies. These species occur preferentially in tropical and subtropical environments [6, 7]. Among the main genera belonging to this family, *Jatropha*, which is represented by about 200 species. This genus is widely distributed in tropical and subtropical regions of Africa and the Americas [7]. *J. pelargoniifolia* and *J. glauca* are known locally as "Obab" and "Obeeb," respectively. They are widely distributed in Africa (Sudan, Eritrea, Djibouti, Ethiopia, Somalia) and the Arabian Peninsula (Yemen, Saudi Arabia). Native people in the Southern region of Saudi Arabia mostly mix both species for ethnomedical use because they have a similar morphology, although they differ in leaf structure (hairy *versus* smooth surface) and fruit color.

Essential oils are natural products with many applications, e.g., in the food, perfume, cosmetics, and pharmaceutical industries. Essential oils are complex mixtures and have various pharmacological properties, including anti-inflammatory, antibacterial, antifungal, protective of the gastrointestinal tract, anticonvulsant, and antioxidant properties [8]. Thus, essential oils are one of the most promising candidate groups of natural compounds for the development of safer therapeutic agents.

Numerous phytochemical and biological studies on essential oils isolated from various *Jatropha* species, such as, *J. curcas, J. ribifolia, J. gossypifolia and J. mutabilis* have been reported [9, 10, 11, 12]. To the best of our knowledge, there are no reports on the composition of essential oils isolated from *J. pelargoniifolia* and *J. glauca*.

Therefore, this study aimed to explore the effects of ecological and geographical factors on essential oil characteristics, including chemical constituent classes and concentrations, total yield, organoleptics, and physical characters, which can be used as chemotaxonomic markers.

4.3. Materials and methods

4.3.1. Plant material

J. glauca (Figure 1S$_a$) is a frutescent plant of approximately 30 cm in height. Leaves are bluish-green, small, petioled, 3–5 digitately lobed with serrate margin, and fruits are capsules. Female flowers are larger, with larger perianth, than the male flowers. Capsules are pale, straw-colored, scaly, 1 cm long, with smooth black seeds. *J. pelargoniifolia* (Figure 1S$_b$) is similar in height, and the leaves are slightly smaller and are covered with silky hairs [13].

J. pelargoniifolia and *J. glauca* were collected in September 2015 from Wadis Mojasas and Shogare (hot and humid areas), respectively, in the Jazan South area of the Kingdom of Saudi Arabia. Species were identified by Dr. Jacob Thomas, taxonomist at the Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia. Voucher specimens of *J. pelargoniifolia* and *J. glauca* (numbers 23064 and 23063, respectively) have been deposited at the herbarium of College of Science, King Saud University. Aerial parts were separated carefully from roots. After removing the dirt, each part was washed with water and then air-dried. The fresh roots and aerial parts were weighed: *J. pelargoniifolia* roots, 0.83 kg; *J. pelargoniifolia* aerial parts, 0.92 kg; *J. glauca* roots, 0.57 kg; and *J. glauca* aerial parts, 0.43 kg.

4.3.2. Essential oils prepared

Freshly cut roots and aerial parts were subjected to hydrodistillation for 8 h using a Clevenger-type apparatus using an adaption of the procedure described in the European Pharmacopoeia (2004) [14]. The oils were dried over anhydrous sodium sulfate and stored in air-tight, amber-colored glass vials at 4°C for later analysis.

4.3.3. Gas chromatography-flame ionization detector (GC-FID) analysis

Sample components analysis was carried out by using Gas Chromatograph System Series (Agilent 5975MS /6890) combined with a flame ionization type of detector (FID) with fused silica capillary column HP-5 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm inner diameter, 0.25 μm film thickness). Oven temperature was gradually increased from 40 to 280°C at a rate of 3°C min^{-1}. The temperature of the injection port was kept at 230°C while the detector temperature kept constantly at 280°C. The split ratio was (1:20). The injection volume was 1.0 μL. A C7-C21 n-alkanes mixture was mixed with n-hexane and used for determination of the temperature programmed retention indices. The sample was analyzed in n-hexane solution. An internal standard (n-alkanes) was then mixed with the sample to help in the standardization of retention times, and the sample was reanalyzed. Retention indices (RI) for all components were verified. The identity of essential oil components was achieved by comparing their retention indices (RI) and mass spectra with those reported from authentic samples and/ or the Wiley and NBS/NIST libraries and those published by Adams, 2001 [15]. The quantitative data regarding the essential constituents were obtained by peak-area normalization using the chromatographic technique with flame ionization detection (GC-FID) operated under similar conditions to the GC-MS. Compounds with concentrations equal or greater than 0.001% were considered for quantification. The false-positive results were prevented via avoiding coelution phenomena through the identification and deconvolution of coeluting peaks are done with Agilent Chemstation in combination with AMDIS as deconvolution software. For the identification we are using comprehensive internal MS-libraries as well as commercial libraries like NIST. Additionally, a retention index calibration is done to have RI as second factor for a correct identification.

4.4. Results and discussion

The data on the essential oil components of different parts of *J. pelargoniifolia* and *J. glauca* from Saudi Arabia are given in Table 1 and Figure 2S. The essential oil samples from aerial parts were observed as yellowish-red liquids and were obtained with yields of 0.22% and 0.35% (v/w) from *J. pelargoniifolia* and *J. glauca*, respectively. The root essential oils were intense yellow liquids with a yield of 0.54% (v/w) for *J. pelargoniifolia* roots and 0.33% (v/w) for *J. glauca* roots. The essential oil of *J.*

pelargoniifolia roots contained sesquiterpenes (77.31%) and monoterpenes (0.85%). Similarly, *J. glauca* root essential oil consisted of sesquiterpenes (66.74%) and monoterpenes (0.96%). The aerial-part essential oils of *J. pelargoniifolia* and *J. glauca* were abundant in diterpenes (35.61% and 39.74%, respectively), and sesquiterpenes (25.57 and 5.65%, respectively), and monoterpenes (0.76% and 0.98%, respectively). The oils of aerial parts of *J. pelargoniifolia* and *J. glauca* contained diterpenes, which were not observed or present in trace amounts in the oils from root samples. The amount of sesquiterpenes was higher than that of monoterpenes in roots and aerial parts. The majority of compounds of *J. pelargoniifolia* and *J. glauca* root essential oils belonged to oxygenated sesquiterpenes (70.37% and 66.39%, respectively), while oxygenated diterpenes were abundant in aerial-part essential oils (32.23% and 35.30%, respectively). In contrast, fatty acids isolated from essential oils of *J. pelargoniifolia* roots, *J. pelargoniifolia* aerial parts, *J. glauca* roots and *J. glauca* aerial parts represented 14.63%, 20.95%, 19.71%, and 29.36%, respectively, of the total oil content.

The abundant compounds of the essential oils of the roots of *J. pelargoniifolia* and *J. glauca* were γ-eudesmol (35.31% and 28.82%), 5-guaien-11-ol (14.43% and 22.86%), α-linoleic acid (4.20% and 4.97%), bulnesol (4.45% and 1.60%), oleic acid (5.23% and 1.50%,), palmitic acid (2.69% and 8.83%), and 10-*epi*-γ-eudesmol (1.45% and 1.82%). The predominant components of *J. pelargoniifolia* and *J. glauca* aerial-part essential oils were phytol (24.19% and 33.05%), palmitic acid (11.53% and 20.43%), γ-eudesmol (6.94% and 0.43%), 5-guaien-11-ol (5.21% and 0.78%), neophytadiene (3.38% and 4.44%), α-linoleic acid (4.36% and 2.47%), δ-cadinol (1.28% and 1.72%), isophytol (1.41% and 1.67%), and myristic acid (2.62% and 1.28%). Common major volatile components in root and aerial-part essential oils were 10-*epi*-γ-eudesmol, γ-eudesmol, 5-guaien-11-ol, and δ-cadinol. Phytol was detected in aerial-part essential oils of *J. pelargoniifolia* and *J. glauca* and in *J. glauca* root oil but was not detectable in essential oil from the roots of *J. pelargoniifolia*.

Volatile oxygenated sesquiterpenes were present in significant amounts in all four essential oils, especially in those of *J. pelargoniifolia* roots, *J. pelargoniifolia* aerial parts, and *J. glauca* roots. Oxygenated diterpenes were predominant in oils from aerial parts of *J. pelargoniifolia* and *J. glauca*, and at minor concentrations in root oil

from *J. glauca*, but not *J. pelargoniifolia*. On the other hand, oxygenated monoterpene and sesquiterpene hydrocarbons were detected in all four essential oils at low levels. Hydrocarbons derived from monoterpenes and diterpenes were exclusively detected in aerial-part essential oils of both species and represented a small fraction of the total oil content. While major components of essential oils are very important for their biological activity, minor components also play a significant role, as they can strengthen the effects of major components, though antagonistic and additive effects have also been observed [16]

The composition of essential oil isolated from the same plant species but from different organ sources can differ. We clearly observed similarities in the chemical composition of the essential oils, especially, in the major components, isolated from different *Jatropha* species but from the same organ. This indicates the importance of the oil-producing organ (i.e., the source of oil production; leaves, roots, bark, or flowers) in determining the oil chemical composition and yield [17]. Recently, secondary metabolites of essential oils were discussed for various *Jatropha* species, including *J. ribifolia*, *J. mutabilis*, *J. gossypifolia*, and *J. curcas*. The major components in *J. ribifolia* essential oil were β-pinene, iso-eugenol methyl ether, α-gurjunene, endo-8-hydroxy-cycloisolongifolene, α-pinene, and p-menth-1-en-8-ol [11], while phytol, spathulenol, *epi*-α-cadinol, caryophyllene oxide, germacrene D, and α-cadinol were the major constituents in *J. mutabilis* leaves essential oils [10]. *J. gossypifolia* leaves essential oil was composed mainly of phytol, octadecanal, and viridiflorol, while stem oil mainly contains phytol, α-copaene, and limonene [18]. Finally, the main constituents of *J. curcas* seed oil are unsaturated fatty acids (oleic and linoleic acids) and saturated fatty acids (palmitic and steric acids) [12] (Table 2). These findings suggest that essential oil chemical constituents and yields depend on many factors, including geographical, environmental, and genetic factors. We were able to characterize the components of the essential oils of *J. pelargoniifolia* and *J. glauca*, and these chemical profiles can be considered as chemotaxonomic markers.

Table 1: Chemical compositions of roots and aerial parts of *Jatropha pelargoniifolia* (JP) and *J. glauca* (JG) essential oils

Constituents	JPr	JPa	JGr	JGa	RI
	Concentration (%)	Concentration (%)	Concentration (%)	Concentration (%)	
Butyric acid	-	-	-	0.018	780
Isovaleric acid	0.006	-	-	-	834
Diacetone alcohol	-	-	-	0.031	844
Elemyl acetate	-	-	0.112	-	859
8-α-Pinenol	0.016	-	-	-	939
1-mercapto-3-pentanone	-	-	-	0.006	947
Phenol	0.013	-	-	-	965
Capronic acid	0.005	-	-	0.015	987
Eucalyptol	0.010	-	-	-	1033
Benzenacetaldehyde	-	0.030	-	0.081	1043
1,2,4-Trithiolane	-	0.039	-	-	1065
cis-Linalool oxide	0.013	-	-	-	1074
Heptanoic acid	0.010			-	1083
Guaiacol	0.998	0.310	0.557	-	1086
trans-Linalool oxide	0.012	-	-	-	1088
3-mercapto-1-hexanol	-	-	-	0.073	1094
Linalool	0.005	-	-	-	1098
2-Phenyl-1-propanol	-	-	-	0.093	1099
2-Phenylethyl alcohol	0.004	-	-	0.107	1110
Fenchol	0.018	-	-	-	1112
Camphene hydrate	0.065	-	-	-	1148
2,3-Dimethylphenol	-	-	-	0.067	1151
1,8-Epoxy-2-*p*-Exo-Menthanol	0.004	-	-	-	1158
4-Vinylguaiacol	-	-	-	0.139	1162
Borneol	0.003	-	-	-	1165
1-Phenyl-1,2-propanedione	-	-	-	0.009	1166
4-Terpinenol	0.527	-	-	-	1177
8-*p*-Cymenol	0.008	-	-	-	1183
γ-Terpineol	-	-	0.012	-	1185
Caprylic acid	0.024	-	0.073	-	1187
Butyl-*n*-hexanoate	0.006	-	-	-	1188
α-Terpinol	0.128	-	0.036	-	1189
cis-Dihydro carvone	-	-	-	0.443	1193
Myrtenol	0.030	-	-	-	1194
4-Vinyl-phenol	0.108	0.075	-	0.085	1229
Benzylacetone	-	-	-	0.232	1240
p-Anisaldehyde	-	0.299	-	0.133	1252
Pelargonic acid	-	-	0.035	0.074	1260
Bornyl acetate	0.014	-	-	-	1285
Indole	-	0.109	-	0.033	1288
Thymol	-	0.265	-	-	1290
trans-Methylgeraniate	-	-	0.016	-	1323
Methylanthranilate	-	0.100	-	0.109	1337
Dehydroionene	-	-	-	0.095	1342
α-Cubebene	0.511	-	-	-	1351
Eugenol	0.004	-	-	0.270	1356
Octadecanal	0.157	-	0.292	0.150	1357
Cyclosativene	0.006	-	-	-	1368
α-Copaene	0.589	-	-	-	1376
β-Damascenone	-	-	-	0.218	1377

Table 1: Cont.

Constituents	JPr	JPa	JGr	JGa	RI
	Concentration (%)	Concentration (%)	Concentration (%)	Concentration (%)	
β-Elemene	0.061	-	-	-	1382
Vanillin	0.042	0.085	0.045	0.107	1391
α-Cyperone	0.109	-	-	-	1398
Capric acid	0.039	0.067	0.097	-	1399
cis-Isoeugenol	0.104	-	0.080	0.026	1402
Caryophylladiene	0.074	-	-	-	1404
α-Gurjunene	0.030	-	0.023	-	1409
Caryophyllene	0.555	-	-	-	1418
α-Ionone	-	0.135	-	0.092	1426
α-Guaiene	0.125	-	-	-	1439
E-β-Farnesene	0.049	-	-	-	1458
Alloaromadendrene	0.091	-	-	-	1461
β-Ionone	-	-	-	1.254	1463
n-Geranyl propanoate	-	-	0.060	0.101	1475
2,6-di(t-Butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	0.011	0.193	0.039	0.317	1478
β-Selinene	0.071	-	-	-	1485
Epi-Bicyclosesquiphellandrene	0.809	-	-	0.713	1490
Valencene	0.168	-	-	-	1491
cis-Methyl isoeugenyl	0.067	-	-	-	1492
α-Selinene	0.095	-	0.029	0.500	1494
β-Dihydroagarofuran	0.157	-	-	-	1496
δ-Selinene	0.028	-	-	-	1497
α-Muurolene	1.317	-	-	-	1499
δ-Guajene	1.336	-	0.302		1505
Dihydro actinidiolide	-	0.030	-	0.228	1508
Butylated hydroxytoluene	0.032	0.474	0.768	2.551	1512
δ-Cadinene	0.379	-	-	-	1524
α-Calacorene	0.647	-	-	-	1542
Elemol	0.628	0.666	0.688	-	1549
Pseudoionone	-	0.134	-	0.176	1563
E-Nerolidol	-	1.372	-	0.780	1564
Spathulenol	-	-	0.324		1576
Caryophyllenoxide	-	-	0.875	0.247	1581
Guajoxid	0.315	-	-	-	1595
Cedrol	1.140	-	-	-	1596
Germacrene B	-	1.610	-	-	1600
epi-Cedrol	8.191	-	5.592	-	1611
Isolongifolanone	2.679	-	-	-	1618
10-*Epi*- γ-Eudesmol	1.447	0.380	1.821	0.480	1619
5-Guaien-11-ol	14.429	5.212	22.863	0.782	1625
γ-Eudesmol	35.309	6.941	28.817	0.425	1630
δ-Cadinol	0.383	1.279	0.279	1.719	1645
4-α-Hydroxy dihydro agarofuran	0.388	3.221	3.523	-	1648
Bulnesol	4.454	-	1.603	-	1666
Tetradecanol	-	-	0.115	0.336	1676
Pentadecanal	-	-	0.254	0.249	1693
Benzyl benzoate	-	-	-	0.754	1762
Ethyl myristate	0.065	-	-	0.241	1777
1-Octadecene	-	-	0.202	0.579	1793

Table 1: Cont.

Constituents	JPr	JPa	JGr	JGa	RI
	Concentration (%)	Concentration (%)	Concentration (%)	Concentration (%)	
Hexadecanal	-	-	0.076	0.352	1795
Cryptomeridiol	0.510	4.886	-	-	1808
Neophytadiene	-	3.382	-	4.442	1841
Myristic acid	0.760	2.615	1.767	1.282	1842
8,11-Heptadecadienal	0.262	-	0.286	-	1855
Benzylsalicylate	-	-	1.120	-	1863
Pentadecanoic acid	0.350	0.988	0.343	0.328	1902
Methyl hexadecanoate	0.023	0.686	0.650	0.477	1927
Isophytol	-	1.412	-	1.667	1944
3,4-Dimethoxycinnamic acid	3.828	-	-	-	1949
Palmitic acid	2.689	11.529	8.834	20.431	1972
Ethyl palmitate	0.118	0.144	-	-	1993
Phytol	-	24.185	1.771	33.052	1999
Eicosane	1.411	0.365	-	-	2000
Geranyl linalool	0.227	-	0.910	0.359	2020
Methyl linoleate	-	-	-	1.005	2071
Methyl oleate	-	-	-	0.475	2077
Methyl stearate	-	-	-	0.537	2133
4-Vinylguaiacol	0.076	0.075	0.092	0.095	2156
α-Linoleic acid	4.196	4.361	4.970	2.470	2173
Oleic acid	5.232	-	1.504	0.338	2175
Bovolide	-	0.559	-	0.753	2179
Stearic acid	0.732	0.518	0.263	0.819	2192
E-Phytol acetate	-	6.112	-	0.583	2221
5,9-Farnesyl propanone	0.417	0.428	0.074	0.414	2377
Tetracosane	-	-	-	0.122	2400
n-Pentacosane	-	-	0.638	1.857	2500
Dehydrodiisoeugenol	0.041	-	-	-	2723
Squalane	-	0.503	-	0.706	2808
Nonacosane	-	0.659	-	0.608	2900
Triacontane	-	0.487	-	1.530	3000
Number of identified components	80	42	45	68	
Oxygenated monoterpenes	0.849	0.630	0.958	0.802	
Monoterpenes hydrocarbons	-	0.134	-	0.176	
Oxygenated sesquiterpenes	70.366	23.957	66.385	4.433	
Sesquiterpenes hydrocarbons	6.941	1.610	0.354	1.213	
Oxygenated diterpenes	-	32.227	1.771	35.302	
Diterpenes hydrocarbons	-	3.382	-	4.442	
Fatty acids	14.629	20.949	19.706	29.362	
Others	7.205	4.031	3.656	14.110	
Total percentages	**99.99**	**86.92**	**92.83**	**89.84**	

*Retention index (RI) relative to *n*-alkanes on the HP-5 capillary column.

Table 2: Conentration (%) of major components of essential oils form different *Jatropha* species

Major composition	% Concentration							
	JPr	**JPa**	**JGr**	**JGa**	**JRr**	**JCs**	**JG**	**JMl**
γ-Eudesmol	35.30	6.90	28.80	0.40	-	-	-	-
5-Guaien-11-ol	14.40	5.20	22.90	0.80	-	-	-	-
epi-Cedrol	8.20	-	5.60	-	-	-	-	-
Phytol	-	24.20	1.80	33.10	-	-	3.90	41.40
β-pinene	-	-	-	-	9.20	-	-	-
Isoeugenol methyl ether	-	-	-	-	8.50	-	-	-
β-vatirenene	-	-	-	-	8.40	-	-	-
α-gurjunene	0.03	-	0.02	-	7.00	-	-	-
α-pinene	-	-	-	-	6.40	-	-	-
p-menth-1-en-8-ol	-	-	-	-	5.20	-	-	-
Cedrol	-	-	-	-	0.20	-	-	-
Oleic acid	5.20	-	1.50	0.34	-	40.1	-	-
α-linoleic	4.20	4.40	5.0	2.50	-	37.60	-	-
Palmitic acid	2.70	11.50	8.80	20.40	-	15.60	-	-
Stearic acid	0.73	0.52	0.26	0.82	-	8.80		-
Germacrene-4-ol	-	-	-	-	-		23.30	1.62
Hexahydrofarnesylacetone	-	-	-	-	-	-	15.40	-
δ-cadinene	0.38	-	-	-	-	-	7.70	-
tetradecanal	-	-	-	-	-	-	6.80	-
cubenol	-	-	-	-	-	-	6.10	-
Spathulenol	-	-	0.32	-	0.70	-	-	12.80
α-Copaene	0.59	-	-	-	-	-	4.1	-
epi-α-cadinol	-	-	-	-	-	-	-	9.30
Germacrene D	-	-	-	-	-	-	-	4.50
α-cadinol	-	-	-	-	-	-	-	3.90
β-Ionone	-	-	-	1.25	-	-	2.00	-
Elemol	0.63	0.67	0.69	-	-	-	-	3.60
α-Muurolene	1.317	-	-	-	-	-	0.90	-
Caryophyllene oxide	-	-	0.87	0.25	-	-	-	4.80
Number of identified components	80	42	45	68	49	N	28	20
Oxygenated monoterpenes	0.85	0.63	0.96	0.80	12.70	N	2.80	-
Monoterpenes hydrocarbons	-	0.13	-	0.18	26.80	N	1.40	-
Oxygenated sesquiterpenes	70.37	23.96	66.39	4.43	22.10	N	56.60	42.86
Sesquiterpenes hydrocarbons	6.94	1.61	0.35	1.21	20.90	N	17.70	12.14
Oxygenated diterpenes	-	32.23	1.77	35.30	-	N	-	41.38
Diterpenes hydrocarbons	-	3.38	-	4.44	-	N	3.90	-
Fatty acids	14.63	20.95	19.71	29.36	-	N	10.00	-
Others	7.21	4.03	3.66	14.11	8.90	N	5.5	3.62
Total percentages	**99.99**	**86.92**	**92.83**	**89.84**	**91.40**	**N**	**97.9**	**100**

(-) = Unidentified, (N) = Not reported, *J. pelargoniifolia* root (JPr), *J. pelargoniifolia* aerial (JPa), *J. glauca* root (JGr), *J. glauca* aerial (JGa), *J. ribifolia* root (JRr), *J. curcas* seed (JCs), *J. gossypifolia* (JG) and *J. mutabilis* leaves (JMl).

4.5. Conclusions

To our knowledge, this is the first report of chemical profiles of essential oils from roots and aerial parts of two plants, *J. pelargoniifolia* and *J. glauca*, native to Saudi Arabia. The clearly different chemical composition of the essential oils of the two *Jatropha* species indicates that the chemical profile represents a valuable taxonomic character that can be useful as a support in solving problems in terms of classification of *Jatropha* species. The distribution of sesquiterpene, diterpene, and monoterpene hydrocarbons and their oxygenated derivatives could be used as differentiating parameters for the various species. However, the existence of intraspecific chemical differences (chemical races) must be kept in mind when making use of chemical characters in plant taxonomy.

4.6. References

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Chapter 5

Phytochemical and Biological Investigation of *Jatropha pelargoniifolia* Roots Native to the Kingdom of Saudi Arabia

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Hanan Yahya Aati (H.A), Ali A. El-Gamal (A.A.G) and Oliver Kayser (O.K) conceived and designed the experiments; H.A performed the experiments; H.A., A.A.G analyzed the data; H.A contributed reagents/materials/analysis tools; H.A wrote the paper; A.A.G participated in the experiments design and coordination and helped to draft the manuscript; O.K participated in the experiment design and supervised; Atallah F. Ahmed (A.F.A) contributed to the structure elucidation of some isolated natural products and to writing a part of the manuscript.

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5.1. Abstract

Extensive phytochemical analysis of different root fractions of *Jatropha pelargoniifolia* Courb. (Euphorbiaceae) has resulted in the isolation and identification of 22 secondary metabolites. 6-hydroxy-8-methoxycoumarin-7-*O*-*β*-D-glycopyranoside (**15**) and 2-hydroxymethyl *N*-methyltryptamine (**18**) were isolated and identified as new compounds along with the known diterpenoid (**1**, **3**, **4**, and **7**), triterpenoid (**2** and **6**), flavonoid (**5**, **11**, **13**, **14**, and **16**), coumarinolignan (**8**–**10**), coumarin (**15**), pyrimidine (**12**), indole (**17**, **18**), and tyramine-derived molecules (**19**–**22**). The anti-inflammatory, analgesic, antioxidant and antipyretic activities were evaluated for fifteen of the adequately available isolated compounds (**1**–**6**, **8**–**11**, **13**–**14**, **16**, **21**, and **22**). Seven (**4, 6**, **10**, **5, 13**, **16**, and **22)** of the tested compounds showed a significant analgesic effect ranging from 40% to 80% at 10 mg/kg in two *in vivo* models. Compound **1** could also prove its analgesic property (67.21%) when it was evaluated on a third *in vivo* model at the same dose. The *in vitro* antioxidant activity was also recorded where all compounds showed the ability to scavenge nitric oxide (NO) radical in a dose-dependent manner. Moreover, eight compounds (**1**, **4**, **5**, **6**, **10**, **13**, **16**, and **22**) out of the fifteen tested compounds exhibited considerable *in vivo* anti-inflammatory activity which reached 64.91% for compound **10** at a dose of 10 mg/kg. Moreover, the tested compounds exhibited an antipyretic effect in a yeast-induced hyperthermia in mice. The activity was found to be highly pronounced with compounds **1**, **5**, **6**, **10**, **13**, **22** and **16** which decreased the rectal temperature to about 37 °C after 2 h of the induced hyperthermia (~39 °C) at a dose of 10 mg/kg. This study could provide scientific evidence for the traditional use of *J. pelargoniifolia* as an anti-inflammatory, analgesic, and antipyretic.

5.2. Introduction

Euphorbiaceae is considered as one of the largest families of flowering plants which includes approximately 7800 species that are distributed among 300 genera and five subfamilies in tropical and subtropical regions [1, 2]. Among the main genera of this family, *Jatropha* L. is represented by approximately 200 species [2]. *Jatropha* species are used in folk medicine to treat various diseases, such as skin inflammation, eye infection, chest pain, stomach pain, itching, and as a vermifuge, or as ornamental plants and energy crops in Latin America, Africa, and Asia [3]. *J. gossypiifolia*, *J. elliptica*, *J. curcas*, and *J. mollissima*, among other species of *Jatropha*, have been reported for their chemical constituents, biological activities, and medicinal uses [4]. *Jatropha glauca*, *J. curcas*, *J. spinose*, and *J. pelargoniifolia* are the only four species that are distributed in Saudi Arabia and are employed as traditional herbal medicines, owing to their anti-inflammatory, antioxidant, antiseptic, and analgesic properties [5, 6].

Jatropha pelargoniifolia Courb. of the current study is grown as a shrub and is widely known as "Obab" in Arabic. It is widely distributed in East Tropical Africa (Sudan, Eritrea, Ethiopia, Somalia, and Kenya) and the Arabian Peninsula (Yemen, Oman, and Saudi Arabia) [7]. The plant is sometimes collected from the wild for local medicinal use, especially the petiole sap which is applied to treat ulcers, severe skin inflammation, and for wound healing [7].

Previous phytochemical studies on the plants belonging to the genus *Jatropha* revealed a broad range of isolated secondary metabolites, such as diterpenoids, triterpenoids, non-conventional coumarino-lignans, alkaloids, coumarins, flavonoids, cyclic peptides, and steroids [8, 9]. However, accordingly reviewed by Zhang *et al*. [8], the main compounds isolated from *Jatropha* genus are the terpenoids. *Jatropha gossypiifolia* was subjected to extensive phytochemical studies that resulted in the isolation of many secondary metabolites, such as propacin, venkatasin, citlalitrione, ricinine, apigenin, jatropholones A & B, and jatrophone [4]. Moreover, curcusones A–D, taraxerol, nobiletin, curacyclines A & B, as well uracil, have been isolated from *J. curcas*. [5, 6, 8]. Additionally, many reported studies showed the isolation of multidione, multifidone, multifolone, and multifidol glucoside from *J. multifida*, while from *J. podagrica, there* was japodic acid, erythrinasinate, γ-sitosterol, japodagrin, and podacyclines A & B [3, 8, 9]. This is indeed a reflection of the versatility of the

enzymatic system that is present in Euphorbiaceous plants, however nothing was reported regarding *J. pelargoniifolia*. Thus, it was of interest to explore the active constituents and their biological activity to provide evidence to support the old traditional use of *J. pelargoniifolia.*

5.3. Result and Discussion

5.3.1. Isolation of compounds

The alcoholic extract of *J. pelargoniifolia* roots powder was successively partitioned with petroleum ether (60 °C), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and then *n*-butanol (*n*-BuOH) to give the correspondent organic fractions. Each fraction was subjected to chromatographic separation on normal and reversed phase (RP) silica gel to yield compounds **1**-**16** from petroleum ether, CH_2Cl_2, and EtOAc fractions. Furthermore, the organic extract that was obtained after an acid-base treatment of the roots powder was isolated on a normal silica gel column which was followed by purification on RP-HPLC and/or crystallization to afford compounds **17**-**22** (Figure 1).

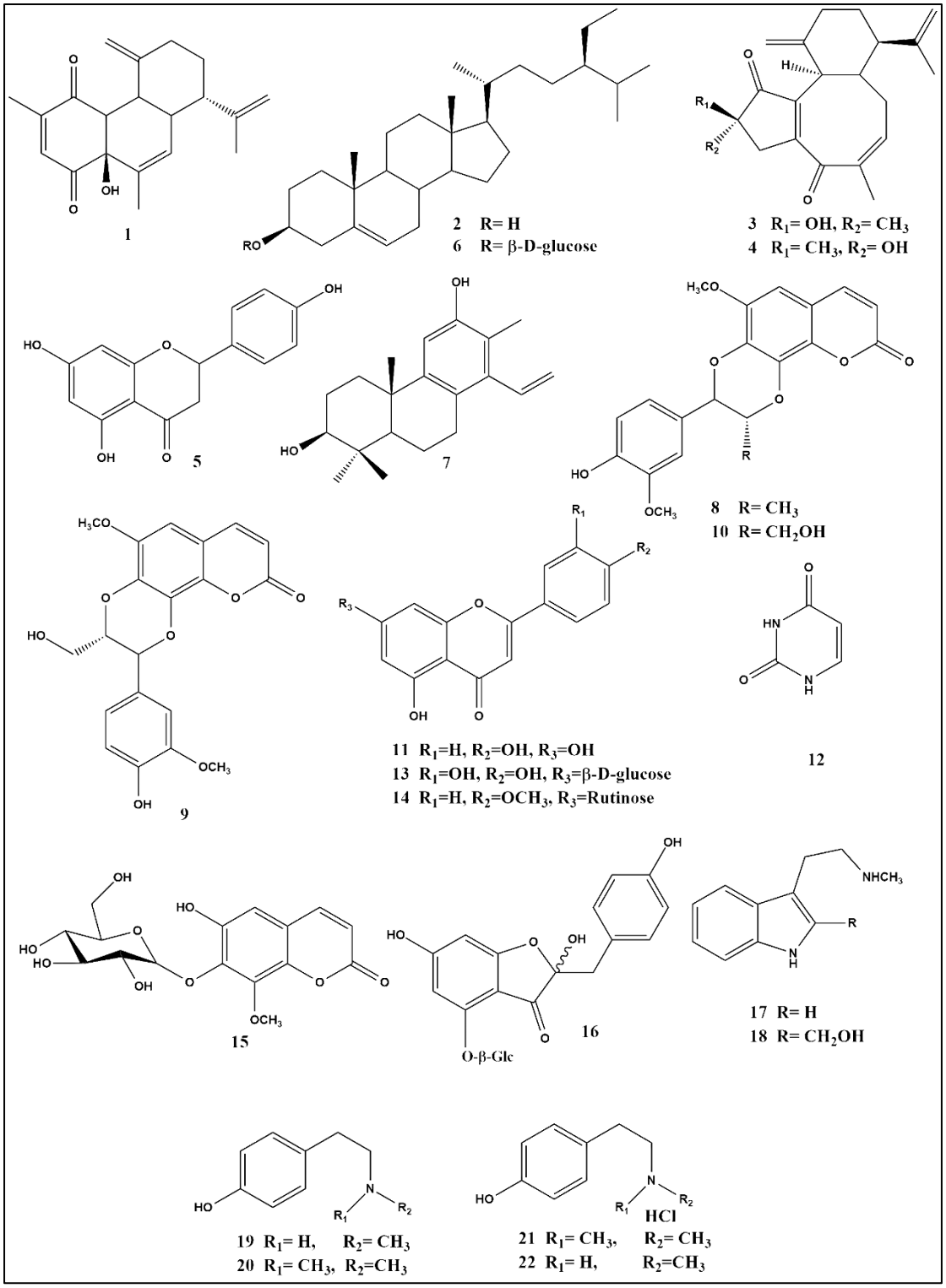

Figure 1. Chemical structures of the compounds isolated from the roots of *Jatropha pelargoniifolia*

5.3.2. Structure elucidation

The new compound **15** was obtained as white crystals. The NMR and ESIMS (Electronspray Ionization Mass Spectrometry) data established the molecular formula of **15** to be $C_{16}H_{18}O_{10}$. The IR absorption bands at max 3349, 1719, and 1625 cm^{-1} suggested the presence of hydroxyl, ester carbonyl, and aromatic functionalities, respectively. Furthermore, the ^{13}C nuclear magnetic resonance (NMR) spectrum of **15**, which was measured in deuterated methanol (CD_3OD, displayed sixteen signals of nine sp^2 and seven sp^3 carbons (including that of a methoxyl group). Its ^1H NMR spectrum showed a pair of ortho-coupled protons at δ_H 6.26 and 7.88 (each, 1H, d, J = 9.5 Hz) as was observed by ^1H-^1H correlated spectroscopy (COSY) that was assignable to H-3 and H-4 of an α-pyrone ring system of a coumarin, respectively [10]. This was further evidenced from the ^{13}C NMR carbon signals of the pyrone at δ_C 163.5 (C, C-2), 146.5 (CH, C-4), 144.4 (C, C-8a), 116.2 (CH, C-3), and 112.7 (C, C-4a) (Table 1). Moreover, the single aromatic singlet appearing at δ_H 7.00 (1H, s) suggested **15** to be a trisubstituted coumarin. Six proton signals at δ_H 3.30–4.99 ppm, a doublet of an anomeric proton at δ_H 4.99 (1H, d, J = 7.8 Hz), and six ^{13}C NMR signals at δ_C 106.2 (CH, C-1`), 75.5 (CH, C-2̀), 77.8 (CH, C-3`), 71.0 (CH, C-4̀), 78.5 (CH, C-5`), and 62.2 (CH_2, C-6̀) indicated the presence of a β-D-glucopyranosyl substituent. An aromatic methoxy substituent (δ_H/δ_C 3.91/57.0) was also revealed. The $^3J_{CH}$ correlations that were observed in the heteronuclear multiple bond correlation (HMBC) spectrum linked these two substituents to the coumarin carbons at δ_C 133.2 and 147.5, respectively (Figure 2). Thus, a hydroxy group should represent the third substituent on the coumarin carbon at δ_C 145.7. The long-range correlations (HMBC) that were found from H-5 (δ_H 7.00, 1H, s) to the carbons at δ_C 146.5 (C-4), 145.7, C-8a (δ_C 144.4), and δ_C 133.2 (C-7) indicated that C-8 (δ_C 147.5) is the position of the methoxyl group. To confirm the locations of the glucosyl and hydroxyl groups, the NMR data of **15** were further compared to those of 5-hydroxy-7-methoxycoumarin-8-O-β-D-glucoside and other closely related coumarin derivatives that were previously isolated from *Daphne pseudo-mezereum* [11] and *Tetraphis pellucida* [12], respectively. The structure of compound **15** was thus established as a new natural product and was identified as 6-hydroxy-8-methoxy coumarin-7-*O*-*β*-D-glycopyranoside.

Table 1. The ^{1}H (600MHz, δ in ppm, *J* in Hz) and ^{13}C NMR (125MHz, δ in ppm) spectral data for compound **15** in deuterated methanol (CD_3OD)

Position	**δ_H**	**δ_C**
2	-	163.5
3	6.26 (d, *J* = 9.5 Hz, 1H)	116.2
4	7.88 (d, *J* = 9.5 Hz, 1H)	146.5
5	7.00 (s, 1H)	106.1
6	-	145.7
7	-	133.2
8	-	147.5
4a	-	112.7
8a	-	144.4
1'	4.99 (d, *J* = 7.8 Hz, 1H)	106.2
2'	3.57 (dd, *J* = 9.4, 9.4, 1H)	75.5
3'	3.46 (d, *J* = 1.9, 1H)	77.8
*4'	3.47 (brs, 1H)	71.0
*5'	3.30 (brs, 1H)	78.5
6'	3.72 (d, *J* = 4.9, 1H) 3.80 (d, *J* = 2.4, 1H)	62.2
OCH_3-8	3.91 (s, 3H)	57.0
OH-6	10.53	-

* Overlapped with solvent signal.

Compound **18** was isolated from the organic extract of the acid-base treated root powder as white needle-shaped crystals. It produced a positive Dragendorff's test, indicating its alkaloid nature. The IR absorption band with a spike at max 3309 cm^{-1} suggested the presence of hydroxyl and/or secondary amine functionality. The UV absorptions at max 295, 287, 279, 230 nm in MeOH were characteristic to an indole chromophore. The COSY correlations (Figure 2) disclosed the ABCD system of the aromatic protons at δ_H 7.41/7.00 (Table 2), which is consistent with 2,3-disubstituted indole alkaloids. A side chain of an ethylene and a *N*-methyl was linked to C-3 of the indole as it was manifested by 2D NMR correlations. However, comparison of ^{1}H NMR data of **18** with those of *N*-methyltryptamine (**17**) revealed that the ^{1}H proton singlet at position 2 in **17** was replaced in **18** by a 2H singlet of a hydroxymethyl proton at δ_H 3.91 ppm. The ^{13}C NMR spectroscopic and ESIMS data of **18** was thus consistent with a molecular formula $C_{12}H_{16}N_2O$ of 30 mass units more than that of **17** ($C_{11}H_{14}N_2$). Furthermore, the HMBC correlation that was found from the methylene protons (δ_H 3.91, 2H, s) to C-2 and C-3 confirmed its C-2 location of the hydroxymethyl group. Finally, a full analysis of th COSY and HMBC spectral correlations (Figure 2) assigned the structure of compound **18** to be 3-(2-(methylamino) ethyl)-1H-indol-2-yl) methanol or 2-hydroxymethyl *N*-methyltryptamine, a new indole alkaloid.

Table 2. The ^{1}H (700MHz, δ in ppm, J in Hz) and ^{13}C NMR (125MHz, δ in ppm) spectral data for compound **18** in deuterated methanol (CD3OD)

Position	**δ_H**	**δ_C**
2	-	128.0
3	-	107.2
3a	-	130.6
4	7.29 (d, *J* = 7.8 Hz, 1H)	112.0
5	7.07 (t, *J* = 7.8 Hz, 1H)	122.4
6	7.00 (t, *J* = 7.8 Hz, 1H)	120.0
7	7.41 (d, *J* = 7.8 Hz, 1H)	118.6
7a	-	138.1
αCH_2	3.10 (t, *J* = 5.8 Hz, 2H)	54.1
βCH_2	3.00 (t, *J* = 5.8 Hz, 2H)	21.5
CH_3	2.69 (s, 3H)	44.9
CH_2OH	3.91 (s, 2H)	52.9

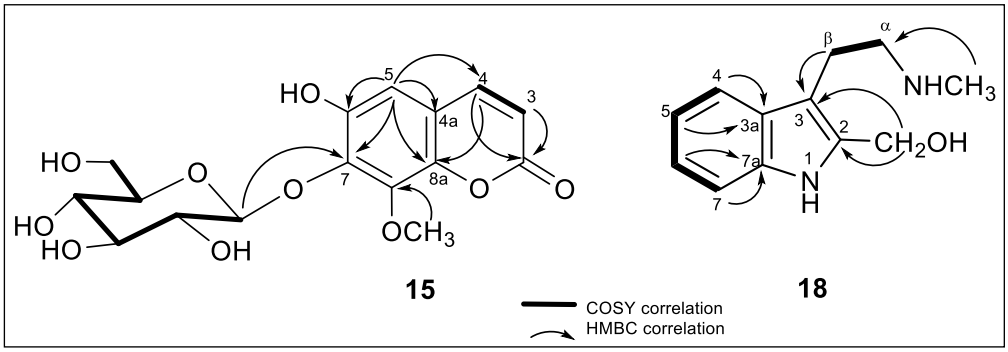

Figure 2. Selected heteronuclear multiple bond correlation (HMBC) and correlation spectroscopy (COSY) correlations of compounds **15** and **18**.

Compounds **1-14**, **16-17** and **19-22**, which were also isolated from *J. pelargoniifolia* roots, were found to be identical to the previously reported natural products by comparison of their spectroscopic (IR, MS, and NMR) data and were identified as jatrophadiketone (**1**) was isolated from the roots of *J. curcas* [13], β-sitosterol (**2**) isolated from *J. curcas* seed kernels and from the methanolic extract of the root bark of *Calotropis gigantean* (Linn)., [14, 15], curcuson D (**3**) and curcuson C (**4**) were isolated from *J. curcas* root extract [16], naringenin (**5**) was isolated from the root extract of *J. gossypifolia* [17,18], β-sitosterol glucoside (**6**) was isolated from the leave and twig extract of *J. curcas*, [19], spruceanol (**7**) was isolated from both the aerial extract of *J. divaricate* and the bark extract of *Aleurites moluccana* [20, 21], propacin (**8**), cleomiscosin B (**9**), cleomiscosin A (**10**) compounds **8** and **10** were

isolated from whole plant extracts of *J. gossypifolia*, while compound **9** was isolated from *Mallotus apelta* [22–26], apigenin (**11**) was identified in *J. gossypifolia* [4,27], uracil (**12**) was isolated from the leaves of *J. curcas* [28–30], cynaroside (**13**) was identified in *Scabiosa atropurpurea* aerial parts extract [31], linarin (**14**) was isolated from the extracts of aerial parts of both *Bupleurum chinense* and *Valeriana officinalis* [32,33], hovetricoside C (**16**) was separated from *Artocarpus tonkinensis* [34], *N*-methyltryptamine (**17**) was isolated from *Zanthoxylum arborescens* [35], *N*-methyltyramine (**19**) was isolated from a beer [36], and hordenine (**20**) was separated and identified from *Ephedra aphylla* that was growing in Egypt [37]. Compounds **21** (hordenine HCl) and **22** (*N*-methyltyramine HCl) were identical to the authentic samples that were purchased from Sigma-Aldrich (St Louis, MO, USA), and on the basis of their ^{1}H NMR and TLC co-chromtographic data, they were isolated previously from *Ariocarpus kotschoubeyanus* [38].

5.3.3. Biological activity

The alcoholic extract of *J. pelargoniifolia* was found to possess significant anti-inflammatory, analgesic, and antipyretic activities when it was tested on *in vivo* models in a dose-dependent manner [39]. This prompted us to extend the study of these activities on the isolated compounds. The anti-inflammatory, analgesic, antipyretic, and antioxidant activities for the compounds which have been isolated in good yields (**1**–**6**, **8**–**11**, **13**–**14**, **16**, **21**, and **22**) were thus evaluated for their analgesic, anti-inflammatory, antipyretic, and antioxidant activities using *in vivo* and *in vitro* models. The analgesic activities were assessed in mice via acetic acid-induced writhing, hot-plate, and tail-flick methods.

In the acetic acid-induced writhing method, compounds **1**, **5**, **4**, **6**, **10**, **13**, **14**, **16**, and **22** showed a dose-dependent analgesic activity by the reduction in the number of writhings. However, the diterpenoids (**1** and **4**), β-sitosterol glucoside (**6**), flavonoids (**5** and **13**)**,** and tyramine HCl (**22**) exhibited the strongest analgesic activity by inhibiting writhing in mice (49.07-65.74% inhibition) at a dose of 10 mg/kg compared with the standard antinociceptive drug (indomethacin), which showed 72.68% reduction in the number of writhings at a concentration of 4 mg/kg (Table 9S). The coumarinolignan (**8**) and hordenine HCl (**21**) did not show any inhibition either at 5 or at 10 mg/kg.

In the hot plate method, the thermal responses in the mice that were treated with selected compounds after half, one, and two hours were significantly reduced ($p < 0.01$). Especially in the mice that were treated with a dose 10 mg/kg of compounds **22, 10, 6, 4, 5, 13, 16**, and **14**, the antinociceptive effects were reduced by 78.57, 76.19, 74.46, 73.33, 69.56, 59.57, 53.48, and 34.88%, respectively (Table 10S). While in the tail-flick method, the tested animals that were treated with 10 mg/kg of compounds **22, 1, 10, 4, 13, 16, 5, 6, 21, 3**, and **9** showed a significant ($p < 0.01$ and 0.05) reduction in antinociceptive activity (67.32, 67.21, 60.45, 57.59, 54.04, 53.59, 48.58, 40.30, 21.75, 20.39, and 10.87%, respectively) compared with indomethacin (95.61%), as depicted from Table 11S. The obtained results confirmed that the strong analgesic activity that is exhibited by the roots of *J. pelargoniifolia* could be due to its bioactive compounds that may exert their analgesic activities through different CNS (Central Nervous System) mechanisms (peripheral and central). Therefore, further studies with purified compounds should be conducted in the future for further pharmacological and toxicological characterization in order to elucidate the mechanisms that are involved in the central analgesic effect of these compounds.

The anti-inflammatory activities of the major isolated compounds were evaluated by using the carrageenan-induced paw edema model in rats. It was found that the size of the edema was significantly reduced ($p < 0.05, 0.01$) in the animals that were treated with the low doses of 5 and 10 mg/kg compared with the standard anti-inflammatory drug (phenylbutazone) at a high dose of 100 mg/kg. The rats that were treated with compounds **10**, **16**, **1**, **5**, **6**, **22**, **4**, **13**, **3**, **8**, **9**, **14**, and **21** exhibited a significant reduction in their hind paw edema in a dose-dependent manner. Therefore, at a dose of 5 mg/kg, the edema size was reduced by 20.44, 47.23, 17.95, 45.85, 33.97, 44.47, 13.53, 26.51, 7.18, 2.20, 5.52, 6.35, and 6.35, respectively, while at 10 mg/kg, the edema size was reduced by 64.91, 55.24, 54.94, 51.38, 51.10, 50.27, 49.17, 48.61, 13.53, 12.98, 10.22, 10.22, and 8.01%, respectively relative to that reduced by phenylbutazone at 100 mg/kg (69.06%) which was almost similar to that produced by a dose of 10 mg/kg of compound **10** (64.91%; Table 12S). These anti-inflammatory results were almost compatible with those of the above mentioned antinociceptive activity for the tested compounds.

In addition, the isolated compounds from the *J. pelargoniifolia* roots were tested for their antipyretic activity against yeast-induced hyperthermia in mice. All tested compounds, which were administered at doses of 5 and 10 mg/kg, showed a considerable reduction in the rectal temperature of the hyper-thermic mice, ranging between 36.733 ± 0.136 °C and 38.650 ± 0.111°C as compared with the hypothermic effect (36.333 ± 0.112°C) resulting from indomethacin administration (Table 13S). Moreover, compounds **5**, **6**, **10**, **13**, and **16** displayed about a 1°C reduction in temperature less than that of the yeast-induced hyperthermia control (~ 38.8 °C) in the first 30 min. of the experiment.

The percentage inhibition ± SD of the nitric oxide-scavenging activity was determined for the selected compounds at concentrations of 20, 40, 60, 80, and 100 µg/mL, and the obtained results were compared with a standard antioxidant drug (ascorbic acid). Compounds **22**, **4**, **2**, **10**, **1**, **14**, **21**, **9**, **8**, **11**, **6**, **3**, **13**, **5**, and **16** exhibited significant free radical-scavenging potency when compared with the free radical-scavenging activity of a strong known antioxidant drug (ascorbic acid) 87.230 ± 0.980. The ability of the tested compounds to produce antioxidant effects was found to be concentration-dependent. At a 100 µg/mL dose, the % inhibition ± SD of the tested compounds were 77.600 ± 4.220, 77.360 ± 4.220, 76.830 ± 5.010, 75.260 ± 5.540, 71.660 ± 0.700, 70.360± 14.730, 67.670 ± 5.750, 63.670 ± 12.850, 63.670 ± 12.850, 57.000 ± 10.210, 56.540 ± 6.030, 38.300 ± 5.630, 33.060 ± 1.860, 27.000 ± 7.850, and 25.610 ± 5.180, respectively (Table 14S). The significant antioxidant activity that was associated with the administration of *J. pelargoniifolia* roots was perhaps due to its content of several phenolic and polyphenolic compounds which play an important role in free radical-scavenging activity with less cytotoxicity.

It is important to mention here that in our previous study, which was carried out on the crude alcoholic extract of *J. pelargoniifolia* roots, we observed a significant anti-inflammatory activity and analgesic potency [39], likely resulting from the presence of cleomiscosin A, hovetricoside C, jatrophadiketone, naringenin, β-sitosterol glucoside, *N*-methyltyramine HCL, curcuson C, cynaroside, curcuson D, propacin, cleomiscosin B, linarin, and hordenine HCL in good yield. Undoubtedly, a synergistic effect between these bioactive constituents produces significant antinociceptive and anti-inflammatory effects. These results justify the use of this plant in folk medicine for the treatment of

pain and several inflammatory conditions. Further study will be conducted on the pure isolated compounds to investigate the exact mechanisms underlying their promising biological activities.

Our study proved that *J. pelargoniifolia* roots can be considered as a source of several biologically- active compounds such as hordenine, which exhibited various biological activities like inhibiting melanogenesis in human melanocytes, increasing the respiratory and heart rates [40], the stimulation of gastrin release, inhibition of monoamine oxidase B, and antibacterial properties [41]. Furthermore, Chrisitine *et al*. reported that *N*-methyltyramine increases blood pressure in an anaesthetized rat, relaxes guinea pig ileum, and increases both the force and the rate of contraction of guinea-pig right atrium by inducing the release of noradrenaline [42]. Additionally, naringenin has been reported to have several pharmacological properties, including anti-dyslipidemic, anti-obesity and antidiabetic, and antifibrotic [43]. Moreover, cleomiscosin A showed strong anti-inflammatory activity and has analgesic and antipyretic potencies [44]. Curcuson C has been reported to have antipyretic activity *in vivo* [45].

5.4. Materials and Methods

5.4.1. Chemicals and Analytical Instruments

The high-resolution electron spray ionization-mass spectrometry (HRESI-MS) analyses were carried out on an Agilent Triple Quadrupole 6410 LC-MS mass spectrometer (Central Lab. College of Pharmacy, King Saud University (KSU). The infra-red spectra were generally recorded in the potassium bromide pellets, unless otherwise specified, using the FTIR spectrophotometer (FT-IR Microscope Transmission, company, Waltham, MA, USA). The melting points were recorded by using a Mettler FP 80 Central Processor that was supplied with a Mettler FP 81 MBC Cell Apparatus. The spectral data for proton and carbon were measured by using Bruker AVANCE 700, 500, and 600 (College of Pharmacy, KSU and Department of Chemistry in TU Dortmund) (Bruker, Fallanden, Switzerland), resonating at either 700, 500, and 600 MHz for proton or at 125 MHz for carbon. The chemical shift values were expressed in ppm with respect to the internal standard tetramethyl silane (TMS) or residual solvent peak, and the coupling constants (*J*) were recorded in Hertz (Hz). The two-dimensional NMR experiments (COSY, HSQC, and HMBC) were performed

using the standard Bruker program (Bruker, Fallanden, Switzerland). The silica gel 60/230–400 mesh (Qingdao Oceanic Chemical Co., Qingdao, China), RP C18 silica gel 40–63/230–400 mesh (Merck, Darmstade, Germany), and sephadex LH-20 with particle size 18–111 μm (GE Healthcare, USA) were used for column chromatography, while the silica gel and reversed phase 60 F254 (Merck, Germany) were used for thin-layer chromatography (TLC). The detection was achieved by using 10% H_2SO_4 in ethanol or ceric sulfate followed by heating. Alkaloids were tested with Mayer's reagent, Hager's reagent, and Dragendorff's reagent. All of the solvents for analytical purposes (HPLC and analytical grade) and the drugs for biological investigation (sodium nitroprusside, sulphanilamide, λ-carrageenan, acetic acid, ascorbic acid, and phenylbutazone) were procured from Sigma Chemical Company (Sigma-Aldrich, St Louis, MO, USA), and the solvents were distilled prior to use. The preparative and semipreparative Shimadzu HPLC were performed, characterized by Rp-18 (ODS-80 TM, TSK, Tokyo, Japan), 10 μm PS, 30 cm L × 2.15 cm i.d. fitted with a guard column (10 μm PS, 7.5 cm L × 2.15 cm i.d.) (ODS-80 TM, TSK, Tokyo, Japan), and VP 250/10 NUCLEODUR C18 HTec, 6 μm PS, 25 cm L × 2 cm i.d., respectively which both used a PDA detector.

5.4.2. Plant Material

The roots of *J. pelargoniifolia* were harvested from Wadi Mojasas, Jazan district (South of Saudi Arabia) in September, 2015. The plant was authenticated by Dr. Jacob Thomas, a botanist of the Science College Herbarium, KSU, where a voucher specimen (#23064) was deposited.

5.4.3. Animals

Male Wistar rats and white male Swiss albino mice with approximate body weights of 200 g and 20–25 g, respectively, were divided into groups of six animals. The animals were obtained from the Experimental Animal Care Center, College of Pharmacy, KSU. After a 7-day period in animal accommodation, they were divided into groups and were maintained at 12 h:12 h light-dark conditions at 55% humidity. Purina chow rat diet (UAR-Panlab, Barcelona, Spain) and drinking water were supplied to the animals *ad libitum*. The protocols for the present study were based on the recommendations of the Ethical Committee of the Experimental Animal Care Center of KSU (approval number CPR-7569).

5.4.4. Extraction, Fractionation, and Purification

The air-dried powder of the *J. pelargoniifolia* roots (2.5 kg) was divided into two parts—A and B. The 2.0 kg of part A was subjected to solvent extraction, while the remaining 500 g of the root powder (part B) was exposed to the acid-base treatment. Part A was extracted by maceration with 80% ethanol (3 L × 5) for three successive days. This process was repeated until complete exhaustion of the plant material [46]. The alcoholic extract was then concentrated to dryness under reduced pressure at 40°C using a rotary evaporator to give 270 g of the dried alcoholic extract. The dried alcoholic extract was suspended in H_2O and was successively partitioned with petroleum ether, dichloromethane, ethyl acetate, and *n*-butanol (600–700 mL × 3) of each to obtain 13.3, 10.3, 5.1, and 33.6 g, respectively.

A part of the petroleum ether fraction (12.8 g) was chromatographed over silica gel CC (Column Chromatography) using a gradient of petroleum ether/EtOAc followed by methanol (MeOH). The 100 ml fractions of each were collected and screened by TLC, and similar fractions were combined together to give six fractions (A–F). Fraction A which was eluted by 15% EtOAc in petroleum ether (609.8 mg) was further subjected to CC and was eluted by petroleum ether/acetone gradient elution, sub fraction A1 (188.9 mg) which was eluted by 6% acetone in petroleum ether was further purified by preparative HPLC gradient elution using acetonitrile: H_2O: TFA to yield 25.0 mg of compound **1**. Direct crystallization of fraction B, which was eluted by 20% EtOAc in petroleum ether, yielded 302.7 mg of compound **2**. Fractions C and D which were eluted with 30 and 40% EtOAc in petroleum ether, respectively, were crystallized with acetone to yield compounds **3** and **4** (14.3 and 25.4 mg, respectively). Additionally, fraction E (287.3 mg) which was eluted by 50% EtOAc was also crystallized from acetone to give 20.2 mg of compound **5**, while fraction F which was eluted with 40% MeOH in EtOAc yielded 330.4 mg of compound **6**, which was purified by crystallization with acetone.

The dichloromethane (DCM) fraction (9.8 g) was subjected to silica gel CC using a column that was packed by the wet method with petroleum ether. The polarity of the column was gradually increased by treating it with DCM, followed by MeOH to give 142 fractions, and similar fractions were pooled together depending on their TLC similarity. Fraction 48–64 which was eluted by 10% MeOH in DCM was concentrated

(4.6 g) and was then subjected to repeated silica gel CC, followed by a preparative revered phase TLC using MeOH: H_2O (3:1) as a solvent system, leading to the isolation of white crystals of compound **7** (7.5 mg). Moreover, subfractions that were obtained using 90% acetone in petroleum ether, 100% acetone, and 10% acetone in MeOH, followed by crystallization with MeOH, afforded compounds **8** (15.1 mg), **9** (15.9 mg), and **10** (16.4 mg), respectively.

The EtOAC extract (4.6 g) was subjected to silica gel CC using a gradient of DCM/MeOH to give six fractions (I–VI). Fractions I which were eluted with 84% DCM afforded 17 mg of compound **11** after crystallization with MeOH. Fractions II which were eluted with 70% DCM afforded 8.6 mg of compound **12**. The fractions that were eluted with 35 and 40% MeOH in DCM (II and IV) were further purified by repeated acetone crystallization to give 14.9 and 13.2 mg of compounds **13** and **14**, respectively. Fractions V which were eluted with 45% MeOH were further subjected to CC using DCM/MeOH, followed by a semi-preparative HPLC (Rp-18) using MeOH: H_2O: TFA as a solvent system afforded 8.6 mg white crystals of compound **15**. Finally, fractions VI which were eluted with 50% MeOH in DCM were subjected to further purification over sephadex LH-20 (using water and methanol as an eluent in the gradient mode). The subfraction VI–A, which was eluted by 20% H_2O/MeOH was further purified over a reversed-phase column to give 12 mg of compound **16**.

Furthermore, Part B was subjected to an acid-base treatment according to the Stas-Otto method I which was described by Mandhumitha and Fowsiya [47]. The crude alkaloidal fraction was subjected to silica columns using gradient elution with solvent system DCM: MeOH: NH_4OH, resulting in five fractions. The first fraction which was eluted using 17% MeOH in DCM with an addition of 1% NH_4OH was followed by further purification by reversed-phase semipreparative HPLC using MeOH: H_2O: TFA to give compound **17** (6.3 mg). The second fraction which was separated by 20% MeOH in DCM to afford a subfraction, which was further purified by a semipreparative HPLC gradient elution using MeOH: H_2O: TFA as a solvent system, afforded white needle crystals of compound **18** (9.4 mg). The third and fourth fractions which were eluted by 23 and 26% MeOH in DCM, followed by an addition of a few drops of NH_4OH, afforded 6.8 and 8.8 mg of compounds **19** and **20**, respectively. The fifth fraction was eluted by 60% MeOH in DCM with an addition of a few NH_4OH drops to yield 86.7 mg of a mixture of two compounds, which were subjected to further

purification using the reversed-phase semipreparative HPLC in gradient mode with MeOH: H_2O: TFA as the mobile phase, resulting in the production of the white crystals of compounds **21** and **22** (20.3 and 21.5 mg), respectively.

6-hydroxy-8-methoxycoumarin-7-*O*-*β*-D-glycopyranoside (compound **15**): White crystals; m.p. 219–220 °C; UV (MeOH) λ_{max} nm 325 and 250; IR (KBr) νmax (cm^{-1}): 3349, 1719, 1625, 1520, 1465, 829; ^1H NMR, ^{13}C NMR, and HMBC data, see Table 1 and Figure 2; HRESIMS (positive) *m*/*z* 371.0900 $[M + H]^+$ (calculated for $C_{16}H_{18}O_{10}$, 371.097825).

3-(2-(methylamino)ethyl)-1H-indol-2-yl)methanol (compound **18**): White needle crystals; m.p. 179–189 °C; UV (MeOH) λ_{max} nm: 295, 287, 279, 230; IR (KBr) ν_{max} (cm^{-1}): 3309,1140, 1120, 1105, 1011, 855. ^1H NMR, ^{13}C NMR, and HMBC data, see Table 2 and Figure 3; HRESIMS (positive) *m*/*z* 205.1293 $[M + H]^+$ (calculated for $C_{12}H_{16}N_2O$, 205.134088).

5.4.5. Biological tests:

5.4.5.1. Antinociceptive Activity Test

5.4.5.1.1. Hot-plate Method

The hot-plate method that was described by Turner was used to determine the antinociceptive activity of the compounds that were isolated from the *J. pelargoniifolia* root [48].

5.4.5.1.2. Acetic Acid-induced Writhing in Mice Test

The method of Koster *et al*. was used to evaluate the analgesic effect of the pure compounds that were isolated from the *J. pelargoniifolia* root [49].

5.4.5.1.3. Tail-Flick Method

Acute nociception was induced using the tail-flick apparatus (Tail flick Apparatus Harvard), following the method that was recommended by D'amour and Smith [50].

5.4.5.2. Anti-Inflammatory Activity Test

5.4.5.2.1. Carrageenan-Induced Edema in the Rat Paw Method

The method that was described by Winter *et al*. was used to evaluate the anti-inflammatory potency of the isolated compounds [51].

5.4.5.3. Antipyretic Activity Screening

5.4.5.3.1. Yeast-Induced Hyperthermia in Rats

Hyperthermia was induced in the mice followed by the administration of the isolated compounds, and their hypothermic activity was determined by applying the method described by Loux [52].

5.4.5.4. Antioxidant Effect

5.4.5.4.1. Nitric Oxide Radical-Scavenging Assay

This assay was carried out according to the procedure that was described by Green *et al*. [53].

5.4.5.5. Statistical Analysis

Data are expressed as mean ± standard error (SE). The results were first checked for normality by Kolmogorov and Smirnov test and homogeneity by Bartlett's test, and then analyzed using the Student's t test and analysis of variance (ANOVA) test. Dunnett posttest was used to determine which groups significantly differed from the control group. The statistical analysis was performed using GraphPad Prism version 3. Results were considered significantly different if the $p < 0.05$ [54].

5.5. Conclusions

The wide traditional use of *Jatropha* species as anti-inflammatory and analgesics has prompted us to investigate the chemistry and bioactivity of *J. pelargoniifolia* growing in Saudi Arabia. The phytochemical study of the plant roots resulted in the isolation of six terpenoids, five flavonoids, three coumarinolignans, two tryptamines, and four tyramines (including their HCl salts), a coumarin, and a pyrimidine. The new compounds were identified as 6-hydroxy-8-methoxy coumarin-7-*O*-*β*-D-glycopyranoside and 2-hydroxymethyl-*N*-methyltryptamine. To the best of our knowledge, the results indicate that hovetricoside C and *N*-methyltryptamine were isolated from the Euphorbiaceae family for the first time, while cleomiscosin B, hordenine, and *N*-methyltyramine with their salts, cynaroside, and linarin were characterized in the *Jatropha* species for the first time.

On the basis of the significant anti-inflammatory, analgesic, antipyretic, and antioxidant activities that were observed in the experimental animals for the alcoholic extract of *J. pelargoniifolia*, fifteen of the adequately isolated compounds were consequently biologically evaluated. Eleven of these compounds exhibited strong analgesic activity. Twelve out of the fifteen compounds succeeded to reduce the chemically-induced inflammatory marker in the animals in a dose-dependent manner. Moreover, five of the compounds showed an anti-pyretic effect by about a 1°C reduction in the temperature in an induced hyperthermia model. The isolated compounds also exhibited varying degrees of nitric oxide-scavenging activity. The significant antioxidant activity that was associated with the administration of *J. pelargoniifolia* roots was thus perhaps due to its phenolic content such as flavonoid, coumarins, and coumarinolignans. The synergistic effect between these bioactive constituents might explain the significant antinociceptive and anti-inflammatory effect of the alcoholic extract of *J. pelargoniifolia* roots and may scientifically justify the use of this plant in folk medicine for the treatment of pain and several inflammatory conditions.

5.7. References

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Chapter 6

Traditional Use of Ethnomedicinal Native Plants in Kingdom of Saudi Arabia

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H.A carried literature search, participated in writing, data analysis and helped to draft the manuscript and submitted finally. A.G conceived of the study and participated in its design and coordination and helped to draft the manuscript. H.S. helped to draft the manuscript, corrected the manuscript and gave revise on ethnobotanical use. O.K conceived of the study and participated in its design and coordination, supervised.

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6.1. Abstract

The Arabian Peninsula is recognized as an arid area dominated by deserts and poor biodiversity. However, the Kingdom of Saudi Arabia (henceforth abbreviated into KSA) has a wide range of flora, consisting of different species of trees, herbs, and shrubs and containing numerous edible and medicinal plants. The KSA is characterized by its vast area of diverse geographical landscapes and climates. Consequently, there is enormous variation in the distribution of plants across the Kingdom. The traditional use of ethnomedical plants in the KSA represents a strong interconnection among familiar remedies, health, diet, and traditional healing practices characterized by specific cultures.

The present paper reviews a collection of medicinal plants in KSA used in ethnomedicine. This review might be useful in developing strategies for the sustainable use of medicinal plants which are among the threatened important natural resources in folk medicine in the KSA. The present study reports 309 genera which cover 471 species from a total of 2,253 known species belonging to 89 families. The most dominating families are Asteraceae, Fabaceae, Lamiaceae, Euphorbiaceae, Solanaceae, Apiaceae, Brassicaceae, Chenopodiaceae, Poaceae, Amaranthaceae, Boraginaceae, Apocynaceae, Convolvoulaceae, Asclepiadaceae, Capparaceae, Polygonaceae, and Zygophyllaceae.

6.2. Introduction

Plant diversity plays a vital role in serving the ecosystems and in maintaining and preserving ecological balance and stability not only in KSA but in the whole world as well. Different plant species have been used in ethnomedicine since ancient times [1, 2]. Medicines of the Egyptians (3,000 BC; Pharaohs), the Greeks (400 BC; Hippocrates), and the Romans (37 BC.; Dioscorides) have a longstanding history. The continus use of plants in therapy was conducted by Prophet Mohammad (Peace be upon him, 571-632 AD); a practice known as The Prophetic Medicine (*Al-Ṭibb al-Nabawi*) by Ibn Qayyim Al-jawziyyah [3]. This period is considered as the golden age for ethnomedicine genesis. The Muslims did not stop at that point, but developed different schools, including the Rhazes (865-925 AD) and Avicenna (980-1037 AD), and their encyclopedias on ethnomedicine: The Container Book in Medicine (*Kitab Al-Hawi Fi Al-Tibb*) and The Law in Medicine (*Al-Qanun Fi Al-Tibb*) respectively, all of which contributed to the development of herbal medicine [4, 5]. Several medications have been extracted from natural resources, including plants in the nineteenth-century. However, Prophetic Medicine is still a major reference for all Muslims in the Arabian Peninsula and the rest of the world. Many medicinal plants that have been reportedly used in Prophetic Medicine are currently used in folk medicine in the Arabian Peninsula. Scientific studies have proven that these plants, including garlic, pomegranate, black seeds, costus, miswak, henna, ginger, and fenugreek are effective for treating human diseases. Such plants have been widely used in the form of low cost and almost zero-side-effect products pharmaceutically manufactured and marketed under such trademarks as Black seed plus®, Fenugreek 610MG®, Ginkago Biloba Plus TM, Kyolic®, and Alvita®.

The KSA, Kuwait, Bahrain, Yemen, Qatar, United Arab Emirates, and Oman formulate what is known as the Arabian Peninsula which is located in the Asian southwest. Its West and Southwest border is the Red Sea, its southern one is the Gulf of Aden, its southern and southeastern border is the Arabian Sea, and its eastern one is the Gulf of Oman and the Arabian Gulf [6]. Figure 1 highlights the geographical merge of the peninsula with the Syrian desert across the northern border, but the Northern boundaries of Saudi Arabia and of Kuwait are generally considered as marking the limit of Arabia there.

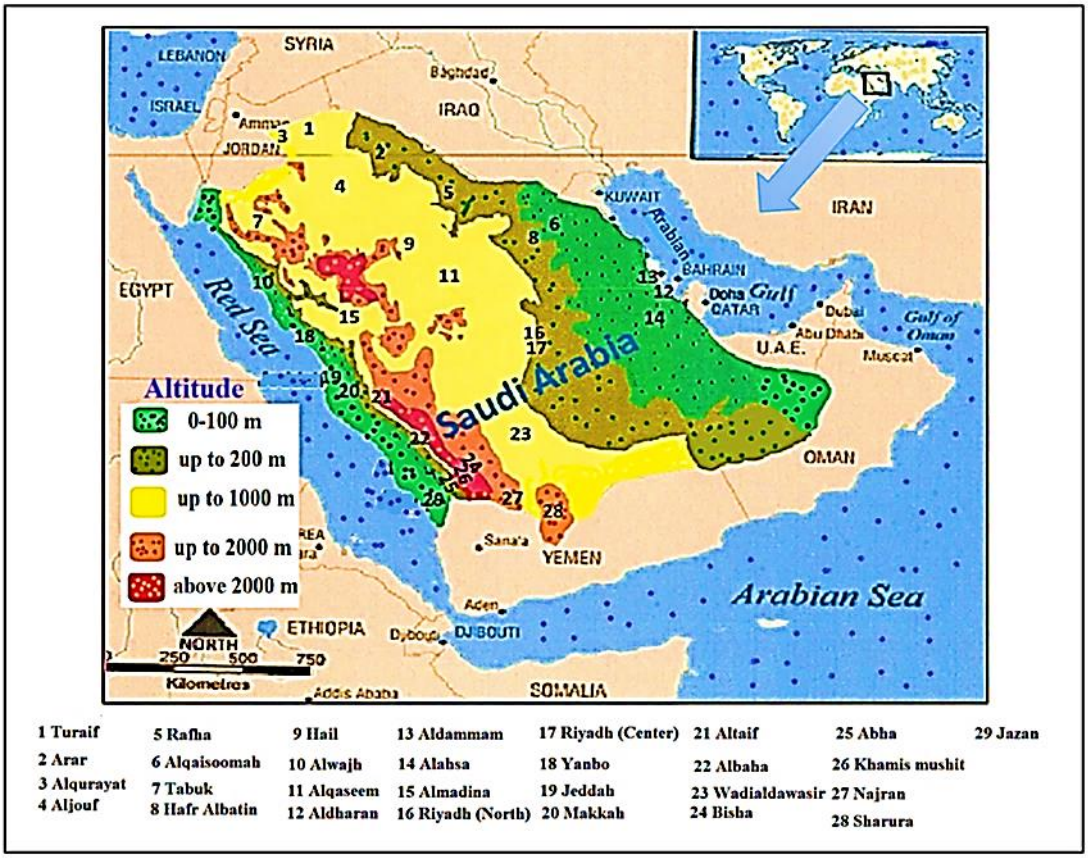


Figure 1: Geographical areas of Saudi Arabia

The 2,250,000 km^2 covered by KSA represent the largest part of the Arab Peninsula. Geographically, it is characterized by a variety of habitats including mountains, valleys, lava areas, meadows and rocky deserts. Therefore, KSA is made up of two areas: the rain fed zones of the western and southwestern highlands and the arid region of the interior area [6].

As for the first division, the Asir highlands constitute a flowing series of cliffs together with similar southwestern highlands, extending far unto the Yemeni borders. They rise up to 2000 m in some areas (Taif) and over 3000 m in others (Abha). The same continuity is to be found in the western continuous chain across the Tihama coastal plain. This continuity comes to an end on the northwestern sides where discontinuity dominates. On the other hand, the eastern part comprises a large amount of sand with much lower mountains and plains. These deserts extend to the northern Great Nafud and the southern Rub AlKhali (The Empty Quarter), with Dahna sands connecting both regions. As regards the Central Region, patchy, stone deserts, hillocks and valleys color the scene versus the complex of metamorphic rocks that characterize

the northern and northern central region. remarkable still is that the altitudes of the plains of the Central Region rise high up to 500-800 m as compared to such mountains and escarpments Jabal Shammar and Jabal Tuwayq which range from 300-600 m [6]. Forests cover 2.7 million hectares (about 1.35% of the total area) of KSA. Most of the forests are in the southwestern part of the Kingdom on the Sarawat mountain.

With regard to the location of KSA, it is located in a climatic transitional zone of tropical and temperate climates. Moreover, the rains falling upon KSA are neither constant in amount and nor equal in distribution so much that the arid areas right in the interior of KSA the underscore no rain in some areas and over 200 mm in some others on an annual basis. Even in winter, there is rainfall, however irregular.

It is noteworthy that winter in KSA ranges from cool to warm (2-20 ^{o}C) as opposed to the high summer temperature ranging from 35 to 50 ^{o}C, high humidity along the Red Sea coast and the Arabian Gulf coast; temperature becomes lower right in the interior.

Geographical and climatic diversity in the kingdom lead to the diversity in the flora, examined for the first time in 1974 [7]. This was followed by two volumes of Medicinal Plants of Saudi Arabia that were published in 1987 and 2000 by Mossa *et al*. [8, 9].

Collenette (1998) asserts that KSA produces about 837 genera manifesting themselves in 2253 species distributed among 132 families. Nearly 20 % of these families stands for totally new and uncommon plants. The southwestern part of KSA embraces about 70% of the floristic elements grown across Taif to the Yemeni border. This is accounted for the regularity of rainfall upon the region [10].

Equally important is KSA flora provide a remarkably rich source for agriculture and medicinal plants. Although many items of the flora are grown locally and nationally, KSA still grows a curious amalgam of Africa, Asia, and the Mediterranean region.

According to Al-Yahya (1984), employing and resorting to traditional medicine is still a fashionable practice nowadays in KSA [11]. It has become customary for KSA citizens to resort to natural herbs and traditional remedies in the hope that these will certainly heal their illness. Such practices are found in using such natural resources as

bee-honey, black seed, myrrh, fenugreek, kawajawa, among others. It has been to their good fortune that these practices have been handed from one generation to another. Mossa *et al.* (1987) spotlight the extensive range of flora that covers almost the whole KSA and offers sample opportunity for Saudis to make the best use of herbs and flora for their medications to local individuals for use in and therapeutic practices [8, 9].

The KSA is currently implementing an overall plan with a view to regulating and organizing the use of traditional medicine on a national basis. Thus, there is a tendency to legalize what is known as Complementary and Alternative Medicine (TM/CAM) via issuing organizing acts and issuing laws. This has been consolidated by the establishment of the National Office in 1995 on behalf of the Saudi Ministry of Health. Another important step came with the TM/CAM Committee in collaboration with the National Research Institute on herbal medicines studies established by King Saud University Medicinal, Aromatic and Poisonous Plant Research Center (MAPPRC), and the Department of Pharmacognosy, both of the College of Pharmacy.

Historically, these practices in KSA date back to ancient times when using traditional medicine was the only way out for the treatment of many illnesses and diseases that were usually unknown and non-diagnosed. However, these medicines were too much respected as they were prescribed by those traditional healers known as Al-Hokama. It is worthy of notice, however, that such practices are beginning to vanish and pass away. That is why it has become a prerequisite to conduct folk and ethnobotanical surveys and studies in KSA on the vanishing of these practices before they become history. As result, this review can serve as a reference document on traditional uses of medicinal plants in Saudi Arabia and increase the possibility of discovering new drug resources.

6.3. Materials and methods

Journals, textbooks, proceedings, websites, periodicals, and databases dealing with medicinal plants used to treat human diseases in Saudi Arabia, Arabian Peninsula, and other parts of the world were checked for related information. Dictionaries of English/Arabic and Arabic/English were also consulted for accuracy.

6.4. Results and discussion

Literature survey showed that a total of 309 genera containing 471 species in 89 families are used in ethnomedicine (Table 1 and Fig. 2). Asteraceae and Fabaceae families have the highest number of ethnomedicinal species (54 and 49, respectively) in Saudi Arabia. Table 1S summarizes ethnobotanical data on all medicinal plants used in KSA [12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41].

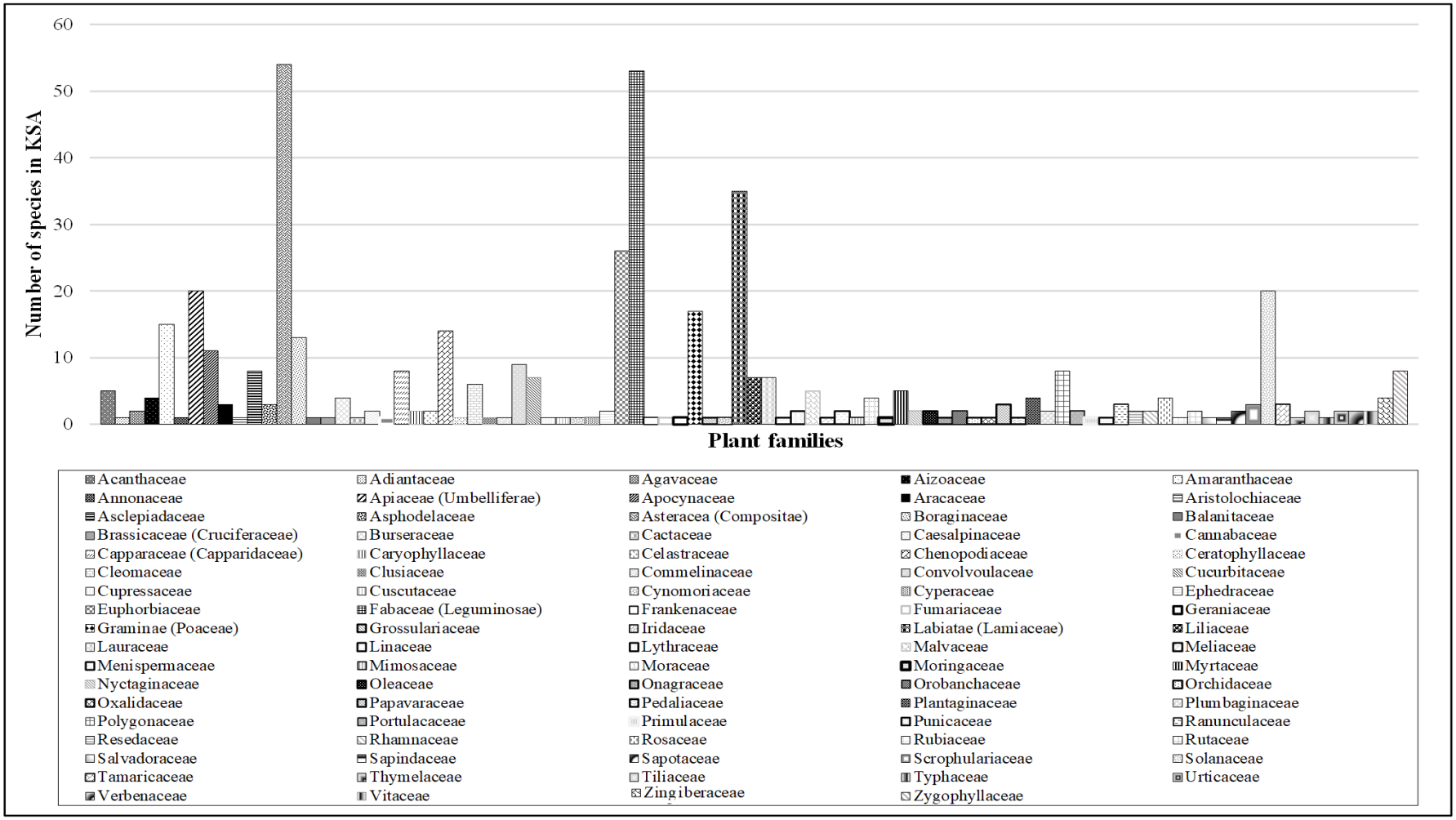
Table 1 shows numerous plant species used for various illnesses associated with gastrointestinal problems, pains, rheumatism inflammations, ulcers, respiratory, circulatory, urological, and skin diseases, and some for toothache, diabetes, allergy, and gynecology. The most mentioned medicinal plant families were Asteraceae, Fabaceae, Lamiaceae, Euphorbiaceae, Solanaceae, Apiaceae, Brassicaceae, Chenopodiaceae, Poaceae, Amaranthaceae, Boraginaceae, Apocynaceae, Convolvoulaceae, Asclepiadaceae, Capparaceae, Polygonaceae, and Zygophyllaceae. All these families as well as other families mentioned in this review are already represented in Saudi Arabia flora [7, 42].

Resorting to traditional medicinal plants for treating some illnesses has resulted in many the production of many promising drugs and medications [43, 44]. The present paper reviews these practices from an ethnopharmacological perspective by targeting 471 medicinal plants used on a regular basis by almost all Saudis (Table 1S).

Table 1: Number of traditionally used species per family in Saudi Arabia

Family name	Number of species in KSA	Family name	Number of species in KSA
Acanthaceae	5	Lauraceae	1
Adiantaceae	1	Linaceae	1
Agavaceae	2	Lythraceae	2
Aizoaceae	4	Malvaceae	5
Amaranthaceae	13	Meliaceae	1
Annonaceae	1	Menispermaceae	2
Apiaceae (Umbelliferae)	18	Mimosaceae	1
Apocynaceae	10	Moraceae	4
Aracaceae	3	Moringaceae	1
Aristolochiaceae	1	Myrtaceae	5
Asclepiadaceae	9	Nyctaginaceae	2
Asphodelaceae	4	Oleaceae	2
Asteracea (Compositae)	54	Onagraceae	1
Boraginaceae	13	Orobanchaceae	2
Balanitaceae	1	Orchidaceae	1
Brassicaceae (Cruciferaceae)	17	Oxalidaceae	1
Burseraceae	5	Papavaraceae	3
Cactaceae	1	Pedaliaceae	1
Caesalpinaceae	1	Plantaginaceae	4
Cannabaceae	1	Plumbaginaceae	2
Capparaceae (Capparidaceae)	8	Polygonaceae	8
Caryophyllaceae	2	Portulacaceae	2
Celastraceae	2	Primulaceae	1
Chenopodiaceae	15	Punicaceae	1
Ceratophyllaceae	1	Ranunculaceae	3
Cleomaceae	6	Resedaceae	3
Clusiaceae	1	Rhamnaceae	2
Commelinaceae	1	Rosaceae	4
Convolvoulaceae	9	Rubiaceae	1
Cucurbitaceae	6	Rutaceae	2
Cupressaceae	2	Salvadoraceae	1
Cuscutaceae	1	Sapindaceae	1
Cynomoriaceae	1	Sapotaceae	2
Cyperaceae	2	Scrophulariaceae	2
Ephedraceae	1	Solanaceae	20
Euphorbiaceae	26	Tamaricaceae	3
Fabaceae (Leguminosae)	49	Thymelaceae	1
Frankenaceae	1	Tiliaceae	2
Fumariaceae	1	Typhaceae	1
Geraniaceae	1	Urticaceae	2
Graminae (Poaceae)	13	Verbenaceae	2
Grossulariaceae	1	Vitaceae	2
Iridaceae	1	Zingiberaceae	4
Labiatae (Lamiaceae)	33	Zygophyllaceae	7
Liliaceae	4		

Figure 2: Number of traditionally used species per family in Saudi Arabia



According to Ali *et al.* (2017), these therapeutic practices and use of medicinal plants appear on the Use Index (UI), [UI = (na/NA ×100), where na is the number of questioners who refer to the species as valuable and NA is the total number of people met]. Three vital plants were at the core of Saudis' practices: *Juniperus procera, Rumex nervosus,* and *Ziziphus spina-chris* [33]. Therefore, these three important and commonly used plants have been discussed here in detail.

Juniperus procera Hochst. ex Endl is known as "Arar" in KSA. It is a long tree that reaches up to 8m tall with needle-shaped leaves, extending from the south reigon of the Arab Peninsula across the Red Sea into Africa [45]. *Juniperus* is one of the major genera of Cupressaceae family consisting of approximately 70 species [46]. Traditionally in Saudi Arabia, *J. procera* used for treating hepatic diseases, jaundice, gastrointestinal disturbances, pharyngitis, as antirheumatism, for gout and several inflammatory conditions [21, 47]. In ethnomedicine, the resinous material of *J. procera* was added to bee-honey and used as a remedy for curing hepatic and skin diseases [48].

A previous phytochemical study of different parts of *J. procera* resulted in the isolation of different classes of sesquiterpenes and diterpenes. Where the stem bark of *J. procera* afforded two lignans identified as β-peltatin and deoxypodophyllotoxin [95]. In addition, the stem barks and leaves of same plant gave two diterpenes designated as totarol and sugiol with strong antimicrobial activity [49, 50]. Abietane, pimarane, and labdane types of diterpenes have been isolated from *J. procera* fruits which showed antiparasitic and nematicidal activities [51]. Moreover, totarol exhibited a synergistic effect with isoniazide (INH) towards four *Mycobacterium* species [52]. In addition, its essential oil possesses significant antioxidant/free radical scavenging activity [53].

The less polar fraction of the aerial part of *J. procera* exhibited significant hepatoprotective effect against liver toxicity induced by CCl_4. The hepatoprotective activity has been referred to the presence of terpenes in good yield; 4-*epi*-abietol, ferruginol, hinokiol, sugiol, *Z*-communic acid and hinokiol-1-one, 3-*β*-12-dihydroxyabieta-8,11,13-triene-1-one, in addition to 8-*α*-acetoxyelemol sesquiterpene [54]. A toxicity study of *J. procera* extract revealed the safety of the extract even at high doses no acute or chronic toxicity appeared [48]. Figure 1S shows the structures of the major compounds isolated from *J. procera*.

Rumex nervosus Vahl. is known locally as “Ithrib”. Some people in the Arabian Peninsula especially in Yemen and Saudi Arabia consume *R. nervosus*, which makes it an edible plant. However, if eaten in large quantities, it can produce toxic effects because of its high calcium oxalate content [55]. *Rumex nervosus* belongs to the family Polygonaceae and is a large annual herb that can grow up to 1.5 m tall, its leaves are usually sagittate, the inflorescence is considerably branched, and it has a leafless panicle. It has a light brown nut and its fruits are cordate-orbicular. It is widely distributed in Yemen, Saudi Arabia, Ethiopia, Somalia, Kenya, and Tanzania. Ithrib is a very common plant that is used by native people as a diuretic, antipyretic, antirheumatic and to treat gonorrhea, leprosy, lung tuberculosis, liver illness, as antihypertension, antihemorrhoids, antiscabies, antiemetic, aphrodisiac, antitussive, antirabies, for dermatitis, antiacne, hypoglycemic, antiinfective, and headache. Decoction of the leaf or root powder produces a substance that is used as a vermifuge. Moreover, the leaves of *R. nervosus* are utilized to treat skin rashes and young leaves are roasted to decrease the acid content before being eaten. The material formed after the burning of the stem is mixed with egg yolk or butter and applied to burns [55 – 59].

There are some reports on the anthelmintic [55], anti-inflammatory and antiviral [57], analgesic [58], diuretic and laxative [59], and antioxidant [60] effects of *R. nervosus*.

It has been confirmed that *R. nervosus* extracts contain alkaloids, flavonoids, phenols, amino acids, furanocoumarins, and saponins by preliminary phytochemical screening [61].

Moreover, the genus *Rumex* is characterized by the presence of anthraquinones, naphthalene-1,8-diols, flavonoids, and stilbenoids [62]. Many phenolic compounds have been isolated from the *R. nervosus* ethyl acetate fraction, including kaempferol, quercetin, gallic acid, hyperoside, quercetin 3-*O*-(6″-acetyl)-galactoside, hesperidin, quercetin, *p*-hydroxy benzoic acid, catechol, and pyrogallol [63]. Figure 2S shows the structures of the major compounds isolated from *R. nervosus*.

Ziziphus spina-christi is commonly called Jujube" and is locally known as “Sidr” or “Nabuk”. It is a multiuse tree belonging to the family Rhamnaceae. Sidr is a native plant that grows in tropical and subtropical regions, and it is widely distributed

throughout the Mediterranean regions, Africa, Asia, and tropical America. In Saudi Arabia, Sidr is wildly distributed throughout the southern and southwestern area and has been used as an ornamental plant and for shade. It is a tall tree that can reach 20 m in height. Its leaves are glabrous on the upper surface, finely pubescent on the lower surface, and ellipsoid or ovate lanceolate in shape with an obtuse or acute apex [64].

Ziziphus spina-christi is greatly respected by Muslims since it is mentioned in the Sunnah and the Holy Quraan twice [65]. From ancient times, in Chinese traditional medicine, suan zao ren (*Z. spinosa*) has been used to increase blood flow to the heart and liver, and it is used to control irritability, insomnia, and palpitations [66]. In Saudi folk medicine, the leaves of *Z. spina-christi* (jujube) are used to heal wounds, treat some skin diseases and sores, cure ringworm, antipyretic, gonorrhea, sex diseases, some inflammatory conditions, and ulcers. Furthermore, it has been reported that *Z. spina-christi* leaves are used in folk medicine as antidiabetic remedy [67]. In the Bedouin, the decoction of the stem bark and fresh fruits is used as a body rinse, to cure fresh wounds, and is also used for the management of dysentery, bronchitis, coughs, and tuberculosis [68].

Previous phytochemical investigation revealed it contains biologically active secondary metabolites including, tannins, flavonoids, terpernoids, saponin glycosides, and alkaloids [68].

Pharmacological studies have demonstrated that the total alcoholic extract of the current plant leaves and stem bark has showed significant antioxidant, antimicrobial and antidiarrheal activities [66, 69]. Additionally, the aqueous extract from the root bark has an antinociceptive activity [70] and central nervous system depressant effect in mice [71]. The butanol extract of *Z. spina-christi* leaves has shown potent hypoglycemic/antidiabetic activities [72]. The aqueous and ethanolic extracts of stem bark of *Z. spina-christi* have been studied and an anticholinergic effect was observed [73], which proved the traditional use of the plant as antispasmodic. A cytotoxic effect was observed for the aerial part of *Z. spina-christi* against cervical, breast and colon cancers [66].

A phytochemical study of *Z. spina-christi* indicated the presence of betulic and ceanothic acids [74]. Cyclic peptide alkaloids, franaganine, mauritine C, and sativanine

A have been isolated and fully characterized from the stem bark of *Z. spina-christi* [75]. Triterpenoidal saponins were recently isolated from the leaves of the same plant and screened for their antidiabetic activity [76]. Additionally, four saponin glucosides identified as christinin A-D have been isolated from the leaves *n*. butanol extract leaves and their structures were fully characterized by using spectroscopic technique [77]. Furthermore, quercetin, hyperoside, ruin, and quercetin-3-*O*-[*β*-xylose-(1–2)-*α*-rhamnose] 4′-*O*-*α*-rhamnose have also been isolated from the leaves and fruits of *Z. spina-christi* [78]. Figure 3S shows the structures of the major compounds isolated from *Z. spina-christi*.

6.5. Conclusions

Ethnomedicinal knowledge is not transferred from the older generations to the young age generations, meaning it will soon be erased especially as most younger individuals prefer to visit clinics and hospitals more regular than older people and the Bedouin. Moreover, folkloric healers (Hakeem) use wild herbs randomly and without any restriction, which increases the chance of extinction of certain medicinal plants. This requires greater consideration because more than 15,000 plant species might face extinction worldwide due to over-harvesting and misuse [2, 14]. Therefore, owing to the high diversity of medicinal plants in the KSA, the present review recommends the following:

- Phytochemical and pharmacological studies on the different flora plants.
- Creation of distribution maps for important medicinal plants using GPS coordinates.
- Data analysis of medicinal plants based on their phytochemical, chemotaxonomical, and pharmacognestical characteristics.
- Monographs of some pharmacopoeial medicinal plants.
- Continuing the surveying of plants and identification of their exact locations.
- Plants provided with sequence bank and fingerprinting.

- Seed library for the plants or seed bank to preserve genetic diversity and to preserve rare plant species.

A total of 471 plant species belonging to 89 families were recorded in the present review from the KSA, which means that this country has a large number of medicinal plants that need to be discovered and have their chemical and pharmacological properties studied.

The findings from the present review paper lend support to the extensive traditional medicinal knowledge in the KSA, which provide the basis for further medicinal research on a scientific evidence basis.

The current study has reviewed a total of 471 plant species belonging to 89 families grown in KSA. This highlights the vast wealth of medicinal plants in KSA. These plants have been used as medications for healing and treating many diseases on an ethnomedicinal, folk medicinal, and national basis. Practicing traditional medicine by resorting to these plants in KSA dates back to ancient times so that it has become fashionable among almost all Saudis.

Therefore, it is suggested that further studies be conducted on unveiling the pharmaceutical, pharmacological, chemical, and laboratory properties of these medicinal plants that have never been targeted before via studies from this perspective. Thus, this tremendous treasure of medicinal plants in KSA needs a greater deal of attention so that researcher would direct future studies to dig out the undiscovered secrets of these plants from a scientific angle of vision. It is hoped these future studies, once scientifically conducted, will come up with new discoveries in the form of new drug leads extracted from natural resources that would utterly changes the medication shelves all over the world.

6.6. References

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Chapter 7

Disscusion, summary and outlook

7.1. General discussion

Skin wound ulceration represents a difficult clinical problem and a major source of morbidity in the Kingdom of Saudi Arabia (KSA) and world wide, especially in-patient suffering from chronic disease such as diabetes mellitus (DM). The patient's quality of life is also significantly affected by pain, swelling, and management of wound drainage. Wound ulcer, skin inflammation could be cause systemic symptoms lead to increase the risk of morbidity and mortality [1]. Wound care and therapy can be very challenging, and the major dream is discovering cheapest, safest and 100% effective wound ulcer and skin inflammation treatment.

Since ancient times, nature has been an important source of medicinal agents. This fact is illustrated by the large number of natural products currently used medically. The flora of Saudi Arabia provides a rich resource of medicinal plants and endemic species for traditional medicine. Medicinal plants are essential in traditional health systems and are an important economic component of the biodiversity in Saudi Arabia [2]. An inventory of the medicinal plants of KSA is essential [3] to prioritize a scientific assessment of the native ethnomedicine, and to advance new derivative medicines and natural health products. The number of medicinal plants and trees among Saudi Arabia's flora is more than 837 useful plants out of 2,253 species [2]. The majority of these flora species can be found in Asir and Hijaz in the western region of the KSA near the border of the Red Sea [2]. Based on some of the reported traditional uses of these herbal plants, it can be seen that many plants in the KSA (native or naturalized) are part of the natural healthcare system and should be scientifically studied [3] Identification and isolation of the bioactive compounds have possible promising pharmacological activities such as wound healing, skin ulcer curing and treatment for skin inflammation from native Saudi plants, can further the search for novel therapeutics for such medical conditions.

Sap of the *J. pelargoniifolia* petioles was traditionally used as a healing agent for treatment of ulcers, wounds and skin inflammation, while the whole plant of *J. glauca* is taken to treat constipation and as ear drops [4].

The toxicity study revealed that 24-hours LD_{50} was approximately more than 1.2 ml/kg b.w. for the essential oils and 2 g/kg for the extracts, which encourage us to

do further biological investigation. The selected biological activities were an analgesic, anti-inflammatory and hepatoprotective activities as well hypoglycemic activity based on the above folkloric use.

The root extracts from *J. pelargoniifolia* exhibited greater anti-inflammatory (50.63%) activity than *J. glauca* when compared with phenylbutazone (64.63%). While the roots of *J. glauca* showed higher hepatoprotective and antinociceptive (63.85%) activities in comparison to indomethacin (69.87%).

Additionally, the oil of *J. pelargoniifolia* roots showed anti-inflammatory, antipyretic, and antinociceptive activities and showed significant ($p < 0.01$) effect as compared to a standard drug.

Phytochemical study of root fractions of *J. pelargoniifolia* resulted in the isolation and identification of 22 compounds. Compounds 6-Hydroxy-8-methoxycoumarin-7-O-*β*-D-glycopyranoside **(15)** and 2-hydroxymethyl-*N*-methyltryptamine **(18)** were isolated and identified as new compounds along with known diterpenoid, triterpenoid, flavonoid, coumarinolignan, coumarin, pyrimidine, indole, and tyramine-derived molecules. The anti-inflammatory, analgesic, and antipyretic activities were evaluated for fifteen of the adequately available isolated compounds, most of them showed significant biological activity.

7.2. Summary

The ethanolic extracts from *J. glauca* and *J. pelargoniifolia* (root and aerial parts) induced analgesic and anti-inflammatory effects; these results justify the traditional use of *J. pelargoniifolia* and *J. glauca* as analgesics for the treatment of inflammatory conditions **(Chapter 2)**.

The chemical composition of the essential oil obtained from *J. pelargoniifolia* roots was determined via GC-FID and evaluated for its potency as an anti-inflammatory, antioxidant, antipyretic, and antinociceptive agent by *in vivo* and *in vitro* models. The findings demonstrated that the investigated essential oil of *J. pelargoniifolia* roots could be used as a natural source for their anti-inflammatory, antinociceptive, antipyretic, and antioxidant effects **(Chapter 3)**.

The different chemical composition of the essential oils of the *J. pelargoniifolia* and *J. glauca* indicate that the chemical profile represents a valuable taxonomic character that can be useful in identification of different *Jatropha* species. The distribution of sesquiterpene, diterpene, and monoterpene hydrocarbons and their oxygenated derivatives could be used as differentiating parameters for the various species **(Chapter 4)**.

Moreover, the phytochemical study of the plant roots resulted in the isolation of six terpenoids, five flavonoids, three coumarinolignans, two tryptamines, and four tyramines, a coumarin, and a pyrimidine. The new compounds were identified as 6-hydroxy-8-methoxy coumarin-7-O-*β*-D-glycopyranoside and 2-hydroxymethyl-*N*-methyltryptamine. Hovetricoside C and N-methyltryptamine were isolated from the Euphorbiaceae family for the first time, while cleomiscosin B, hordenine and N-methyltyramine with their salts, cynaroside, and linarin were characterized in the *Jatropha* species for the first time. The synergistic effect between these bioactive constituents might explain the significant antinociceptive and anti-inflammatory effect of the alcoholic extract of *J. pelargoniifolia* roots and scientifically justify the use of this plant in folk medicine for the treatment of pain and several inflammatory conditions **(Chapter 5)**.

The last part of the thesis is dealing with a review of the widely-distributed medicinal plants used in ethnomedicine in KSA. This review might be useful in developing strategies for the sustainable use of medicinal plants which are among the threatened important natural resources in folk medicine in the KSA **(Chapter 6)**.

7.3. Outlook

Because *J. pelargoniifolia* Courb. produced many natural secondary metabolites with interesting pharmacological activities. More efforts should be done to prove their efficacies and their exact mechanism (*in vitro* and *in vivo*) before clinical recommendation. Not only their efficacies but their safeties should be observed. Beside doing toxicity tests by cell culture and brine shrimp test, *in vivo* studies in higher animal can be an option. The tests include acute toxicity test to know LD_{50} of each compound and subchronic test to know the safety of those compounds if they were given in repeated doses. Beside general toxicity test, we have done special test for mutagenicity. Further investigation is still needed for oil and extract safety because direct contact of *J. pelargoniifolia* latex and oil to the skin and eye cause severe irritation.

Finally, the genus *Jatropha* comprises of 200 species, so far only a few species have been investigated chemically. There are still many species that have received no or little attention. So, phytochemical and biological studies should focus now on those plants to search for more potential bioactive components used as natural remedies for treatment of various diseases.

7.4. References

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Appendix A: Glossary of abbreviations

ABTS	2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate Aminotransferase
brs	Broad singlet
b.w.	Body weight
CD_3OD	Deuterated methanol
cm	Centimeter
^{13}C NMR	Carbon Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy
°C	Degree centigrade
d	Doublet
DCM	Dichloromethane
dd	Double doublet
DPPH	1,1-diphenyl-2-picrylhydrazyl
EtOAc	Ethyl acetate
EtOH	Ethyl alcohol
Fig.	Figure
FRAP	Ferric reducing antioxidant power
Fr.	Fraction
g	Gram
GC-FID	Gas chromatography-flame ionization detector
GC-MS	Gas Chromatography- Mass Spectrometry
GGT	Gamma glutamyl transpeptidase
HMBC	Heteronuclear Multiple Bond Correlation
^{1}H NMR	Proton Nuclear Magnetic Resonance
HPLC	High Pressure Liquid Chromatography
hr	Hour
HRESIMS	High Resolution Electron Spray Ionization -Mass Spectrometry
HSQC	Heateronuclear Single Quantum Correlation
Hz	Hertz
i.d.	Internal diameter
IR	Infrared spectrometry
i.p.	Intraperitoneal
J	Coupling constant
m/z	Mass over charge ratio
$[M]^+$	Molecular ion peak
m	Meter
MDA	Malonaldehyde
m.p.	Melting point
mg	Milligram
mg/kg	Milligram per kilogram
mg/ml	Milligram per milliliter
MHz	Mega Hertz

min	Minute
ml	Milliliter
ml/min	Milliliter per minute
NP-SH	Non-protein sulfhydryl groups
OECD	Organization for Economic Co-operation and Development
p.o.	Per os
ppm	Part per million (10^{-6})
Rp	Reversed phase
RI	Retention indices
R_t	Retention time
s	Singlet
s.c.	Subcutaneous
TCA	Trichloroacetic acid
TLC	Thin layer chromatography
TP	Total protein
UV	Ultra-violet spectroscopy
δ	Chemical shift value
λ_{max}	Maximum fluorescence emission wavelength
µ	Micron
µl	Microliter
µm	Micrometer

Appendix B: Supplementary material for chapter 3

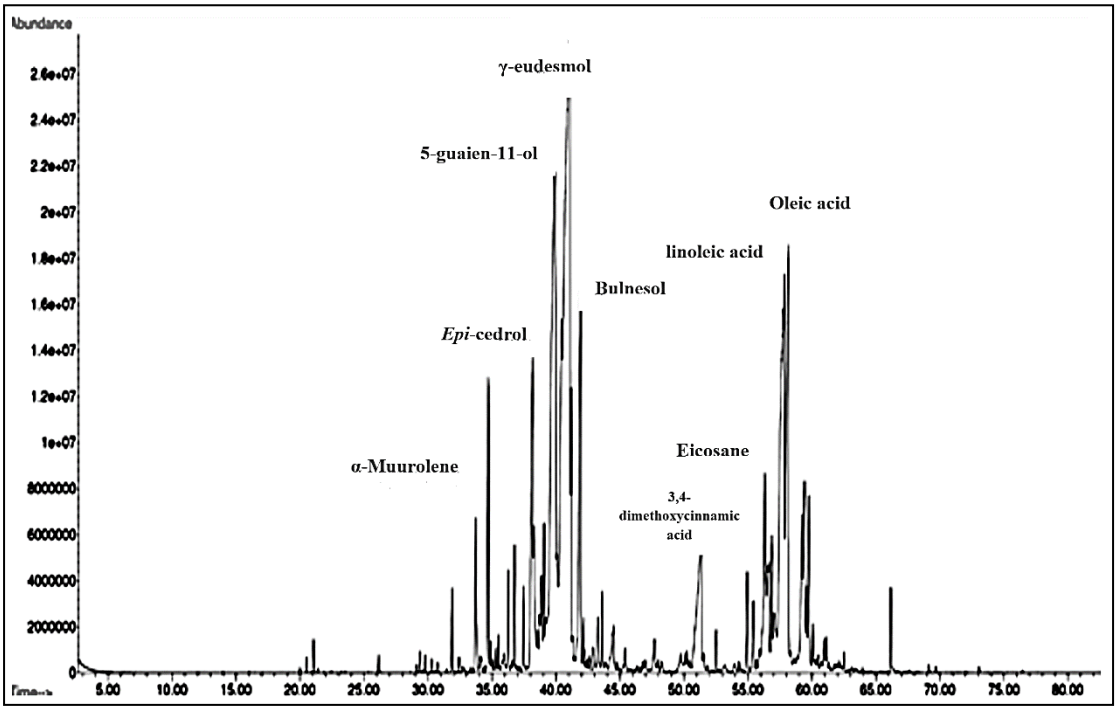
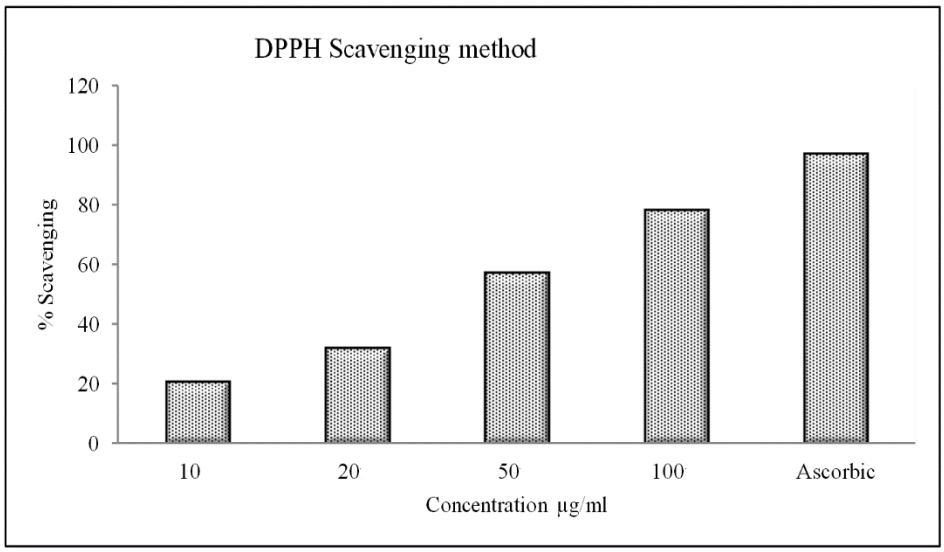
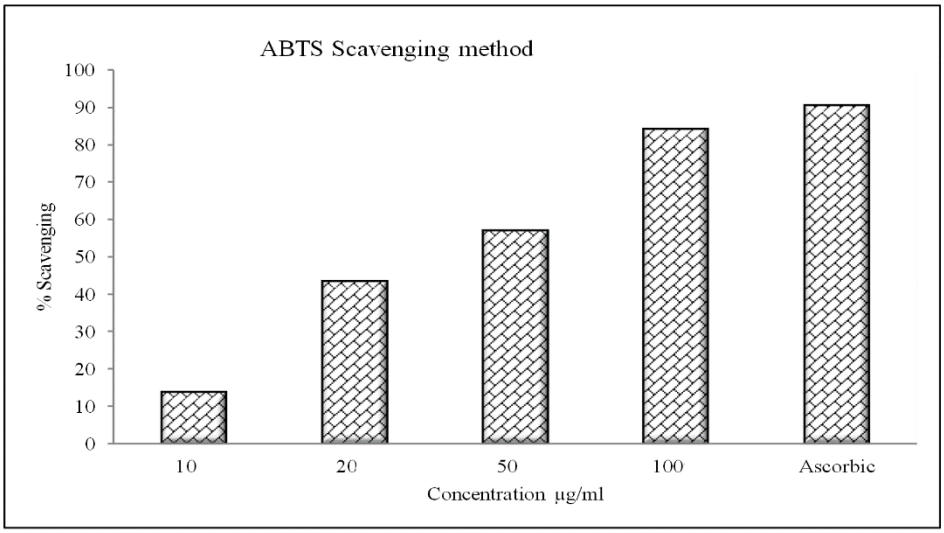
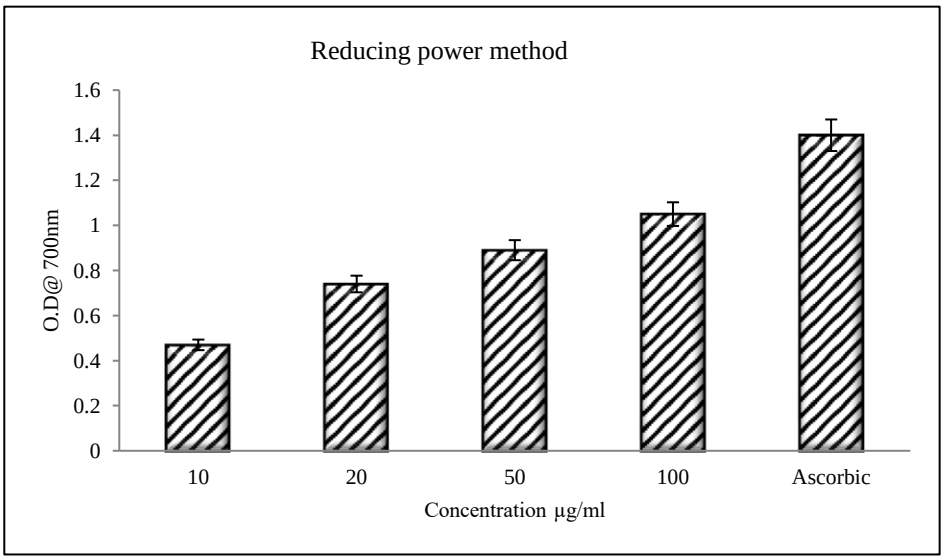


Figure 1S: GC chromatogram of *J. pelargoniifolia* root oil







Figures 2S $_{a, b, c}$: Antioxidant activity of *J. pelargoniifolia* root essential oil

Table 1S: Structures of major components of *J. pelargoniifolia* roots essential oil

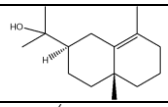
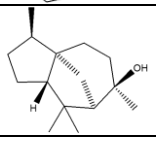
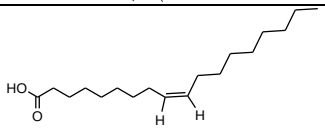
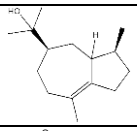
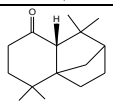
Component names	Concentration	Structure
γ-Eudesmol	35.31 %	
5-Guaien-11-ol	14.43 %	
Epi-Cedrol	8.19 %	
Oleic acid	5.23 %	
Bulnesol	4.45 %	
trans-Isolongifolanone	2.68 %	

Table 2S: Percentages of classes of compounds in the essential oil of *J. pelargoniifolia* roots

Class	Percentage
Oxygenated monoterpenes	0.849
Oxygenated sesquiterpenes	70.366
Sesquiterpene hydrocarbons	6.941
Fatty acids (saturated)	4.939
Fatty acids (unsaturated)	9.690
Other	7.205
Total	99.99

Table 3S: Effect of *J. pelargoniifolia* essential oil on cotton pellet granuloma in Wistar rats

Group (n=6)	Dose (µl/kg)	Initial weight of sterilized cotton pellet (mg)	Mean increase in weight of pellet (mg±SE)	Net	% Inhibition
Control	-	30	81.900±1.912 ‴	51.900±1.912	-
Essential Oil	120	30	69.667±1.787‴	39.667±1.787**	23.57
Essential Oil	240	30	51.217±1.463‴	21.217±1.463**	59.12
Phenylbutazone	100 mg/kg	30	41.700±1.065‴	11.700±1.065###	77.45

All values represent mean ± SE. ***p* < 0.01 (one-way ANOVA, followed by Dunnett's test) *versus* the control group, ###*p* < 0.001 (unpaired t-test) *versus* the control group, ‴*p* < 0.001 (paired t-test) *versus* initial cotton weight.

Table 4S: Analgesic effect of *J. pelargoniifolia* essential oil on tail flick in mice

Treatment (n=6)	Dose (µl/kg)	Reaction time (seconds) pre -drug	Reaction time (seconds) post-drug administration					
			30 min.	% Inhibition	60 min.	% Inhibition	120 min.	% Inhibition
Essential Oil	120	4.667±0.333	5.500±0.224	17.85	6.333±0.333**	35.71	6.500±0.428**	39.28
Essential Oil	240	5.167±0.307	6.667±0.422*	29.00	7.833±0.307**	51.61	8.500±0.428**	64.51
Indomethacin	4 mg/kg	4.833±0.307	7.500±0.428###	55.17	8.667±0.333###	79.31	10.667±0.307###	110.34

All values represent mean ± SE. **p* < 0.05; ***p* < 0.01 (Repeated measures ANOVA, followed by Dunnett's test) *versus* pre-drug group, ###*p* < 0.001 (unpaired t-test) *versus* pre-drug group.

Table 5S: Analgesic effect of *J. pelargoniifolia* essential oil in mice via the hot plate method

Treatment (n=6)	**Dose (µl/kg)**	**Reaction time (seconds) pre - drug**	**Reaction time (seconds) post-drug administration**					
			30 min.	**% Inhibition**	**60 min.**	**% Inhibition Inhibition**	**120 min.**	**% Inhibition**
Essential Oil	120	6.333±0.211	7.000±0.365	10.52	7.833±0.477*	23.68	8.667±0.333**	36.84
Essential Oil	240	6.667±0.333	9.500±0.224**	42.50	11.333±0.333**	70.00	11.667±0.333**	75.00
Indomethacin	4 mg/kg	6.667±0.333	11.000±0.258$^{###}$	65.00	13.167±0.307$^{###}$	97.50	14.667±0.333$^{###}$	120.00

All values represent mean ± SE. *$p < 0.05$; **$p < 0.01$ (Repeated measures ANOVA, followed by Dunnett's test) *versus* pre-drug group, $^{###}p < 0.001$ (unpaired t-test) *versus* pre-drug group.

Table 6S: Determination of LD_5

Group	**Dose ml/kg**	**Dose difference (a)**	**Dead**	**Mean mortality (b)**	**Product (a*b)**
1	0.2	-	0	-	-
2	0.4	0.2	1	0.5	0.1
3	0.8	0.4	2	1.5	0.6
4	1	0.2	4	3	0.6
5	2	1	5	4.5	4.5
6	4	2	6	5.5	11
					16.8
LD_{50}					**1.2**

LD_{50}= Lethal dose-(Total Product/No. of animals), 4-(16.8/6)= 1.2 ml/kg.

Appendix C: Supplementary material for chapter 4

Figure 1S$_a$: *J. glauca* aerial parts

Figure 1S$_b$: *J. pelargoniifolia* aerial parts

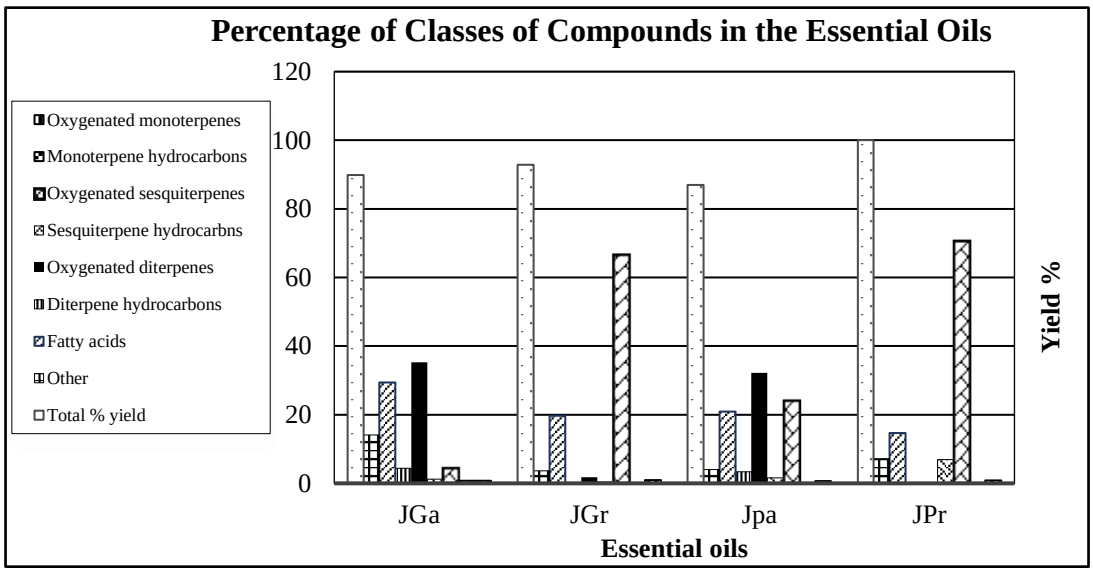


Figure 2S: Contents (%) of classes of compounds in the essential oils of roots and aerial parts of *J. pelargoniifolia* and *J. glauca*

Appendix D: Supplementary material for chapter 5

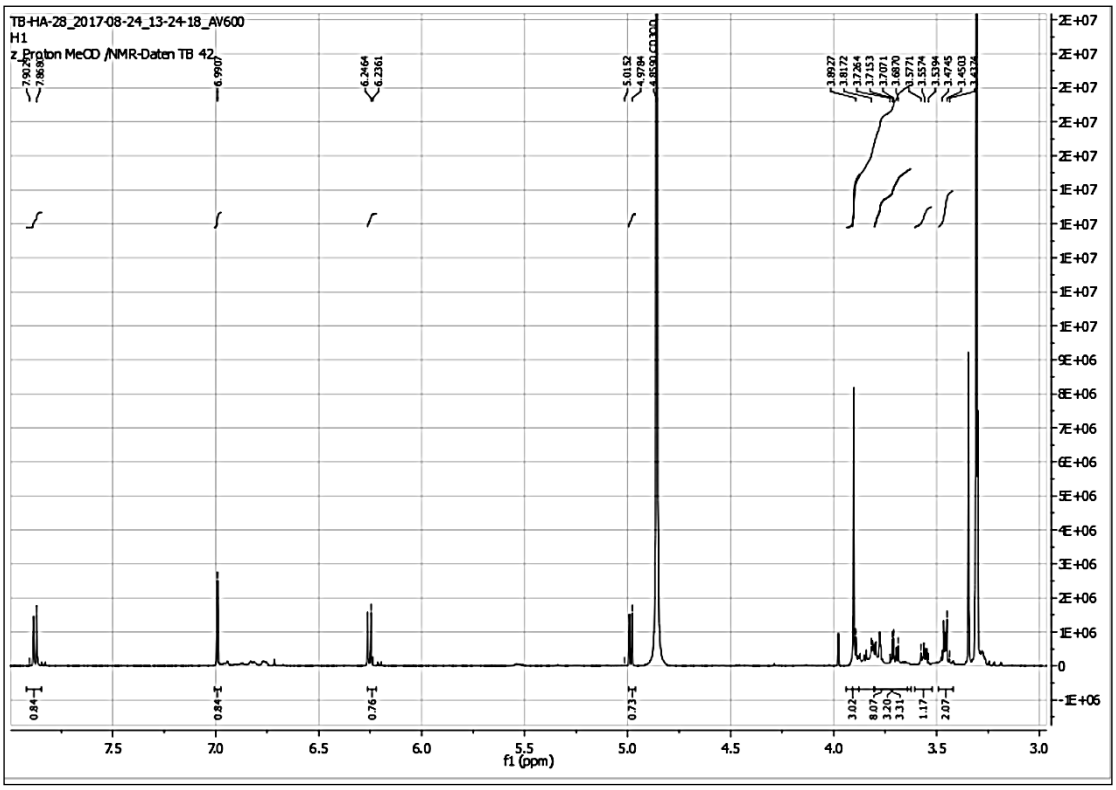


Figure 1S: ^{1}H NMR spectrum for compound **15** in CD_3OD (600 MHz)

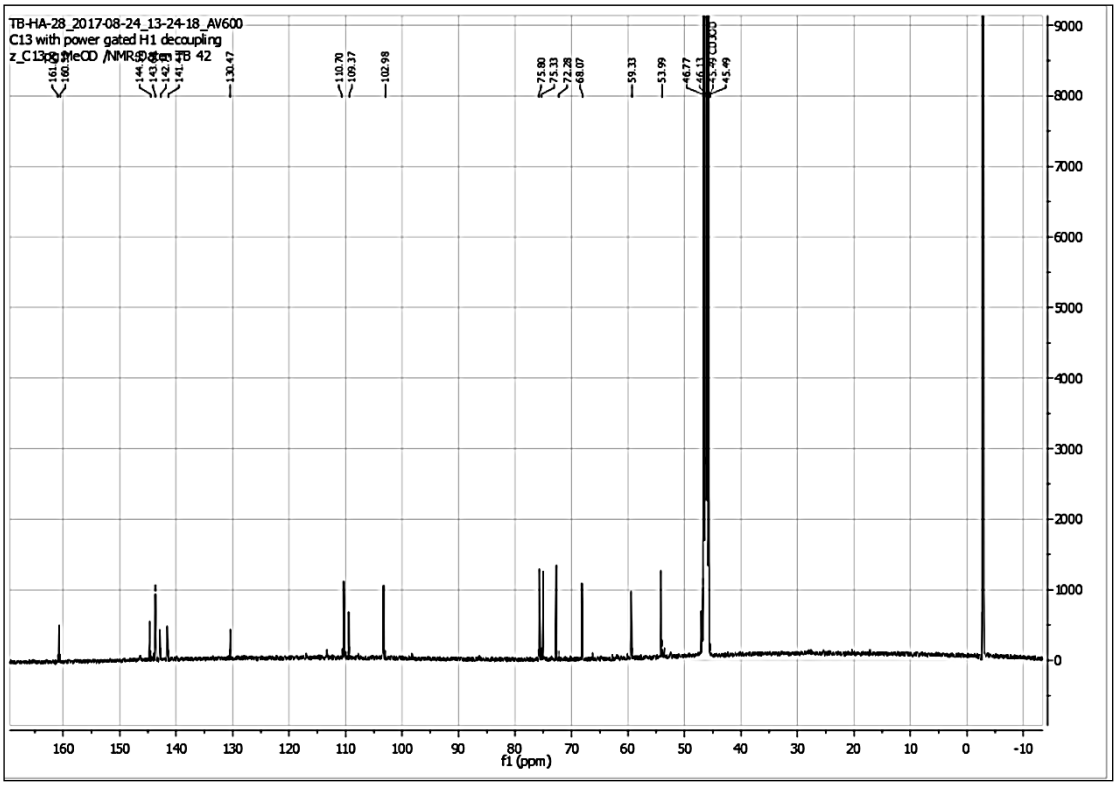


Figure 2S: ^{13}C NMR spectrum for compound **15** in CD_3OD (125 MHz)

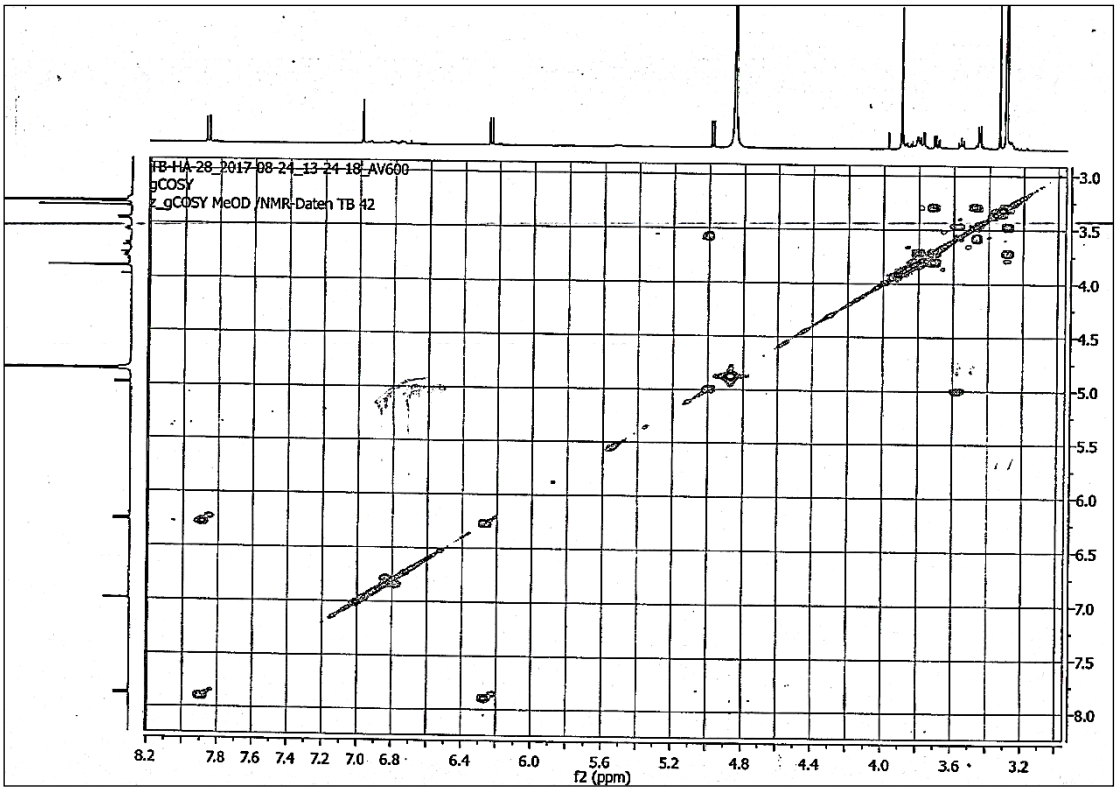


Figure 3S: COSY spectrum for compound **15** in CD_3OD

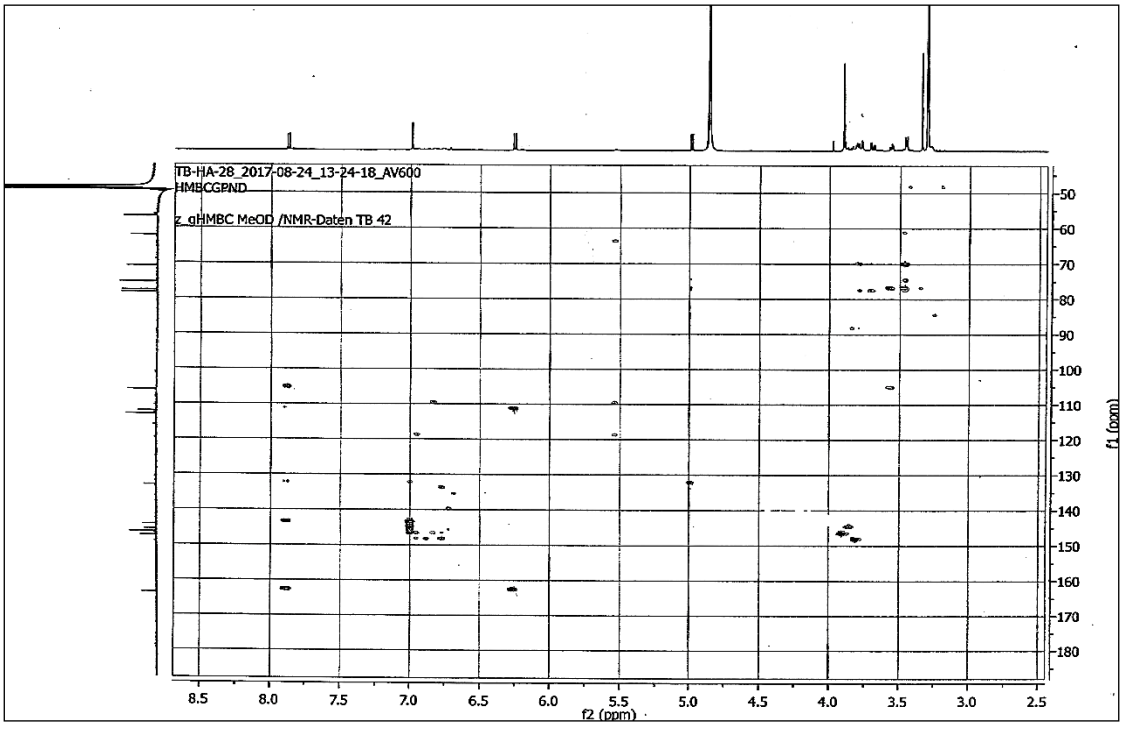


Figure 4S: HMBC spectrum for compound **15** in CD_3OD

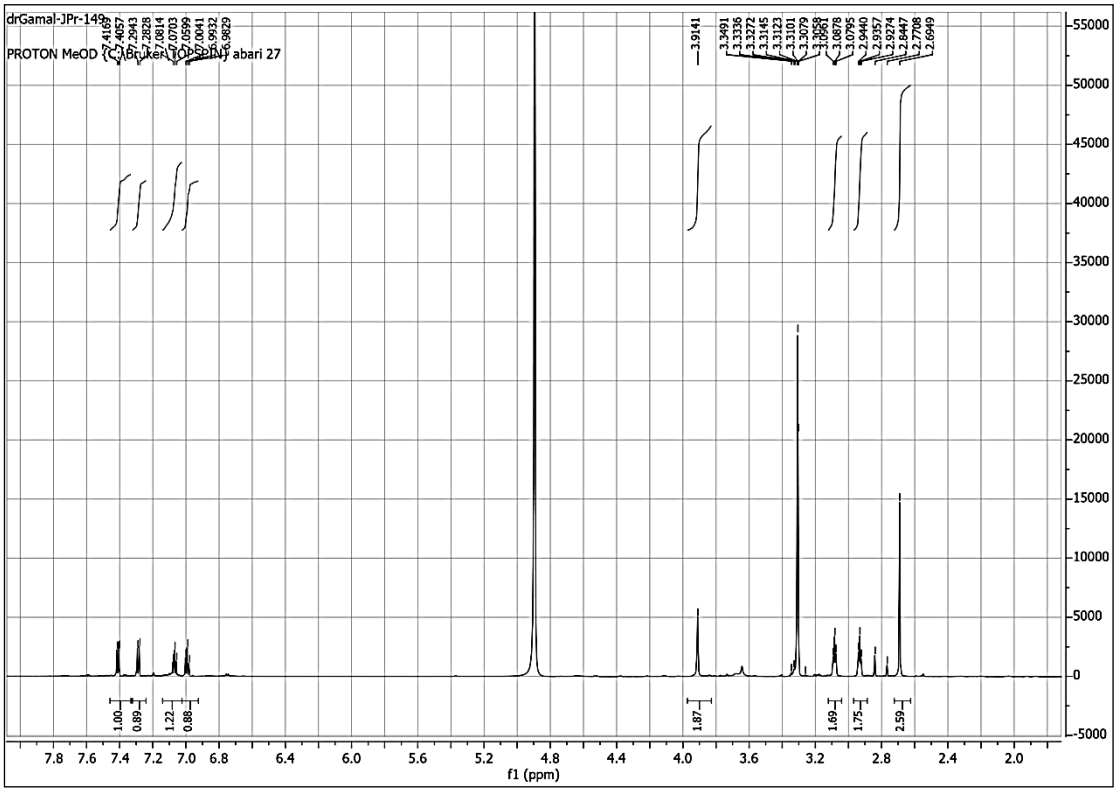


Figure 5S: ^{1}HNMR spectrum for compound **18** in CD_3OD (700 MHz)

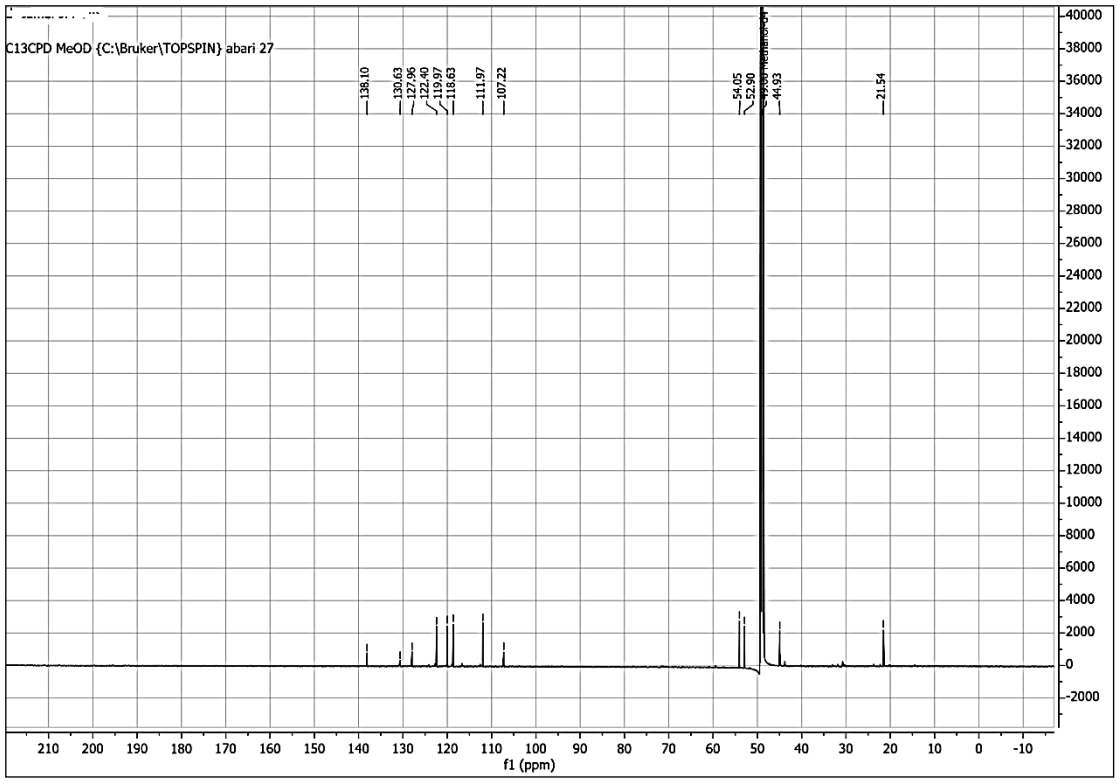


Figure 6S: ^{13}CNMR spectrum for compound **18** in CD_3OD (125 MHz)

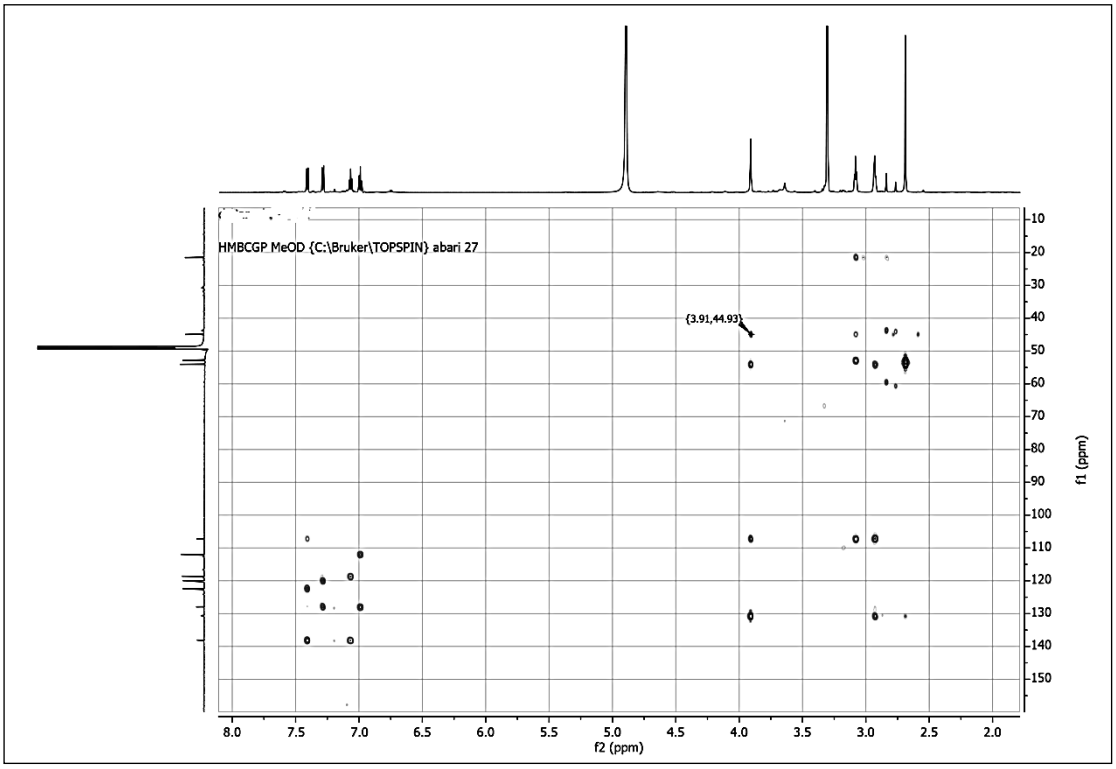


Figure 7S: HMBC spectrum for compound **18** in CD_3OD

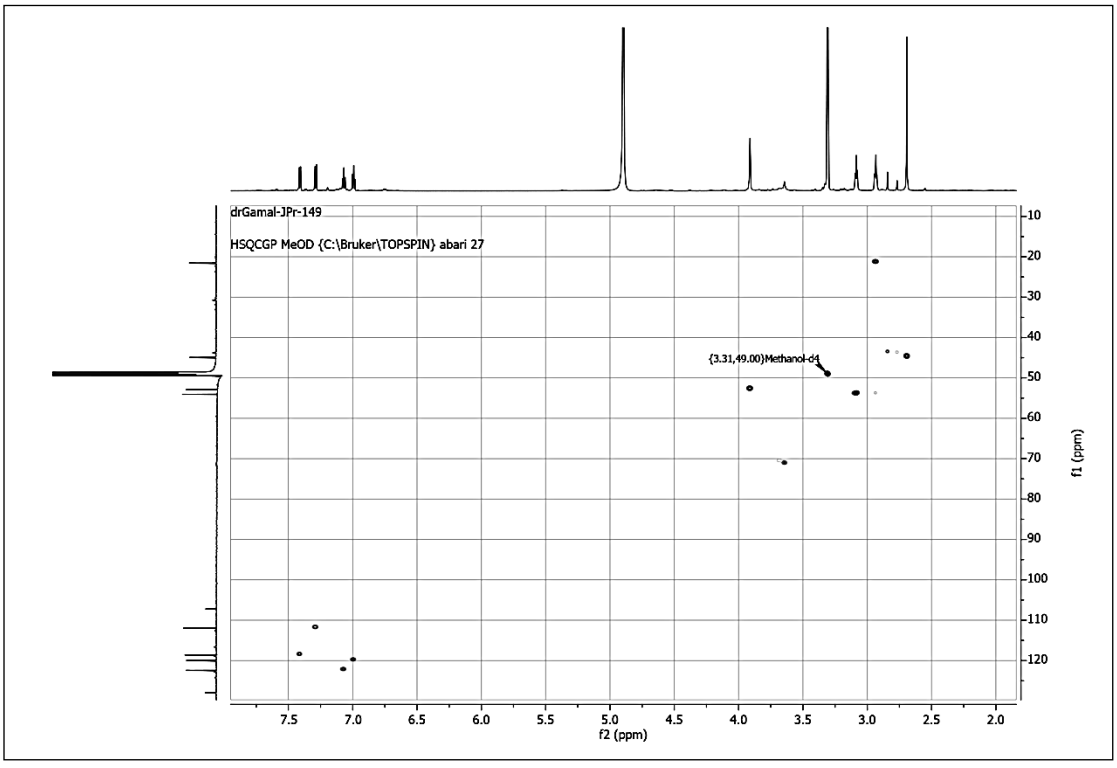


Figure 8S: HSQC spectrum for compound **18** in CD_3OD

Table 9S: Analgesic effect of isolated compounds by using acetic acid-induced writhing in mice

Treatments (n=6)	Dose (mg/kg)	Number of writhing in 20 min	% Inhibition
Control (20% acetic acid)	0.1 ml	36.000±1.155	-
No-2	5	34.333±1.282	4.62
No-2	10	34.500±1.310	4.16
No-6	5	24.667±1.202**	31.48
No-6	10	17.000±0.856**	52.77
No-5	5	18.167±0.477**	49.53
No-5	10	13.333±0.494**	62.96
No-11	5	36.667±0.667	1.85
No-11	10	32.500±0.922*	9.72
No-14	5	30.833±1.138**	14.35
No-14	10	27.500±0.764**	23.61
No-13	5	21.167±0.980**	41.20
No-13	10	15.000±0.577**	58.33
No-16	5	26.833±1.046**	25.46
No-16	10	25.333±0.667**	29.62
No-3	5	35.667±1.706	•
No-3	10	32.333±1.430	10.18
No-4	5	23.167±1.014**	35.64
No-4	10	18.333±0.882**	49.07
No-1	5	24.333±1.308**	32.40
No-1	10	18.167±0.946**	49.53
No-8	5	37.833±1.014	•
No-8	10	37.167±1.327	•
No-9	5	34.000±1.366	5.55
No-9	10	30.000±1.065**	16.16
No-10	5	26.500±0.885**	26.38
No-10	10	24.167±0.946**	32.87
No-21	5	38.000±0.966	•
No-21	10	37.000±0.966	•
No-22	5	24.000±1.483**	33.33
No-22	10	12.333±0.558**	65.74
Indomethacin	4	9.833±0.601$^{###}$	72.68

All values represent mean ± SE. *$p < 0.05$; **$p < 0.01$ *versus* control group (one-way ANOVA, followed by Dunnett's test), $^{###}p < 0.001$ *versus* control group (unpaired t-test).

•% inhibition is very low or no effect.

Table 10S: Analgesic effect of isolated compounds by using hot plate method in mice

Treatments	Dose mg/kg	Reaction time (sec.) pre-drug	Reaction time (sec.) post-drug					
			30 min.	% Change	60 min.	% Change	120 min.	%Change
No-2	5	7.167±0.307	6.830±0.307	4.65	7.667±0.333	6.97	7.667±0.333	6.97
No-2	10	7.007±0.365	7.833±0.307	11.90	7.667±0.333	9.52	8.333±0.422*	19.04
No-6	5	8.000±0.365	8.667±0.337	8.33	10.167±0.307**	27.08	11.667±0.422**	45.83
No-6	10	7.833±0.307	11.000±0.365**	40.25	12.667±0.333**	61.70	13.667±0.422**	74.46
No-5	5	7.833±0.307	9.333±0.494*	19.14	10.500±0.428**	34.04	11.333±0.333**	44.68
No-5	10	7.667±0.494	11.000±0.365**	43.47	12.667±0.333**	65.21	13.000±0.516**	69.56
No-11	5	7.833±0.307	7.333±0.422	•	7.667±0.333	•	8.333±0.333	6.38
No-11	10	7.333±0.333	8.000±0.365	•	7.833±0.307	•	8.833±0.303*	20.45
No-14	5	7.500±0.428	7.833±0.307	4.44	8.167±0.307	8.88	9.167±0.477*	22.22
No-14	10	7.167±0.307	7.833±0.307	9.30	8.333±0.333	16.27	9.667±0.422**	34.88
No-13	5	8.000±0.364	9.333±0.333	16.66	10.667±0.422**	33.33	11.000±0.365**	37.50
No-13	10	7.833±0.307	11.000±0.365**	40.42	11.667±0.426**	48.93	12.500±0.619**	59.57
No-16	5	7.167±0.307	8.000±0.365	11.62	9.000±0.365**	25.58	10.167±0.307**	41.86
No-16	10	7.167±0.307	8.667±0.422	20.93	10.833±0.401**	51.16	11.000±0.365**	53.48
No-3	5	7.000±0.365	7.333±0.333	4.76	7.167±0.307	2.38	8.333±0.422	19.04
No-3	10	7.000±0.365	7.333±0.333	4.76	7.833±0.307	11.90	9.000±0.365**	28.57
No-4	5	7.167±0.307	7.667±0.333	6.97	8.500±0.428	18.60	9.833±0.477**	37.20
No-4	10	7.500±0.428	10.000±0.577**	33.33	10.500±0.428**	40.00	13.000±0.365**	73.33
No-1	5	7.667±0.333	7.500±0.428	•	7.667±0.333	•	8.000±0.447	4.34
No-1	10	7.500±0.428	7.667±0.494	2.22	7.833±0.401	4.44	8.333±0.494	11.11
No-8	5	7.000±0.365	7.333±0.333	4.76	7.500±0.428	7.14	8.167±0.401	16.16
No-8	10	7.167±0.307	7.667±0.494	6.97	8.167±0.307	13.95	9.000±0.365*	25.58
No-9	5	6.833±0.307	7.000±0.365	2.43	7.833±0.307	14.63	8.167±0.401	19.51
No-9	10	7.667±0.333	7.833±0.307	2.17	8.667±0.422	13.04	9.833±0.477**	28.26
No-10	5	7.167±0.307	8.000±0.258	11.62	8.667±0.422*	20.93	10.167±0.307**	41.86
No-10	10	7.000±0.365	9.000±0.258**	28.57	10.500±0.342**	50.00	12.333±0.494**	76.19
No-21	5	6.833±0.307	7.000±0.365	2.43	7.167±0.307	4.87	8.000±0.365	17.07
No-21	10	7.000±0.365	7.667±0.333	8.52	7.500±0.428	7.14	7.833±0.401	11.90
No-22	5	7.000±0.365	7.833±0.307	11.90	8.667±0.422*	23.89	10.333±0.422**	47.61
No-22	10	7.000±0.365	8.500±0.342*	21.42	9.167±0.401**	30.95	12.500±0.428**	78.57
Indomethacin	4	7.167±0.307	10.167±0.307***	41.86	12.833±0.401***	79.06	14.667±0.333***	104.65

All values represent mean ± SE. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ versus the corresponding pre-drug trated group (Repeated measures ANOVA, followed by Dunnett's test).
•% inhibition is very low or no effect

Table 11S: Analgesic effect of isolated Compounds by using tail flick method in mice

Treatments	Dose mg/kg	Reaction time (s) pre-drug	Reaction time (s) post-drug					
			30 min.	% Change	60 min.	% Change	120 min.	% Change
No-2	5	5.200±0.195	5.117±0.189	•	5.300±0.235	•	5.167±0.126	•
No-2	10	5.100±0.277	5.133±0.123	•	5.283±0.206	3.59	5.083±0.087	•
No-6	5	5.150±0.134	5.450±0.136	5.82	5.883±0.138**	14.23	5.967±0.156**	15.85
No-6	10	5.417±0.160	5.983±0.108	10.45	6.700±0.106**	23.69	7.600±0.372**	40.30
No-5	5	4.933±0.133	6.067±0.076**	22.97	6.483±0.160**	31.41	7.000±0.097**	41.89
No-5	10	5.283±0.125	6.783±0.119**	28.39	7.333±0.152**	38.80	7.850±0.173**	48.58
No-11	5	5.200±0.153	5.017±0.130	•	5.117±0.108	•	5.250±0.099	•
No-11	10	5.250±0.100	5.117±0.130	•	5.050±0.115	•	5.267±0.112	•
No-14	5	4.933±0.126	4.867±0.084	•	4.983±0.087	•	5.183±0.114	•
No-14	10	5.050±0.134	5.133±0.141	•	5.383±0.119	6.60	5.767±0.189**	14.19
No-13	5	4.967±0.112	5.300±0.110	6.71	6.150±0.112**	23.82	6.533±0.180**	31.54
No-13	10	5.150±0.106	6.017±0.162**	16.82	6.950±0.195**	34.95	7.933±0.145**	54.04
No-16	5	5.217±0.135	5.967±0.120	14.37	7.260±0.568**	39.55	7.000±0.124**	34.18
No-16	10	5.100±0.113	6.800±0.141**	33.33	7.250±0.134**	42.15	7.833±0.171**	53.59
No-3	5	5.100±0.129	5.133±0.105	•	5.350±0.152	4.90	5.767±0.148*	13.07
No-3	10	5.067±0.145	5.467±0.214	7.89	5.417±0.183	6.90	6.100±0.129**	20.39
No-4	5	5.117±0.095	6.017±0.209**	17.58	6.633±0.174**	29.64	7.183±0.149**	40.39
No-4	10	5.267±0.198	6.700±0.265**	27.21	7.683±0.158**	45.88	8.300±0.232**	57.59
No-1	5	4.833±0.253	6.033±0.133**	28.82	6.317±0.158**	30.68	7.000±0.097**	44.82
No-1	10	5.083±0.122	7.217±0.147**	41.96	7.867±0.176**	54.75	8.500±0.270**	67.21
No-8	5	4.867±0.123	5.083±0.119	4.45	5.367±0.136	10.27	5.500±0.169*	13.01
No-8	10	5.150±0.195	5.167±0.095	•	5.417±0.125	5.17	5.750±0.099*	11.65
No-9	5	4.983±0.145	5.167±0.143	3.67	5.300±0.106	6.35	5.850±0.128**	17.39
No-9	10	5.517±0.170	5.867±0.112	6.34	6.050±0.123*	9.66	6.117±0.095*	10.87
No-10	5	5.050±0.134	5.883±0.151**	16.50	6.683±0.117**	32.34	7.100±0.086**	40.59
No-10	10	5.100±0.146	6.733 ±0.161**	32.02	7.150±0.250**	40.19	8.183±0.119**	60.45
No-21	5	5.033±0.251	5.183±0.182	2.98	5.267±0.143	4.63	5.157±0.182	9.60
No-21	10	5.133±0.131	5.233±0.128	•	5.967±0.080**	16.23	6.250±0.089**	21.75
No-22	5	5.467±0.199	6.633±0.282**	21.34	7.000±0.250**	28.04	7.550±0.128**	38.10
No-22	10	5.050±0.143	7.067±0.246**	39.93	7.983±0.108**	58.08	8.450±0.165**	67.32
Indomethacin	4	5.317±0.154	7.650±0.246***	43.48	8.050±0.251***	51.41	10.400±0.427***	95.61

All values represent mean ± SE. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ *versus* the corresponding pre-drug trated group (Repeated measure ANOVA, followed by Dunnett's test).
•% inhibition is very low or no effect.

Table 12S: Effect of isolated compounds on carrageenan-induced paw edema in albino rats

Treatments	Dose mg/kg	Before carrageenan	3 h after carrageenan	Net	% Inhibition
Only carrageenan	0.2 ml	1.063±0.023	1.667±0.017‴	0.603±0.007	-
No-2	5	1.067±0.021	1.673±0.020‴	0.607±0.008	•
No-2	10	1.053±0.027	1.645±0.020‴	0.592±0.046	•
No-6	5	1.083±0.027	1.482±0.021‴	0.398±0.009**	33.97
No-6	10	1.072±0.024	1.367±0.025‴	0.295±0.017**	51.10
No-5	5	1.087±0.030	1.383±0.019‴	0.327±0.021**	45.85
No-5	10	1.093±0.011	1.387±0.017‴	0.293±0.014**	51.38
No-11	5	1.047±0.027	1.652±0.011‴	0.605±0.022	•
No-11	10	1.075±0.021	1.675±0.015‴	0.600±0.009	•
No-14	5	1.103±0.011	1.668±0.025‴	0.565±0.021	6.35
No-14	10	1.098±0.007	1.640±0.019‴	0.542±0.025	10.22
No-13	5	1.063±0.026	1.507±0.027‴	0.443±0.022**	26.51
No-13	10	1.027±0.036	1.337±0.036‴	0.310±0.010**	48.61
No-16	5	1.063±0.025	1.507±0.027‴	0.443±0.022**	47.23
No-16	10	1.027±0.036	1.337±0.036‴	0.310±0.010**	55.24
No-3	5	1.090±0.009	1.650±0.020‴	0.560±0.018	7.18
No-3	10	1.047±0.033	1.568±0.027‴	0.522±0.021**	13.53
No-4	5	1.078±0.023	1.600±0.021‴	0.522±0.018**	13.53
No-4	10	1.088±0.013	1.412±0.012‴	0.323±0.014**	49.17
No-1	5	1.052±0.032	1.547±0.025‴	0.495±0.043*	17.95
No-1	10	1.095±0.014	1.367±0.014‴	0.272±0.009**	54.94
No-8	5	1.097±0.010	1.687±0.011‴	0.590±0.016	2.20
No-8	10	1.132±0.013	1.657±0.023‴	0.525±0.031*	12.98
No-9	5	1.047±0.025	1.617±0.013‴	0.570±0.019	5.52
No-9	10	1.085±0.025	1.627±0.017‴	0.542±0.012*	10.22
No-10	5	1.002±0.033	1.482±0.018‴	0.480±0.036**	20.44
No-10	10	1.103±0.007	1.315±0.013‴	0.212±0.012**	64.91
No-21	5	1.097±0.010	1.661±0.009‴	0.565±0.013	6.35
No-21	10	1.065±0.026	1.622±0.022‴	0.555±0.018*	8.01
No-22	5	1.100±0.008	1.435±0.012‴	0.335±0.007**	44.47
No-22	10	1.087±0.015	1.387±0.009‴	0.30±0.010**	50.27
Phenylbutazone	100	1.038±0.027	1.225±0.024‴	0.187±0.007###	69.06

All values represent mean ± SE. *$p < 0.05$; **$p < 0.01$ *versus* only carrageenan group (one-way ANOVA, followed by Dunnett's test), ‴$p < 0.001$ *versus* the corresponding before carrageenan group (paired t-test), ###$p < 0.001$ *versus* only carrageenan group (unpaired t-test).

•% inhibition is very low or no effect.

Table 13S: Effect of isolated compounds on yeast-induced hyperthermia in mice

Treatments	Dose mg/kg	Normal temperature	Rectal temperature after yeast administration	Rectal temperature after drug administration		
				30 min	60 min	120 min
No-2	5	35.067±0.099	38.833±0.109‴	38.800±0.121	38.733±0.143	38.200±0.121*
No-2	10	35.167±0.123	38.650±0.126‴	38.517±0.154	38.450±0.138	38.083±0.125*
No-6	5	35.20±0.11	38.767±0.143‴	38.250±0.118*	38.267±0.126*	37.783±0.119**
No-6	10	35.167±0.105	38.817±0.127‴	37.917±0.111**	37.567±0.174**	36.98±0.15**
No-5	5	35.533±0.117	38.883±0.105‴	38.333±0.056**	37.933±0.076**	37.817±0.162**
No-5	10	35.333±0.112	38.783±0.192‴	37.917±0.168**	37.400±0.179**	37.000±0.097**
No-11	5	35.383±0.087	38.850±0.161‴	38.717±0.185	38.683±0.119	38.567±0.169
No-11	10	35.283±0.087	38.700±0.115‴	38.600±0.129	38.533±0.133	38.250±0.092
No-14	5	35.400±0.163	38.817±0.125‴	38.683±0.158	38.617±0.158	38.433±0.139
No-14	10	35.933±0.582	38.967±0.120‴	38.817±0.114	38.567±0.112	38.433±0.123*
No-13	5	35.533±0.141	38.700±0.165‴	38.417±0.158	37.967±0.120**	37.367±0.126**
No-13	10	35.567±0.120	38.850±0.118‴	37.900±0.093**	37.283±0.101**	37.033±0.099**
No-16	5	35.350±0.118	38.750±0.214‴	38.217±0.079	37.900±0.093**	37.583±0.0162**
No-16	10	35.383±0.185	38.800±0.200‴	37.833±0.123**	37.117±0.095**	36.833±0.109**
No-3	5	35.650±0.112	38.933±0.133‴	38.933±0.154	38.800±0.132	38.667±0.112
No-3	10	35.717±0.114	38.883±0.135‴	38.733±0.136	38.517±0.079	38.600±0.148
No-4	5	35.833±0.120	38.900±0.086‴	38.917±0.128	38.600±0.115	38.483±0.145*
No-4	10	35.767±0.133	38.917±0.079‴	38.883±0.149	38.367±0.112*	38.317±0.117**
No-1	5	35.667±0.088	38.883±0.133‴	38.283±0.095**	37.983±0.154**	37.883±0.158**
No-1	10	35.550±0.169	38.883±0.117‴	38.133±0.131**	37.583±0.176**	37.067±0.173**
No-8	5	35.567±0.230	38.883±0.119‴	38.800±0.132	38.783±0.135	38.650±0.111
No-8	10	35.617±0.187	38.750±0.152‴	38.533±0.150	38.617±0.114	38.333±0.184
No-9	5	35.667±0.126	38.767±0.169‴	38.750±0.118	38.617±0.130	38.300±0.106
No-9	10	35.600±0.129	38.817±0.125‴	38.767±0.112	38.633±0.133	38.000±0.097**
No-10	5	35.833±0.095	38.867±0.141‴	38.317±0.098*	37.817±0.130**	37.267±0.092**
No-10	10	35.783±0.125	38.850±0.154‴	37.833±0.112**	37.400±0.171**	37.133±0.284**
No-21	5	35.633±0.163	38.850±0.138‴	38.850±0.123	38.717±0.114	38.533±0.123
No-21	10	35.683±0.128	38.867±0.171‴	38.667±0.123	38.450±0.145	38.200±0.124**
No-22	5	35.700±0.115	38.817±0.114‴	38.100±0.103**	37.800±0.159**	37.200±0.115**
No-22	10	35.750±0.099	38.867±0.123‴	38.233±0.102**	37.117±0.101**	36.733±0.136**
Indomethacin	4	35.717±0.189	38.950±0.106‴	37.100±0.093***	36.850±0.180***	36.333±0.112**

All values represent mean ± SE. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ *versus* the corresponding yeast administration group (Repeated measure ANOVA, followed by Dunnett's test), ‴$p < 0.001$ *versus* the corresponding normal group (paired t-test).

Table 14S: % Inhibition of nitric oxide scavenging activity for isolated compounds at different concentrations

Treatments	(% Inhibition ± SD)				
	Concentration				
	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
Compound-2	30.150±14.360	54.430±3.140	63.960±10.700	71.600±10.480	76.830±5.010
Compound-6	10.560±5.340	23.090±3.040	33.500±8.520	50.030±4.000	56.540±6.030
Compound-5	5.160±2.480	10.430±3.250	19.360±8.720	21.630±10.280	27.000±7.850
Compound-11	7.400±4.560	20.060±8.580	36.710±16.530	50.500±10.210	57.000±10.210
Compound-14	17.860±15.850	28.100±22.280	42.400±23.650	59.160±14.840	70.360±14.730
Compound-13	10.400±6.320	14.160±4.350	25.610±5.180	30.040±3.350	33.060±1.860
Compound-16	6.660±2.100	11.260±0.280	19.200±6.400	21.860±5.350	25.610±5.180
Compound-3	7.400±4.560	12.630±5.430	19.430±6.210	28.460±12.000	38.300±5.630
Compound-4	23.860±8.910	41.430±7.770	48.160±7.160	66.030±8.240	77.360±4.220
Compound-1	11.460±5.000	27.900±0.450	44.300±6.760	61.530±2.560	71.660±0.700
Compound-8	7.500±1.550	15.750±0.440	42.610±10.720	50.170±9.220	63.670±12.850
Compound-9	15.060±7.500	29.300±2.980	38.270±2.980	50.430±7.230	63.670±12.850
Compound-10	33.310±19.720	45.380±20.110	56.550±9.420	64.730±4.350	75.260±5.540
Compound-21	39.970±7.660	54.970±6.680	61.700±2.950	63.180±1.660	67.760±5.750
Compound-22	23.860±8.910	41.430±7.770	48.160±7.160	66.030±8.240	77.600±4.220
Ascorbic Acid	64.380±15.470	66.640±15.420	78.130±3.220	85.680±1.690	87.230±0.980

All parameters are expressed as mean ± SD of three independent measurements.

Appendix E: Supplementary material for chapter 6

Table 1S: Ethnobotanical information on some medicinal herbs used in different regions of Saudi Arabia

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Blepharons ciliaris L.	Shook aldabb	Acanthaceae	Leaves & whole plant	Toothache & skin wound.	M, W, S	12, 13, 14, 15, 16
Blepharis maderaspatensis	Athrarh		Leaves	Used as treatment for scorpion sting.	-	17
Ecbolium viride (Forssk)	Khoseer		Leaves powder	Treat pimples & when applied around genital area it improves urination.	S	15, 16
Ecbolium gymnostachyum (Nees)	Syhan		Leaves	Wound healing & bone fracture.	M	13
Hypoestes forsskalii (Vahl)	Nadqah		Leaves	Anti-inflammatory & wound healing in cattle.	M	13
Adiantum capillus-veneris	Kizbratal Baer	Adiantaceae	Whole plant	Antipyretic, antitussive, diuretic, emmenagogue, expectorant & pulmonary catarrh.	M, W	12, 13
Dracaena ombet Kotschy & Peyr.	Azef	Agavaceae	Resin	Used as antihemorrhagic & for skin infections.	S	15, 18
Sansevieria ehrenbergii Schweinf. ex Baker	Salaf		Leaves	Used for treating blisters.	S	15, 18
Aizoon canariense L.	Hodaq	Aizoaceae	Whole plant	Used to treat indigestion flatulence & hypertension.	M	13
Mesembryanthemum crystallinum	Nabat Al-thalg		Whole plant	Antimicrobial.	S	19
Mesembryanthemum forsskalei	Samh		Seeds	Making bread.	S	19
Trianthema portulacastrum	Laani		leaves	Used as treatment for scorpion sting.	-	17
Achyranthes aspera L.	Mahwat	Amaranthaceae	Leaves, roots, seeds & barks	Stomachache, bowel complaints piles, boils, scorpion sting & skin eruptions.	S, W	15, 17
Aerva javanica (Bunn.f.)	Tuwain		Whole plant	Toothache, hemorrhage, snake & insect bites.	M, W, S	12, 13, 15
			Roots (juice)	Treat eye diseases in cattle.		
Aerva lanata L.	Al-Athlab		Whole plant	Diuretic, scorpion sting & demulcent.	M, S	13, 15, 8, 17
Alternanthera pungens Kunth.	-		Whole plant	Used as treatment for scorpion sting.	M	17

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Alternanthera sessilis L. R.Br. ex DC.	-	Amaranthaceae	Leaves	Used as treatment for scorpion sting.	M	17
Amaranthus caudatus L.	Kaf-Almehana		Whole plant	Diuretic, strangury, blood purifier & for the treatment of piles.	S	15
			Leaves	Used as an abortifacient.		
Amaranthus graecizans L.	Thaflah		Leaves	Chewed & the liquid swallowed to treat tonsillitis, scorpion sting & used as anthelmintic.	M	13, 17
Amaranthus hybridus Spinach	Sabanih		Whole plant	Treat jaundice, inflammation, blood tonic, laxative & as digestive.	-	20
Amaranthus retrofexus	Oshba alqanzeer		Leaves	Astringent & anthemorrhagic.	M	13
Amaranthus spinosus L.	Da'ad		Whole plant	Antipyretic, diuretic, laxative, scorpion sting & stomachic.	S	15, 17
			Roots	Treat gonorrhea & constipation & jaundice.		
Amaranthus viridis L.	Shae		Whole plant	Antipyretic, diuretic, emollient in scorpion sting, expectorant, laxative & leprosy.	M, S	13, 15, 17
Bassia muricata L.	Hassaniya		Whole plant	Antimicrobial.	S	15, 19
Celosia trigyna L.	Trgana		Leaves & flowers	Treatment of diarrhoea & for excessive menstruation	S	13, 19
Annona squamosa L.	-	Annonaceae	Leaves, roots & barks	Used as treatment for scorpion sting.	-	17
Ammi majus	-	Apiaceae (Umbelliferae)	Whole plant	Skin diseases, leukoderma, contraceptive, diuretic, tonic, angina, carminative, antiasthmatic & toothache.	M	12

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Anethum graveolens L.	Dill, Shibt	Apiaceae (Umbelliferae)	Whole plant, roots, leaves, seeds, fruits	Antimycobactetial, Antifungal, insecticides, psychoactive, hallucinogenic, appetizer, colic gripes, mouth wash, carminative, aphrodisiac, diuretic, astringent, cordial, laxative, stimulant, emmenagogue, anthelmintic, abortifacient, antispasmodic, treating bronchitis, liver and spleen disorder, lumbago & galactagogue.	S, M, W	12, 15, 13
Apium graveolens	Karfas		Flowers roots & seeds	Digestive, antacid, body tonic, laxative, chest ailments, arthritis, jaundice, appetizer, colic gripes, tonic, purgative, vermifuge, antiasthma, liver and spleen disorder, antirheumatic, anasarca, homeopathic, antispasmodic, sedative, high blood pressure, kidney problems, anticonvulsant & calming.	M	12, 20
Bepleurum semicompositum	-		Fruits	Stomach troubles, carminative & mentalis disorders.	M	12
Carum carvi L.	-		Roots	Used as treatment for scorpion sting.	-	17
Conium maculatum L.	Shawkaran		Flowers & leaves	Used as treatment for scorpion sting.	-	17
Coriandrum sativum L.	Kosbarah		Whole plant	Antiflatulence, antidiarrhea, anticolic & as general tonic.	S	15
Cuminum cyminum	Kamun		Seeds & leaves	Diuretic, carminative, anticolic, antioxidant, uterine discharge, hiccup, appetizer, deafness, spasm & digestive.	S	21, 20

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Daucus carota Linn.	Jizr	Apiaceae (Umbelliferae)	Leaves & roots	Mucolytic, chest pain, cough, hepatic and gastric problems, diuretic, brain stimulant, coarseness of voice, digestive, fatigue, hypertension, nervousness & skin diseases.	-	20
Ducrosia ismaelis L.	-		Whole plant	Antimicrobial.	S	15, 19
Echinosciadium arabicum L.	-		Whole plant	Central nervous system stimulation & antimicrobial.	S	15, 19
Eryngium foetidum L.	Cilantro		Whole plant	Used as antipyretic, antiemetic & antidiarrheal.	S	15, 18
Foeniculum vulgare Mill.	Shawmar, Sheeh		Whole plant seeds, leaves & stems	Used to relieves digestive problems, increases lactation, antiflatulence, reduces inflammation, antimicrobial, used for skin disorders, antitussive, stomach problems, as toothbrush, used for conjunctivitis & used for blepharitis of the eye.	W	13, 22
					S	15, 16, 23
Petroselinum crispum L.	-		Whole plant	Used against kidney stones.	S	15
Petroselinum sativum L.	Maqdunes		Whole plant & root	Used against kidney stones, diuretic & liver disease.	S	15, 23, 20
Pimpinella anisum Lnn.	Yanisun		Leaves & seeds	Purgative, cough, mucolytic, headache, dejection & earache.	-	20
Pituranthus triradiatus L.	-		Whole plant	Treat hypertension &as antimicrobial.	S	15, 19
Trachyspermum ammi L.	Kemyon, Nahwa		Seeds & stem	Used against kidney stones & used as treatment for scorpion sting.	S	15, 23
Adenium arabicum Balf.	Adnah	Apocynaceae	Barks & whole plant	Used in bones dislocations, painful joints, wounds & skin infections.	S	15, 21
Adenium obesum L.	Aden		Whole plant & barks	Applied on wounds as antimicrobial & used as treatment for scorpion sting.	S	15, 16, 19, 17
Blyttia reticuloses L.	-		Whole plant	Antimicrobial.	S	15, 19

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Canthranthus roseus	Biftah	Apocynaceae	Leaves & roots	Digestive, astringent, emetic, purgative, hemorrhagic, emmenagogue, diabetes, used as treatment for scorpion sting & cancer.	M	12, 17
Carlumab penicillata (Defl.)	Ghaltha		Whole plant & leaves	Central nervous system stimulation, used in diabetes, stomach ulcer & smallpox.	S	15
Carissa edulis Vahl Symb. Bot.	A'rm, Airoon		Whole plant	Anthelmintic, antiscorbutic, astringent, stomachic & toothache.	S	15
Holarrhena pubescens (Buch.-Ham.)	Kurchi		Seeds & barks	Treating painful joints & in diarrhea.	S	21
Nerium oleander L.	Dafla		Leaves & roots	Treat skin disease & steam of boiled leaves inhaled in sinusitis.	W, S	15, 16
Rhazya stricata Decne.	Harmal		Whole plant	Antirheumatic, pain relief, antibacterial & treatment for allergy.	M, S	12, 14, 15, 17
Torularia torulosa L.	-		Whole plant	Lower blood pressure & cardiac stimulation.	S	15, 19
Arisaema flavum	Dakaf	Aracaceae	Rhizome	Used as treatment for scorpion sting.	-	17
Hyphaene thebaica L. Mart., Hist.	Doom		Whole plant	Cardiac stimulation & antimicrobial.	S	15, 19
Phoenix dactylifera L.	Nakhl		Flower & pollen	Used as an aphrodisiac & as a general tonic.	S, W, M	21
Aristolochia bracteolata Lam.	Loiya	Aristolochiaceae	Leaves	Used to treat snake bite & scorpion sting.	S	16, 17
Calotropis procera Ait.	Oshar	Asclepiadaceae	Whole plant & latex	Respiratory system, antiasthmatic, anticancer, joint inflammation, antibacterial, purgative, expectorant, dysentery, emetic & for leshmaniasis.	M, W, S	12, 13, 14, 16

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Caralluma sinaica (Decne.)	Ded-Elkalba	Asclepiadaceae	Whole plant & leaves	Hypoglycemic & antiprotozoal.	S	15, 24
Caralluma tuberculata N. E. Br.	Pawany		Whole plant	Used in the case of diabetes, peptic ulcers، inflammation & its juice as drops for ear inflammation.	S, W	16
Cynanchum acutum L.	Al-Modeed		Leaves & stems	Insecticide & parasiticide.	S	15
Gomphocarpus fruticosus L. Ait.f.	Alqaraa Kabeer		Leaves	Tumors, skin disease, scabies & itching.	S	25
Gomphocarpus sinaicus	Arjal		Whole plant	Used for hemorrhagic disorders, rhinorrhagia & metrorrhagia.	M	12
Leptadenia pyrotechnica (Forssk.)	Markh		Whole plant & seeds	Used for flu, tussive, lactagogue & antimicrobial.	M, S	12, 15
Pergularia tomentosa L.	Gholfa		Whole plant	Skin diseases.	M, W, S	13, 14, 15
Periploca aphylla L.	Sawas		Whole plant	Antiprotozoal, stomachic & purgative.	S	18, 24
Aloe tomentosa Defl.	Sabbar	Asphodelaceae	Leaves & sap	Applied on skin for rashes & sunburn. Sap is applied for inflammation of eyes.	S	16, 21
Aloe vera (L.) Burm. f.	Saqal, Sabar		Whole plant, Juice of leaves, bulbs & roots	As laxative, in asthma, peptic ulcers, burns & diabetes.	S	15, 26
Asphodelus centifolias L.	Broque		Seeds	Used in colds, haemorrhoids & as antirheumatic.	S	15
Asphodelus fistulosus	Towaim,		Whole plant & seeds	Diuretic, ulcers, toothache & antiinflammatory.	W, M, S	12, 14, 15
Achillea biebersteinii Afan.	Thafraa	Asteraceae (Compositae)	Leaves	Strong antimicrobial activity, relieve itching, remove readiness & swelling in eyes.	S	15, 19, 21

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Achillea fragrantissima L.	Qusom	Asteraceae (Compositae)	Whole plant	Central nervous system stimulation & antimicrobial.	S, M	15, 19
Ambrosia maritima L.	Ambrosia		Whole plant	Antiflatulence & diuretic.	S	15, 21
Anthemis melampodina L.	Aqhwan		Whole plant	Antimicrobial.	S	15, 19
Anthemis pseudocotula L.	Aqhwan		Whole plant	Antimicrobial.	S	15, 19
Anvillea gracinii (Burm.f) DC.	Nougd		Whole plant	Used treatment for colds, diabetes.	W, S	13
Artemisia abyssinica L.	Boatheran		Whole plant	Antimicrobial.	S	15, 19
Artemisia herbaalba L.	Chih		Flowery tips	Digestive disorders, abdominal pain, colic & liver failure.	S	15
Artemisia judaica	Boaithran		Whole plant	Antipyretic, menstruation regulator, for nerve system, carminative & emmenagogue.	M	12
Artemisia monosperma	Adar		Leaves & flowers	Constipation, antirheumatic & flu.	M	12
Artemisia scoparia	Slikah		Whole plant	Purgative, earache, antibacterial, hypercholesteremia, antipyretic, antiseptic, cholagogue, diuretic, vasodilator, jaundice, hepatitis, inflammation of the gall bladder & treatment of scorpion sting.	M	12, 17
Artemisia sieberi Besser.	Chih		Leaves	Used as an anthelmintic.	S, M	12, 15, 27
Atractylis carduus	Korshoof		Whole plant	Cardiac depression.	W, S	13, 19
Calandula micrantha L.	-		Whole plant	Central nervous system stimulation.	S	15
Carthamus tinctorius L.	Zafaran		Leaves	Treating red, swollen eye & conjunctivitis.	S	21
Centaurea iberica	-		Leaves	Treatment of scorpion sting.	-	17
Centaurea sinaica DC.	Marar		Whole plant	Central nervous system stimulation.	W, S	13, 19
Centaurothamnus maximus	Byad		Leaves	Wound healing.	-	25

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Chrysanthenum coronarium	-	Asteraceae (Compositae)	Whole plant	Blennorrhagia, purgative, inflammation, syphilis, expectorant, stomachic & gonorrhea.	M	12
Cichorium intybus L.	Hendiban		Leaves, seeds & roots	Antipyretic, dyspepsia, headache, jaundice & as demulcent.	-	21
Cnicus benedictus L.	-		Whole plant	Treatment of scorpion sting.	-	17
Conyza bonariensis L.	Khoa		Whole plant	Antimicrobial.	S	15, 19
Conyza dioscoridis L. (Desf.)	Ain alkatkot		Leaves	Epilepsy in children.	S	15
Conyza incana L.	Arfaj		Whole plant	Central nervous system depression, cardiac stimulation & antimicrobial.	S	15, 19
Echinops galalensis L.	Karshoof		Whole plant	Antimicrobial.	S	15, 19
Echinops hussoni L.	-		Whole plant	Antimicrobial.	S	6, 8
Echinops spinosissimus Tuna.	Shook Al-gamal		Whole plant	Splenic disease & sore throat.	W, M, S	13, 14, 28
Eclipta prostrata L.	-		Whole plant & leaves	Treatment of scorpion sting.	-	17
Euryops arabicus Steud.	Kaboor		Whole plant	Cardiac stimulation & for wound healing.	W, S	13, 19, 21
Francoeuria crispa (Forssk)	Githgath		Whole plant	Used for swellings & as anti-inflammatory.	M, S	14, 28
Gnophalium luteo-album	-		Leaves & stems	Expectorant, astringent, cholagogue, diuretic, haemostatic & breast cancer.	M	12
Jasonia candicans L.	-		Whole plant	Cardiac stimulation & antimicrobial.	S	13, 19
Kleinia pendula (Forssk.) DC.	Laban		Roots	Otitis.	S	25
Lactuca saligna	Odad		Whole plant & seeds	Tonic, carminative, diuretic, colic, catarrh, bronchitis, typhoid, fever & for digestive problem.	M	12
Lactuca serriola L.	-		Latex	Treatment of scorpion sting.	-	17

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Launea nudicaulis L.	Hwwa	Asteraceae (Compositae)	Leaves	Antipyretic & antihemorrhagic after childbirth.	S	21
Matricaria aurea (Loel.) Sch.Bip.	Babunaj		Flowers	Used for making a tea for all stomach ailments & used to treat skin diseases.	S	14, 15, 18
Picris abyssinica L.	-		Whole plant	Antimicrobial.	S	15, 19
Picris cyanocarpa	Hozan		Whole plant	Central nervous system stimulation, used for hypertension & cardiac stimulant.	S	15, 19
Pluchea arabica (Boiss.) Qaiser & Lack	Godot		Whole plant	Treating boils, skin sores & ear infection.	S	15, 18
Psiadia arabica Jaub.	Tubbak		Heated branches	Antirheumatic & heal broken bones.	S	16
Psiadia punctulata DC.	Fotaa		Branches & stems	Relieve muscle pain.	W, S	13, 18
Pulicaria arabica L.	Garaez		Whole plant	For hypertension & antimicrobial.	S	15, 19
Pulicaria crispa	Arararabi		Whole plant	Antimalarial & stomach disorders.	S	15
Pulicaria guestii Rawi	-		Whole plant	Strong antimicrobial.	S	15, 19
Pulicaria jaubertii Gamal-Eldin	Ansif		Leaves & flowers	Used for digestion problem & as tonic.	S	21
Pulicaria undulata (Forssk.) Oliver.	Gathgath		Wole plant	Central nervous system depression & antimicrobial.	W, S	13, 15, 19
Rhanterium epapposum L.	Arfaj		Whole plant	Antimicrobial.	S	15, 19
Senecio asirensis Boulos & J. R. I. Wood	Pedaa, Henna		Leaves	Antipyretic.	S	15, 18
Sonchus oleraceus L.	Uddaid		Leaves & flowers	Promotes menstruation & treatment of scorpion sting.	W, M	13, 14, 17
Tagetes minuta L.	Bard-agoosh		Leaves & flowers	Treatment for cold & constipation.	S	16
Verbesina encelioides (Cav.)	Safeara		Leaves	Wound & skin disease.	S	15
Vernonia schimperii L.	Neeka		Leaves	Treat scorpion bites, antipyretic & bites.	S	15, 21
			Roots & seeds	Anthelmintic.		

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Xanthium spinosum L.	Shobet	Asteraceae (Compositae)	Wole plant	Antimicrobial.	S	15
Alkanna orientalis (L.) Boiss.	Lebbed	Boraginaceae	Whole plant	Central nervous system stimulation, antimicrobial & throat pain.	S	19, 21
Anchusa milleri L.	-		Whole plant	Central nervous system stimulant.	S	19
Arnebia hispidissima (Lehm.) DC.	Kohaeel		Whole plant	Antipyretic.	W, S	13
Cordia myxa L.	Bambar		Leaves & seeds	Used for stomach ailments, wounds healer, tonic, expectorant, refreshing, heart and brain diseases & respiratory system.	M	12, 21
Cordia sinensis Lam.	Tanab		Leaves & stems	Antirheumatic, painful menstruation, bladder diseases, gastric ulcers & malaria.	-	24
Echium horridum L.	-		Whole plant	For hypertension.	S	19
Heliotropium aegyptiacum Lehm.	Ramram		Root	Treatment of scorpion sting.	-	17
Heliotropium arbainense Fresen.	Ramram		Whole plant	For hypertension & antimicrobial.	W, S	13, 19
Heliotropium digynum (Forssk)	-		Leaves	Skin diseases, demonomania & antimicrobial.	M, W, S	12, 13, 19
Heliotropium europaeum L.	-		Whole plant	Antimicrobial.	W, S	13, 19
Heliotropium ramosissimum DC.	Rmram		Whole plant	Used for snake bites.	W	13
Heliotropium strigosum Willd.	Ramram		Whole plant	Used for snake bites.	-	17
Trichodesma africanum L.	Hamham		Whole plant	Treatment for cough & cold.	M, S	14
Balanites aegyptiaca (L.) Del	-	Balanitaceae	Whole plant	Treatment of different ailments such as syphilis, jaundice, liver and spleen problems, epilepsy, as treatment for scorpion sting & yellow fever.	S	15, 17
Anastatica hierochuntica L.	Khaf-Maryam	Brassicaceae (Cruciferaceae)	Whole plant	Facilitate labor & antidiabetic activity.	M, S	14, 29
Brassica rapa L.	-		Whole plant	As aphrodisiac.	S	15

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Diplotaxis acris (Forssk) Boiss	Fegl Algabal	Brassicaceae (Cruciferaceae)	Leaves	Antidiabetic & wound healing.	S	15
Diplotaxis harra L.	Harra		Whole plant	Antimicrobial.	S	15
Eruca sativa L.	Rocka		Seeds	Used in ringworm.	M, S	14
Farsetia aegyptiaca Turra.	Jarbaa		Whole plant	Antirheumatic.	W, S	13, 14
Farsetia longisiliqua Decne	-		Whole plant	Central nervous system depression.	S	19
Lepidium aucherii L.	-		Whole plant	For hypertension & cardiac stimulant.	S	19
Lepidium draba L.	-		Whole plant	Central nervous system stimulation & antimicrobial.	S	19
Lepidium sativum L.	Thuffa, Rashad		Leaves & seeds	Used for aches and pains, emollient, antifungal & for measles.	S	30, 21
Morettia parviflora L.	-		Whole plant	Antimicrobial.	S	15
Nasturtium officinale R.Br.	Jarjir		Whole plant, leaves & roots	Diuretic, digestive, herpetic eruptions, jaundice & renal disease.	-	20
Notoceras bicorne L.	-		Whole plant	Antimicrobial.	S	19
Savignya parviflora L.	-		Whole plant	Central nervous & cardiac systems stimulant.	S	15
Schimpera arabica L.	-		Whole plant	Cardiac stimulant.	S	19
Sisymbrium irio L.	-		Seeds	Antipyretic.	S	18
Zilla spinosa Prant L.	Shibrim		Leaves	Purgative but toxic at high dose.	S	14
Commiphora Africana L.	-	Burseraceae	Whole plant	Anticancer & anti-inflammatory.	S	21
Commiphora habessinica	Mrr		Resins	Used for chest pain & as antiinfective.	-	21
Commiphora molmol (Engl.)	-		Barks	Treatment of snake bites.	-	17
Commiphora myrrha (Nees) Engl.	Myrrha		Resins	Antimicrobial, antiseptic, astringent, carminative, disinfectant, antileishmania & expectorant.	S	15, 27
Commiphora opobalsamum L.	-		Whole plant	Antimicrobial.	S	19, 15
Opuntia ficus-indica Mill.	Barshoom	Cactaceae	Succulent	Treatment for pimples & skin problems.	S	15, 16

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Tamarindus indica L.	Tamr-Hindi	Caesalpinaceae	Fruits	Headache, jaundice, relieve stomach problem, antihypertensive, wound healing & antiemetic.	S	115
Celtis Africana N.L.Burm,	-	Cannabaceae	Leaves & stems	Antirheumatic, toothache & anticancer.	-	24
Cadaba farinose Forssk.	Asaf	Capparaceae (Capparidaceae)	Whole herb	Purgative, anthelmintic, emmenagogue & aperient.	S	8, 24
			Leaves	A remedy for dysentery, fever, cough & lungs problem.		
Cadaba glandulosa Forssk.	Qormot		Leaves & stems	As anthelmintic.	-	24
Capparis cartilaginea	Shafallah		Whole plant	Disinfectant, anti-inflammatory, wound wash, antitumor, tonic & purgative.	M, S	12, 15
Capparis decidua	Tandhab		Whole plant	Antirheumatic, astringent, cough, tremor, wound, sedative, vermifugal, antidiabetic & gout.	M, W, S	12, 13, 15, 31
Capparis spinosa	Kabar		Roots, barks & leaves	Aperient, tonic, diuretic, antidiabetic & expectorant.	M, W, S	12, 13, 15, 31
Dipterygium glaucum	Alqa		Leaves	Expectorant, analeptic & stimulant.	M, S	12, 15
Maerua crassifolia	Sarh		Leaves	Used in toothache & intestinal disease.	W, S	8, 13
Maerua oblongifolia	Maru		Whole plant	Hypocholesterolemic.	S	8
Dianthus deserti	Alhilba	Caryophyllaceae	Roots	Used for sprains.	W	13
Polycarpaea repens	Rokeka		Whole plant	Used as antidote against snake bites.	W, S	13, 15
Catha edulis Forsk.	Khat	Celastraceae	Leaves	Central nervous system stimulant & treatment for diabetic.	S	16
Moytenus ovatus	Hurgran		Leaves	Used for stomach problems.	W, S	13, 20
Anabasis articulate L.	-	Chenopodiaceae	Whole plant	Cardiac stimulation & antihypertension.	S	19

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Anabasis setifera Moq.	Himd	Chenopodiaceae	Leaves	Antidepressant.	M, S	14, 15
Atriplex halimus L.	Rughl		Seeds	Emetic.	W	13, 20
Beta vulgaris	-		Roots	Headache, toothache, liver pain, burns, constipation, emmenagogue, purgative, eye inflammation, itch, scurf & dandruff, tumor, leukemia, anemia, snake bite, vermifugal & antirheumatic.	M	12
Brassia eriophora	-		Whole plant & seeds oil	Antirheumatic, snake bite & vermifugal.	M	12
Brassia muricata	-		Whole plant & seeds oil	Kidney diseases, antirheumatic & ulcer gargle.	M	12
Chenopodium album L.	Atrah		Leaves & fruits	Postnatal problems.	W, M, S	13, 14, 15
Chenopodium ambrosioides L.	-		Leaves	Used as treatment for scorpion sting.	-	17
Chenopodium murale L.	AlZorbiah, Jkheara		Whole plant & Flowers	Stomachache, leishmaniasis, antitumor & antihypertension.	M, W, S	12, 13, 15, 32
Cornulaca monacantha Delile	Had		Leaves	Used for liver problems, jaundice & purgative.	W, S	13, 15
Haloxylon salicornicum	Ramath		Whole plant	Antidiabetic & used for cold.	W, M, S	13, 14, 15
Kochia indica	-		Whole plant	Heart tonic.	M	12
Salsola imbricate	Kha·reet		Whole plant	Anthelmintic.	M, S	14, 15
Seidletzia rosmarinus Bunge ex Boiss	Ushnan.		Leaves	Antimicrobial.	S	19
Suaeda vera L.	-		Whole plant	Cardiac stimulant.	S	15
Ceratophyllum demersum L.	-	Ceratophyllaceae	Whole plant	Treatment for scorpion sting.	-	17
Cleome amblyocarpa	Khunayzah	Cleomaceae	Whole plant	Antimicrobial.	W, S	13, 15
Cleome arabica	Zafrah-Amal		Leaves	Tonic, stimulant, appetizer, purgative & for panicula.	M, S	12, 15
Cleome brachycarpa	Birbran		Whole plant	Itching, antirheumatic, inflammation, leukoderma & skin disease.	M, S	12, 15
Cleome chrysantha	Safaira·a		Whole plant	Anthelmintic & antiseptic.	S	8

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Cleome gynandra L.	-	Cleomaceae	Whole plant	Treatment for scorpion sting.	-	17
Cleome viscosa L.	Om-Hanif		Whole plant	Carminative, anthelmintic & rubefacient.	S	8
Hypericum chrysostrictum L.	-	Clusiaceae	Whole plant	Central nervous system depression & antimicrobial.	S	19
Commelina benghalensis L.	-	Commelinaceae	Whole plant	Treatment for scorpion sting.	-	17
Convolvulus arvensis L.	Olaique	Convolvoulaceae	Leaves & fruits	Foot cracking.	W, M, S	13, 14, 15
Convolvulus fatmensis	Al-oleeq		Leaves	Anti-inflammatory.	S	15
Convolvulus hystrix	-		Whole plant	Antimicrobial.	S	15
Convolvulus oxyphyllus	-		Whole plant	Cardiac depression.	S	15
Convolvulus pilosellifolius	-		Whole plant	Antimicrobial.	S	15
Cressa cretica	Naduoh		Whole plant	Digestive, tonic, vermifugal, antiasthmatic, tuberculous, hematonic, CNS depressant & appetizer.	M, S	12, 19
Evolvulus alsinoides L.	-		Whole plant	Treatment of scorpion sting.	-	17
Ipomoea aquatic Forssk.	-		Leaves	Treatment of scorpion sting.	-	17
Ipomoea eriocarpa R.Br.	-		Leaves	Treatment of scorpion sting.	-	17
Citrullus colocynthis L.	Hunzal	Cucurbitaceae	Roots, seeds & fruits	Used as diuretic, emetic, expectorant, purgative, jaundice, ascites, analgesic, anesthetic, anti-HIV, antiaging, allergy, antiasthmatic, antibacterial, antidiabetic, sedative, antihemolyteic, antimalaria, antimitotic, antioxidant, antiscorbutic, antiseptic, antitumor, carminative, fungicide, herbicide, insecticide, laxative & lubricant.	M, W, S	12, 15
Coccinia grandis L.	Bakhra-a		Leaves	Treat earache & for scorpion sting.	S	16, 17

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Cucumis melo	Shamam	Cucurbitaceae	Leaves & fruits	Astringent, demulcent, laxative, antipyretic, jaundice & renal calculi.	-	20
Cucumis prophetarum	Shree-Elzeeb		Roots, seeds & fruits	Sexual diseases, liver diseases, stomachache, demonomania, emetic, antimicrobial & purgative.	M, S	12, 15
Kedrostis foetidissima Jacq. Cong.	-		Leaves	Used for warts.	S	25
Momordica balsamina L.	Madoda		Whole plant	Antimicrobial.	S	19
Juniperus polycarpos L.	-	Cupressaceae	Whole plant	CNS stimulant & antimicrobial.	S	15
Juniperus procera	Arar		Leaves	Gout, jaundice & cure wounds.	S, W	33
			Fruits	The smoke of fruiting branches used as antirheumatic, headaches & skin diseases.		
			Twigs & buds & small branches	Intestinal worms and a decoction of dry young branches is used as medicine against itch of camels.		
			Resin	Stimulant and for treatment of ulcers and liver diseases.		
Cuscuta campestris	-	Cuscutaceae	Whole plant	Purgative & constipation.	M	12
Cynomorium coccineum L.	Tartooth	Cynomoriaceae	Whole plant	Astringent, aphrodisiac, laxative & tonic.	M	12, 15
Cyperus longus L.	-	Cyperaceae	Whole plant	Treatment of scorpion sting.	-	17
Cyperus rotundus L.	Alsaad		Whole plant	Treatment of scorpion sting.	W	13, 17
Ephedra alata L. (female)	-	Ephedraceae	Whole plant	Central nervous system stimulant & antimicrobial.	S	19
Acalypha ciliata Forssk	-	Euphorbiaceae	Leaves	Antimalaria, anti-scabies & anthelmintic.	S	25
Acalypha fruticosa Forssk	Zohar		Leaves	Used for treating bee stings.	S	15
Acalypha indica L.	Thfelan		Whole plant	Used for cure from bronchitis, pneumonia & asthma.	S	15

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Andrachne aspera Spreng. var. glandulosa A.Rich	Kamas	Euphorbiaceae	Whole plant	Used in eye problems & for eye wash.	S	9
Chrozophora oblique L.	-		Whole plant	Antimicrobial.	S	15
Chrozophora oblongifolia (DC.) A. Juss. ex Spreng	Tannoum		Whole plant	Antimicrobial, cathartic & emetic.	S	15
Chrozophora plicata (Vahl) A. Juss	Tanoom		Whole plant	Depurative & purgative.	S	8, 18
			Leaves	Used for the cure of leprosy.		
Clutia lanceolata Forssk.	Laukh		Whole plant	Hypoglycemic.	S	9
Croton lobatus L.	-		Leaves	Used as treatment of scorpion sting.	-	17
Euphorbia arabica Hochst.	-		Whole plant	Used in skin infection.	S	34
Euphorbia cuneate Vahl.	AL-baky		Whole plant	Sedative & antimicrobial.	W, S	13, 9
Euphorbia cyparissioides L.	-		Whole plant	Antimicrobial.	S	19
Euphorbia dracunculoides Lam.	Yaktin		Leaves & fruits	Purgative, intestinal disorders, antitumor & wart remover.	M, S	12, 8
Euphorbia granulate Forssk.	Lebbein		Whole plant	Blood purifier, diuretic, purgative & vermifugal.	M, W, S	12, 13, 53
			Latex	Used as purgative.		
Euphorbia helioscopia L.	Emaiah		Whole plant	Purgative, ulcer, antirheumatic, vermifugal, cholera, cancer, anthelmintic, catarrh & eruptions, neuralgia.	M, S	8, 12
Euphorbia hirta L.	-		Whole plant	Antiasthmatic, antiasthmatic & bronchitis.	S	15
Euphorbia peplus L.	Khaneez		Whole plant	Antihypertension.	W, S	13, 15

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Euphorbia retusa Forssk.	Ghazalah	Euphorbiaceae	Whole plant	Antitussive, antiasthmatic & mentalia disorders.	M, S	12, 14, 15
			Latex	Eczema, wound healing & for leishmaniasis.		
Euphorbia schimperiana Scheele.	Saibarisodis		Whole plant	Antitussive, antiasthmatic, earache, skin infections & snake bites.	S	35
Euphorbia scordifolia Jacq.	Rummid		Whole plant	Antipyretic & constipation.	S	34
Euphorbia terracina L.	Harmal		Whole plant	Used as a remedy for fever & paralysis.	S	34
Jatropha curcas L.	Kharat		Leaves	Used in wounds, eczema & scabies.	S	9
Jatropha glauca	Obeeb		Whole plant	Treatment of chronic skin diseases.	S	9
Jatropha pelargoniifolia	Obab		Whole plant & petioles	The sap of the petiole is applied to ulcers.	S	15
Phyllanthus maderaspatensis L.	Tamarhindi		Leaves	Used for headache.	S	8
Ricinus communis L.	Kharwah		Whole plant	Treatment of scrofulous sores, boils & rheumatic swellings	W, S	13
Acacia arabica L.	-	Fabaceae (Leguminosae)	Bark, gum, leaves, seeds & fruits	Nutritive, expectorant, antihemorrhagic, diarrhea, cough, blennorrhagia, mouthwash, astringent, dysentery, gargle, tonic, inflammation of urinary tract, hemorrhagic, transudation, aphrodisiac & diabetes.	S, M	18, 12
Acacia ehrenbergianan	Salam		Wood	Used to treating paralysis.	-	20
Acacia farnesiana	-		Whole plant	Vermifugal, dysentery, mouth wash, blood diseases, antipruritic, ulcers, leukoderma, catarrh, vulvovaginitis, astringent, demulcent, aphrodisiac, antispasmodic & insecticidal.	M	12

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Acacia gerardi Benth.	Karat	Fabaceae (Leguminosae)	Resin & pods	Used for burns, toothache & antipyretic.	-	20
Acacia seyal	Talh		Barks, gums & seeds	Astringent, cold, ophthalmia, diarrhea, haemorrhage & leprosy.	M	12
Acacia negrii Pichi.	Salam		Leaves	Treat eye diseases.	S	16
Acacia oerfota Forssk.	-		Whole plant	Treatment of scorpion sting.	-	17
Abrus precatorius L.	Habb shoush		Root	Used to treatment of scorpion sting, emetic, eye disease & purgative.	-	17, 20
Albizia lebbeck	-		Fruits & stems	Used for snake bites, mouthwash, antiparalysis, night blindness, astringent, diarrhea, dysentery, gonorrhea & swelling of cervical glands.	M	12
Alhagi graecorum Boiss	Aqool		Whole plant	Analgesic, antitussive, antihemorrhoids, anti- rheumatic, aphrodisiac, diuretic & laxative.	S	15
Alhagi maurorum Medic.	Al-Agool		Leaves	Antioxidant & analgesic.	S	15
Alhagi melorum	-		Whole plant	Antipyretic, digestive, tonic, purgative, diuretic & catarrh.	M	12
Astragalus atropilosus	-		Leaves	For backache.	S	15
Astragalus mareoticus	-		Leaves	Treatment of scorpion sting.	-	17
Astragalus sieberi	-		Whole plant	Antihypertension.	S	19
Astragalus spinosus (Forssk.) Muschl.	Katad		Whole plant	Treat leukemia & promote wound healing.	W, S	13, 15
Astragalus tribuloides	-		Seeds	Anti-esophagitis, anti-enteritis & peri colitis.	M	12
Cassia holosericea	-		Leaves & fruits	Tonic for digestive system, flatulence & purgative.	M	12
Cassia senaa L.	Senna		Whole plant	Antimicrobial, laxative & purgative.	S	19, 16

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Cassia italic Mill.	Ishriq	Fabaceae (Leguminosae)	Leaves	Laxative, treating influenza and other respiratory disease & urinary tract purifier.	M, S	15, 16
Cicer arietinum L.	Himas		Seeds	Used in abortion, cure ulcer, antitumor, anti-scabies, for pimples, toothache, oedemas, renal calculi, aphrodisiac & diuretic.	-	20
Clitoria ternatea L.	-		Leaves, roots & stems	Treatment of scorpion sting.	-	17
Crotalaria retusa L.	-		Stem	Treatment of scorpion sting.	-	17
Delonix elata L.	Ranf		Leaves & seeds.	Mosquito control agent.	S	15
Desmodium gangeticum	-		Roots	Treatment of scorpion sting.	-	17
Dichrostachys cinereal L.	-		Roots & leaves	Treatment of scorpion sting.	-	17
Glycyrrhiza glabra L.	Irk al hiel		Rhizomes	Treating muscle pain & treatment of scorpion sting.	S	17, 18
Indigofera articulate Gouan.	Khedaish		Roots	Relieve toothache.	M, S	12, 18
			Whole plant	Purgative, diuretic, lithotomy, antitoxicant, swelling of spleen, antitumor, antirheumatic, teeth protection & snake bite.		
Indigofera oblongifolia	Hasar		Roots & Leaves	Analgesic, remove hair dandruff & anti-inflammatory.	S	20
Indigofera tinctorial L.	-		Whole plant	Treatment of scorpion sting.	-	17
Lablab purpureus L.	Lablab		Roots	Laxative, diuretic & regulate menstruation.	S	20

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Medicago sativa L.	Jat	Fabaceae (Leguminosae)	Leaves	Used for bone fracture, bruises reliever & used as an aphrodisiac.	S	20
Melilotus albus Medik.	Otrah		Whole plant	Astringent & as antirheumatic.	W, S	20
Melilotus indicus L.	Handaquq		Whole plant	Emollient.	-	20
Melilotus officinalis	Iklilulmalik		Flowers, stems & roots	Diuretic, scabies, boils, wounds, for edema, insomnia & colic.	S	20
Ononis serrate L.	-		Whole plant	Antimicrobial.	S	19
Prosopis cineraria L.	Ghaf		Whole plant	Treatment of scorpion sting.	-	17
Prosopis juliflora L.	-		Whole plant	Antiprotozoal.	S	18
Psoralea plicata L.	-		Whole plant	Antimicrobial.	S	19
Raetam ratam L.	-		Whole plant	Anticancer & anti-inflammatory.	S	27
Senna alexandrina Mill.	Sana		Leaves & fruits	Stimulant laxative & cathartic.	S	15
Senna italic Mill.	Sana-mekki		Leaves & fruits	For elephantiasis & ophthalmic diseases.	M, W, S	12, 13, 15
Taverniera lappacea L.	-		Whole plant	Central nervous system stimulation & antimicrobial.	S	15, 19
Tephrosia apollinea Del.	Dhafran		Whole plant	Antihypertension & cardiac stimulant.	S	15, 19
Tephrosia nubica ssp. Arabica (Boiss.) Gillet.	-		Whole plant	Antihypertension & cardiac stimulant.	S	15
Tephrosia purpurea	Sakhal		Seeds	Relieve urinary tract problems.	S	16
Trigonella anguina L.	Nafel		Whole plant	Antimicrobial.	S	19
Trigonella foenum-graecum L.	Helba		Seeds	Relieve upset stomach.	S	16
Trigonella stellate Forssk.	Girgas		Whole plant	Hair diseases.	N	14
Frankenia pulverulenta	-	Frankenaceae	Whole plant	Carminative & analgesic.	M	12

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Fumaria parviflora	Humaida	Fumariaceae	Whole plant	Digestive, increase biliary secretion, antipyretic, antitoxic, hematonic, diuretic, appetizer, blood purifier, skin diseases, spleen disorder, purgative & antiemetic.	M	12
Geranium trilophum Boiss.	Zahra'a	Geraniaceae	Whole plant	Relieve backache.	S	16
Avena sativa L.	Shofan	Gramineae (Poaceae)	Colloidal oat extract.	Skin diseases.	S	8
Chloris virgata	-		Whole plant	Tonic.	M	12
Cutandia memphitica L.	-		Whole plant	Central nervous system stimulant & antimicrobial.	S	15
Cymbopogon schoenanthus L.	El-lemad		Whole plant	Antipyretic, colic, antispasmodic, hypotension, carminative, colic gripes, flatulence, polyarthritis, hysteritis, analgesic, sedative & expectorant.	M, S	12, 15
Cynodon dactylon	Thail		Whole plant, roots & juice	Diuretic, astringent, ophthalmic disorders, hemorrhage, rhinorrhagia, dysentery, urinary tract inflammation & dysuria.	M, W, S	12, 13, 14, 15
Dactylotenium aegyptium	Bahma		Whole plant & seeds	Analgesic & wound sepsis.	M, S	12, 14, 19
Echinochloa colona	-		Whole plant	Digestive, constipation, treatment of scorpion sting & increase bile secretion.	M, W	12, 17
Eleusine indica	-		Whole plant	Antispasmodic, antipyretic &liver diseases.	M	12
Heteropogon contortus L.	-		Whole plant	Treatment of scorpion sting.	-	17

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Imperata cylindrica	Halfa	Gramineae (Poaceae)	Roots, flowers, branches, young buds	Antipyretic, diuretic, hemorrhagic, hemolysis, rhinorrhagia, carminative, astringent, tonic, emollient, hematuria, hematemesis, edema, jaundice, antibacterial, cancer & tonic.	M, W	12, 13
Panicum turgidum Forssk.	Tammam		Whole plant	Eye infection.	W, M, S	13, 14, 15
Phragmites australis Cav.	Hajna		Whole plant	Used as antiemetic & antipyretic.	-	20
Setaria viridis (L.) P. Beauv.	-		Leaves	Treatment of scorpion sting.	-	17
Ribes nigrum L.	-	Grossulariaceae	Fruits	For throat inflammation & respiratory tract ailment.	-	24
Iris germanica L.	-	Iridaceae	Roots	For treatment of cancer, inflammation, bacterial & viral infections.	-	24
Lanata camara L.	-	Labiatae (Lamiaceae)	Whole plant	Antipyretic, antimicrobial & antimutagenic.	S	15
Lavandula coronopifolia	Dikta		Whole plant	Antibacterial.	S	9
Lavandula dentata L.	Dhurum		Leaves & flowers	Used in headache, relieve rheumatic pain & cold.	W, S	13, 16
Lavandula pubescens Decne.	Attan		Leaves & flowers	For cold & headache.	W, S	16
Lavandula stoechas subsp. Stoechas	Lavender		Whole plant	antiseptic, antispasmodic, digestive, expectorant & antiasthmatic.	S	9
Marrubium vulgare L.	Frasyoon		Whole plant	Used for the treatment of coughs, and chronic bronchitis, dyspepsia, jaundice amenorrhea, rheumatism & hepatitis.	W, S	13, 19
Mentha lavendulaceae Willd.	Niena·a		Whole plant	Analeptic & carminative.	S	15
Mentha longifolia L.	Haback		Leaves	Used for headache, antipyretic, menstrual cramps & anti-infective.	W, S	13, 16

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Mentha microphylla C. Koch	Niena'a, bariniena'a	Labiatae (Lamiaceae)	Whole plant	Analeptic, appetizer & carminative.	W, S	13, 34
Meriandra benghalensis Benth.	Dharah		Leaves	Used for headache, joint ache & muscle pain & skin problems.	S	16
Nepeta deflersiana	Shya'a		Leaves	Relieve stomach ache, antimicrobial & in burns.	S	16, 19
Ocimum americanum L.	Sims		Leaves	Used in parasitic skin disease.	S	15
Ocimum basilicum L.	Rayhan		Leaves	Allay upset stomach ache, cold & in fever.	W, S	13, 16
Ocimum tenuiflorum L.	Shajrat-azzir		Whole plant	Snake bites & scorpion sting.	S	15
			Leaves	Used in cough & bronchitis.		
Origanum majorana L.	Bardakush		Whole plant	Used in asthma, cough, indigestion, rheumatism & headache.	S	9
Origanum syriacum L.	Al-barda		Leaves	Antitussive & anti-inflammatory.	S	15
Oriranum syriacum var. bevanii L	Oregano		Whole plant	Treating tooth decay, gum infections & cough.	S	15
Otostegia fruticose Forssk.	Shakab		Leaves & flowers	Irritation of eye, as remedy for sun stroke & for gout.	W, S	15, 16
Plectranthus asirensis	Shar Elkrood		Leaves	Used to treat diaper rash & itching.	S	16
Plectranthus barbatus Andres.	Shar Elkarood		Leaves	As deodorant.	S	15
Plectranthus cylindraceus	Khurub		Whole plant	A remedy for sore throat.	S	15
Plectranthus tenuiflorus	Shar		Leaves	Used for earache.	S	16
Rosmarinus officinalis L.	Eklel Aljabal		Whole plant	Antifungal.	S	15
Salvia aegyptiaca	Ghashba		Whole plant	Eye diseases, diarrahoea & blennorrhagia.	W, M, S	8, 12

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Salvia lanigera L.	Jurayba	Labiatae (Lamiaceae)	Whole plant	Carminative & used in indigestion.	S	34
Salvia spinose L.	Harsha		Seeds	Used for the cure of toothache, gonorrhea & urethritis.	S	8
Stachys Sp. Aff. Schimperi Vatke	-		Whole plant	Strong antimicrobial activity.	S	19
Teucrium oliverianum Ging.	Qassapa		Whole plant	Diabetes.	M, S	14, 15
Teucrium polium L.	Jaada		Whole plant	Treat liver disease, jaundice, diabetes, fertility problems & cancer.	W, S	36
Teucrium yemense Defl.	Rechal Fatimah		Whole plant	Used as anti-diabetic & in kidney problems.	S	15
Thymbra spicata subsp. Spicata L.	Za'atar		Whole plant	Antimicrobial.	S	15
Thymus decussates Benth.	Za'atar		Whole plant	Antiemetic.	S	15
Thymus vulgaris L.	Za'atar		Whole plant	Antiseptic, anthelmintic, expectorant, carminative, diuretic, sedative, used in veterinary medicine.	S	15
Allium ampeloprasum	-	Liliaceae	Leaves	Antimicrobial.	S	15
Allium cepa	Basl		Bulb	Diabetes, colic gripes, flu, catarrh, bronchitis, ulcers, dysentery, antiepileptic, rhinorrhagia, jaundice, diuretic, ophthalmia & demonomania.	M	12
Allium sativum	Thom		Bulb	Antiasthmtic, increase blood circulation, muscle relaxation, diabetes, catarrh, bronchitis, flu, dysentery, hypertensive, urinary tract inflammation, liver diseases, antirheumatic, diuretic, emmenagogue, diarrhea & antiemetic.	M	12

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Asparagus africanus Lam.	Khurus	Liliaceae	Leaves	Relieve breathing problems.	W, S	16, 15
Cinnamon zellanicum L.	Qerphah	Lauraceae	Whole plant	Antimicrobial.	S	15
Linum usitatissimum L.	Hab kattan	Linaceae	Seeds	Constipation, painful joint, urinary disorder & venereal diseases.	-	20
Lawsonia inermis L.	Henna	Lythraceae	Branches, leaves, flowers & young buds	Gargle, spleen tumor, skin diseases, hair tonic, headache & jaundice.	M, S	12, 16
Syzygium aromaticum L.	Mesmar		Whole plant	Treat toothache, respiratory disorders, inflammation & gastrointestinal disorders.	S	18
Abutilon pannosum G. Forst.	-	Malvaceae	Whole plant	Antimicrobial.	S	19
Gossypium barbadense L.	Khutin		Seeds	Treatment for earache.	S	16
Hibiscus europaeum L.	Raein		Whole plant	Antimicrobial	S	15
Hibiscus sabdariffa L.	Karkeda		Whole plant	Antihypertension, reduce the testicular damage & ameliorate the drop-in sperm quality.	S	15
Malva parviflora L.	Khobaiza		Whole plant	Laxative & promotes hair growth.	M, S, W	13, 14, 15
Azadirachta indica A.	-	Meliaceae	Whole plant	Antifungal.	W, S	15, 30
Cocculus hirsutus L.	Hamr almajun	Menispermaceae	Leaves & roots	Used as febrifuge, emetic, demulcent, for digestive problem & purgative.	S	20
Cocculus pendulus	-		Whole plant	Antipyretic.	M	12
Albizzia lebbeck L.	Lebbeck	Mimosaceae	Phloem	Anthelmintic.	M	12
Dorstenia foetida Forsskal.	Kartib	Moraceae	Seeds	Stomach disorders.	-	20
Ficus carica L.	Teen		Leaves, fruits & latex	Used for burns, leprosy, tonic & as diuretic.	W	20
Ficus palmata Forssk.	Hamat		Latex	Yogurt production.	W, S	12, 16
Ficus salicifolia	Lithab		Whole plant	Leukoderma & eye wash.	M, S	12, 19

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Moringa peregrine Forssk.	Habb Elyasar	Moringaceae	Seeds	Analgesic, abdominal pain, burns, constipation, laxative & for headache.	S	37
Eucalyptus camaldulensis Dehnh.	Khafour	Myrtaceae	Whole plant	Abortion & perfume.	M, S	14, 15
Eucalyptus dives L.	Eucalyptus		Whole plant	Strong antiseptic & antiviral activity.	S	15
Eucalyptus globules L.	-		Whole plant	Antifungal.	S	15
Myrtus communis L.	Hadas		Leaves	Used for abdominal colic, antipyretic, cough & as insecticidal.	-	20
Pimenta dioica L.	-		Whole plant	Anti-inflammatory, analgesic & antipyretic.	S	15, 38
Boerhavia coccinea	-	Nyctaginaceae	Roots	Diuretic & urinary tract disorders.	M	12
Commicarpus grandifloras	-		Whole plant	Strong antimicrobial activity.	S	19
Jasminum grandiflorum L.	Anbar, Yasmin	Oleaceae	Leaves & flowers	Treatment for dysentery, abdominal pain & colic.	S	20
Olea european L.	Athm		Twigs & branches	Used as tooth brush & keeping gums healthy, liver diseases, ulcer, for edema & diabetes.	W, S	13, 16, 20
Epilobium hirsutum L.	Saqalqurab	Onagraceae	Whole plant	Central nervous system depression & antimicrobial.	S	19
Cistanche tubulosa	Dhanun	Orobanchaceae	Leaves, stems & flowers	Used for jaundice & diarrahoea.	S, M	12, 21
Lindenbergia sinaica L.	-		Whole plant	Antimicrobial.	S	19
Eulophia petersii Reichb.f.	Iseb	Orchidaceae	Basal stem bulbs	Used to treat skin problems.	S	20
Oxalis corniculate L.	-	Oxalidaceae	Leaves & flowers	Antiparasitic, antivertigo & mouth inflammation.	S	25
Argemone Mexicana L.	Argemonia	Papavaraceae	Whole plant	Antimicrobial.	W, S	19
Agemone ochroleuca Sweet	-		Whole plant	Strong antimicrobial activity.	W, S	19
Papaver somniferum Schl.	Hashish		Capsules & seeds	Antitussive & insomnia.	S	15, 20

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Sesamum indicum L.	Gilgilan	Pedaliaceae	Seed oils	Used as treatment for dysentery, colic & urinary problems.	S	20
Plantago ammplexiculis Cav.	Rabal	Plantaginaceae	Whole plant & leaves	Renal disease & urinary tract purifier.	M, S	14
Plantago coronopus L.	Rebla		Whole plant	Used as laxative & wound healing.	-	20
Plantago major L.	Lisan Alkalb		Leaves & seeds	Used for diarrhea, dysentery, ulcer & abscesses.	-	20
Plantago ovata Forssk.	Geneima		Seeds	Used as a laxative, an emollient, demulcent and astringent & particularly in chronic colitis.	S	15
Limonium axillare Forssk.	Kattaf	Plumbaginaceae	Whole plant	Central nervous system depression & antimicrobial.	S	15
Plumbago zeylanica L.	Ensain		Whole plant	Antirhumatic, dysmenorrhea, carbuncles, contusion of the extremities, ulcers & elimination of intestinal parasites.	S	15
Calligonum comosum L.	Arta،a	Polygonaceae	Whole plant	Anti-inflammatory & antiulcer activity.	S	15
Emex spinous L.	Hambazz		Whole plant	Purgative, diuretic, digestive, appetizer, stomach troubles & anthelmintic.	M, S	12, 15, 18
Polygonum argyrocoleum Steud.	Abuzalaf		Whole plant	Used in stomach troubles.	S	34
Rheum palmatum L.	-		Roots & Rhizomes	Antimicrobial.	S	15
Rumex nervosus Vahl.	Ithrib		Leaves & roots	Edible plant, diabetes, skin burns, inflammatory diseases, diarrhea, wounds, typhus, rabies, skin disorders. diuretic, gonorrhoea, lung tuberculosis, leprosy, antipyretic, liver disease, hypertension, haemorrhoids, scabies, antiemetic, aphrodisiac, cough, for rabies, antirheumatic & antimigraine.	S, M	16, 12
Rumex pictus Forssk.	Hamsees		Whole plant	Sedative, relieve spasm & antimicrobial.	S	9, 15

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Rumex steudelii	Tabal	Polygonaceae	Whole plant	Remedy for abdominal pains due to intestinal worms.	S	15
Rumex vesicarius L.	Hammaad		Whole plant	Used for toothache.	W, M, S	13, 14, 15
			Seeds	For the cure of dysentery.		
Portulaca oleracea L.	Regla	Portulacaceae	Whole plant	Anti-inflammatory, antidiabetic and for cold.	W, M, S	13, 14
Portulaca quadifida L.	-		Stems	Treatment of scorpion sting.	-	17
Anagallis arvensis L.	Ayn Algat	Primulaceae	Whole plant	Used for skin rash & ulcers.	-	20
Punica granatum L.	Ruman	Punicaceae	Fruits	Dysentery, microbial infection, diarrhea, haemorrhage & respiratory pathogenesis.	-	24
Clematis simensis Fresen.	Haya-a	Ranunculaceae	Leaves	Relieve rheumatic pain.	S	19
Clematis wightiana L.	-		Whole plant	Cardiac depression & antimicrobial.	S	19
Nigella sativa L.	Habbatus sauda		Whole plant	Carminative, mucolytic, anti-ulcers, anti-diabetes, antidote, antiasthmatic, hypertension, hepatoprotective, antiobesity, migraine, haemorrhoid & expel rodents.	S	20
Reseda muricate Presl.	Danban	Resedaceae	Fruit	Menstruation tonic.	M, S	14
Ochradenus arabicus L.	Kardey		Whole plant	Hypoglycemic activity.	W, S	13
Ochradenus baccatus Del.	Ghorzaa		Whole plant	Backache, fistula & antimicrobial.	W, M	13, 14
Zizyphus nummularia L.	Zyzafun	Rhamnaceae	Whole plant	Antispasmodic, emollient, antitussive, anti-poison, laxative & skin eruption.	S	20

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Ziziphus spina-christi L.	Sidr, Nubak	Rhamnaceae	Whole plant & bark	Duodenum and stomach ache, remover dandruff, weaknesses, diabetes, pharyngitis, bronchitis, anemia, diarrhea, skin infections, antipyretic, sleep irritability, liver problems, digestive disorders, antimicrobial, anti-inflammatory, antiulcer, antitumor & cardiovascular disorders	W, M, S	13, 14
Amygdalus arabica L.	-	Rosaceae	Whole plant	Antimicrobial.	S	19
Cotoneaster nunnularia	-		Whole plant	Purgative, antiflatulence, expectorant, appetizer, digestive, antitussive, aperient & stomachic.	M	12
Prunus amygdalus L.	-		Whole plant	As aphrodisiac.	S	15
Rosa abyssinica Lindley.	Aball		Whole plant	Antimicrobial.	S	19
Coffea arabica L.	Bun	Rubiaceae	Seeds	Relieve stomachache, colds, cough, antipyretic & toothache.	S	16
Haplophylum tuberculatum Forssk.	-	Rutaceae	Whole plant	Used in liver disease	S	34, 39
			Leaves	Used as sedative, treatment of scorpion sting & strengthen week muscle.		
Ruta chalepensis	Shathab		Leaves & stems	Relieve snake bite, headache, sore ears, rheumatism & wound healing.	S	16
Salvadora persica L.	Miswak	Salvadoraceae	Whole plant	Used as toothbrush & treatment of scorpion sting.	S	15, 17
Dodonaea viscosa Jacq,	Shath	Sapindaceae	Leaves	Used for treating chronic ulcers, burns & leishmaniasis.	S	15, 40
Monotheca buxifolia Falc.	But	Sapotaceae	Ripe barriers	General tonic.	S	15, 21
Schweinfurthia pterosperma	-		Whole plant	Antimicrobial.	S	15

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Striga hermonthica Del.	Doosh, Odaar	Scrophulariaceae	Flowers	Used for irritating & infected eyes.	S	16
Verbascum bottae Deflers.	-		Leaves & flowers	Antitussive, skin disease & antirheumatic.	S	15, 25
Datura fastuosa	Benj, Mang	Solanaceae	Roots, leaves, seeds & flowers	Antispasmodic, antiepileptic, headache, eye and ear diseases, epilepsy, madness, astringent, anthelmintic, parasiticide, narcotic, emetic, tuberculosis & antiasthmatic, antirheumatic.	M	12
Datura innoxia	Binj		Leaves & seeds	Colic gripes, carminative, antiasthmatic, antitussive, antitumor, hysteria, antirheumatiod, emmenagogue, flu, headache & anesthetic.	M, S, W	12, 13, 15
Datura metel L.	Binj		Leaves, flowers & seeds	Antiasthmatic, inflammation, antirheumatoid, earache & eye diseases.	S, M	12, 15
Datura stramonium L.	Binj-daturah		Leaves, roots, stems & seeds	Antirheumatic, antiasthma, antitussive, bronchitis, earache, hair fall & treatment of scorpion sting.	S, W	15, 13, 17
Hyoscyamus albus L.	-		Whole plant	Used as treatment for scorpion sting.	-	17
Hyoscyamus muticus L.	Asakran		Leaves, flowers & buds	Antitussive, expectorant, antiasthmatic, carminative, sedative, antispasmodic, sea sickness & toothache.	M	12
Hyoscyamus pusillus L.	Babekh Safaree		Seeds	Toothache.	S, M	12, 15

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Lycium shawii Roem.	Awsag	Solanaceae	Fruits	Mouth ulcers.	S, W, M	13, 14, 15
Nicotiana rustica L.	Al-tabag		Tobacco snuff	Useful in nasal polyp, nasal catarrh, headache, chronic giddiness, fainting, rheumatic swellings & skin diseases.	S	15
Nicotiana tabacum L.	-		Leaves	Used as treatment for scorpion sting.	-	17
Physalis minima L.	-		Whole plant	Used in gonorrhea & earache.	S	15
Solanum anguivi Lam.	-		Stems	Used as treatment for scorpion sting.	-	17
Solanum careens L.	-		Whole plant	Antimicrobial.	S	15
Solanum forskalii Dunal.	Nashbah		Whole plant	Used for treating ulcers.	W, S	13, 15
Solanum glabratum Dunal.	-		Leaves & fruits	Diuretic, for scabies, cough & hemorrhoids.	S	15
Solanum incanum L.	Aeen Elbagar		Fruits	Antimicrobial.	W, S	13, 41
Solanum nigrum L.	Enab-Alzeeb		Whole plant	Used in jaundice, antipyretic, gonorrhea, diarrhea, heart diseases, inflammation, edema, mastitis & hepatic cancer.	S	15
Solanum surattense Burm. f.	Bankum-Bakini		Whole plant	Antipyretic, antiasthmas, antitussive, in sexual diseases & to promote female fertility.	S	34
Solenostemma argel (Del.) Hayne.	Al-Argal		Leaves	Antirheumatic & antitussive.	S	15
Withania somnifera L.	Sem-alfaar		Leaves	Used in ulcers.	W, M, S	13, 14, 17
			Whole plant	Antirheumatic, dyspepsia, appetizer & for edem.		
			Roots	Used as tonic for uterus of women after habitual miscarriage.		
			Stems	Used as treatment for scorpion sting.		

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Tamarix amplexicaulis L.	-	Tamaricaceae	Whole plant & roots	For central nervous system depression, cardiac stimulation & antimicrobial.	S	19
Tamarix aphylla L.	Athel		Leaves & roots	Wound infection & stomachache.	W, M, S	13, 14
Tamarix nilotica Ehrenb.	Tarfaa		Leaves & seed's oil	Used for leg varices.	M, S	14
Gnidia somalensis Franch.	Barha	Thymelaceae	Leaves	As emetics & purgatives.	S	21
Grewia tenax Forssk.	Khadar	Tiliaceae	Wood & barks	Antitussive, analgesic, central nervous system depression & antimicrobial.	M, S	12, 15
Triumfetta flavescens L.	-		Whole plant	Central nervous system stimulant & cardia stimulation.	S	19
Typha domingensis (Pers.) Poir.	Pardey	Typhaceae	Whole plant	Cardiac depression.	S	19
Forsskalea tenacissima L.	Lussaique	Urticaceae	Whole plant	Used for ulcers	M, W	14, 13
Urtica urens L.	Hurraikha		Leaves	Used for muscle pain & antirheumatic.	S	16
Lantana petitiana	Saaf	Verbenaceae	Leaves & roots	Used for abdominal colic.	-	21
Phyla odiflora L.	-		Leaves	Used as treatment for scorpion sting.	-	17
Cissus rotundifolia L.	Ghalaf	Vitaceae	Whole plant	Heated plant used to relieve backache.	S	16
Cyphostemma ternatum	Kum		Leaves & stems	Used to treating infections.	-	21
Alpinia galangal L.	-	Zingeberaceae	Rhizomes	Used against kidney stones.	S	23
Alpinia officinalis L.	-		Roots & rhizomes	Antirheumatic & children whooping cough.	S	15
Curcuma longa L.	Kurkum		Rhizomes	Skin disorders, bronchitis, antitussive & eye infections.	S	15

Table 1S: Cont.

Scientific name	Local name	Family	Part used	Traditional uses	Region	Reference
Zingiber officinalis L.	Zingibil	Zingeberaceae	Rhizomes	Hepatoprotective, clears vision, digestive, aphrodisiac, gout, antirheumatic, voice clearness, antipyretic, antiscorbutic & food condiment.	S	20
Fagonia bruguieri Prod.	Shika'a	Zygophyllaceae	Whole plant	Antipyretic, antiasthmatic, anti-emetic, dysentery, typhoid, anti-toxic, anti-tumor, blood and heart tonic & for ulcers.	W, M, S	12, 13, 14
Fagonia indica Burm.	Showaika		Whole plant	Used for smallpox & gout.	M, W, S	12, 13, 14
Peganum harmala L.	Harmal		Seeds	Sexual stimulation.	W, S	13, 16
Tribulus terrestris L.	Shirshir		Leaves	Renal colic.	W, M, S	12, 13, 14
Zygophyllum album L.	Retret		Whole plant	Antidiabetic & cardiovascular disease.	S	15
Zygophyllum coccineum L.	Harm		Whole plant	Anthelmintic.	M, S	13, 14
Zygophyllum simplex L.	AL-damran		Whole plant	Used in ophthalmic disease.	W, M, S	12, 13, 14

W= west region of KSA, S= south region of KSA, M=middle region of KSA

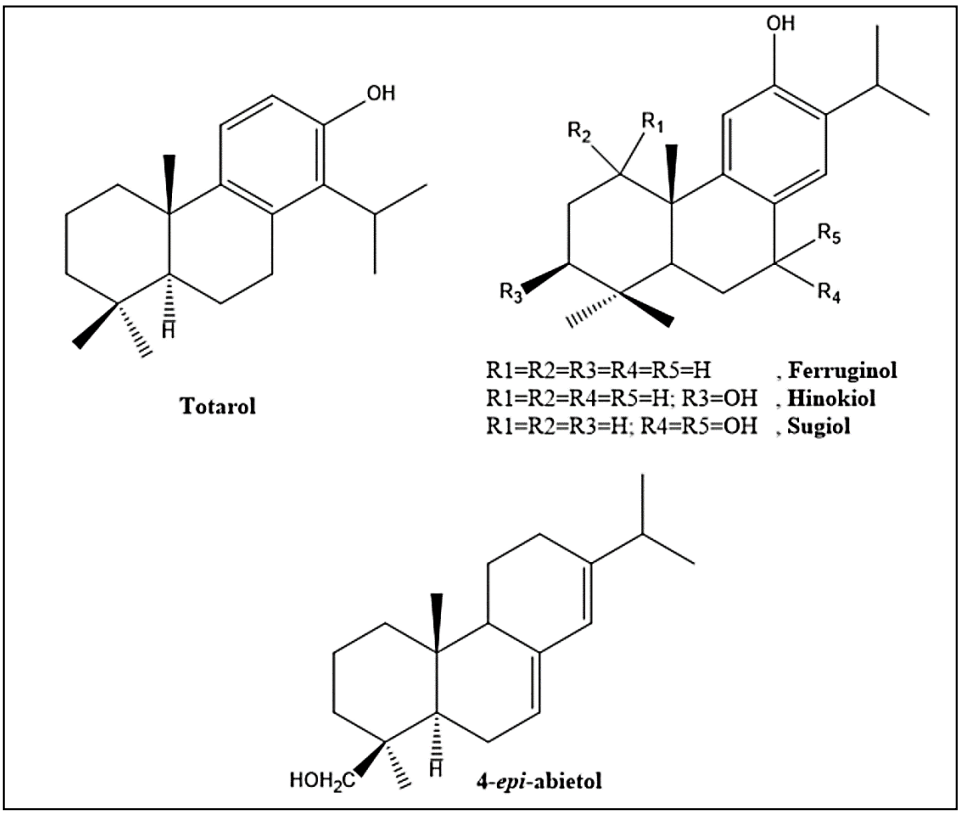


Figure 1S: Structures of the major compounds isolated from *J. procer*

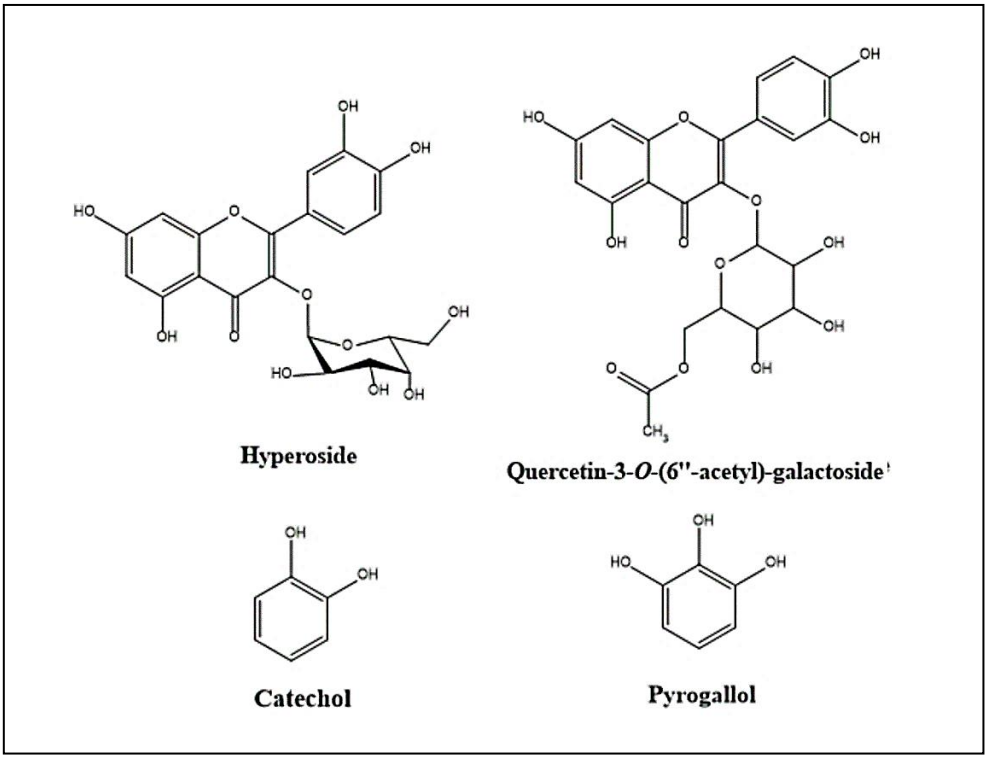


Figure 2S: Structures of the major compounds isolated from *R. nervosus*

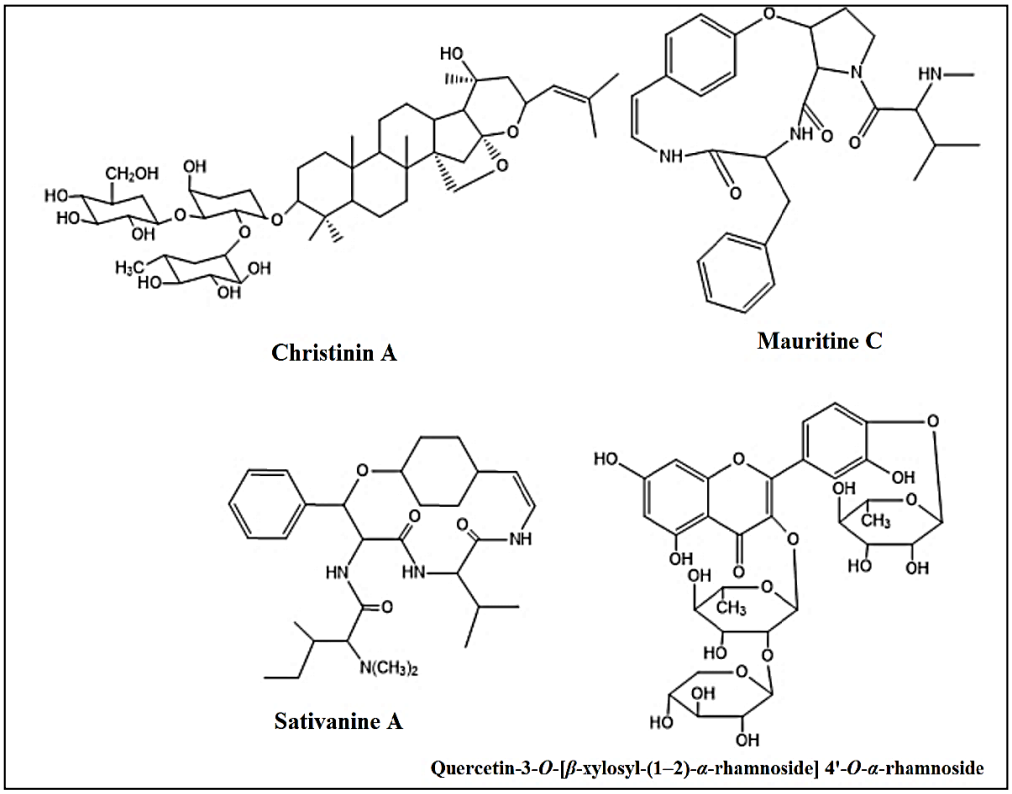


Figure 3S: Structures of the major compounds isolated from *Z. spina-christi*

Appendix F: List of original contributions

A. Oral presentation

1- Hanan Aati. Phytochemical and Biological Investigation of *Jatropha pelargoniifolia* Root Native to Saudi Arabia. Riyadh, King Saud University, Pharmacognosy Department, Mon. 8-1-2018, Annual EJSP Seminar.

B. Poster presentantions

1- Aati Hanan, Oliver Kayser, ElGamal Ali. Phytochemical and Biological Investigation of *Jatropha pelargoniifolia* Root Indigenous to Saudia Arabia. GA 2017, Basel, Switzerland Conference. Mo-Poster Session 1-PO-142. 4th September, 2017.

2- Aati Hanan, ElGamal Ali, Oliver Kayser. Comparative Study of the Biological Activities of *Jatropha pelargoniifolia* and *Jatropha glauca* Native to Saudi Arabia. 66th annual meeting of the GA 2018, Shanghai, China, August 26-29, 2018, (accepted, id.4).

3- Aati Hanan, ElGamal Ali, Oliver Kayser. Chemical Composition and Biological Activity of the Essential Oil from *Jatropha pelargoniifolia* Root Indigenous to the Arabian Peninsula. 66th annual meeting of the GA 2018, Shanghai, China, August 26-29, 2018, (accepted, id.5).

4- Aati Hanan, ElGamal Ali, Oliver Kayser. Studies on the Chemical Composition of Essential Oils of *Jatropha pelargoniifolia* and *Jatropha glauca* endogenous on the Arabian Peninsula. 66th annual meeting of the GA 2018, Shanghai, China, August 26-29, 2018, (accepted, id.6).

5- Aati Hanan, ElGamal Ali, Oliver Kayser. Phytochemical Investigation of Bioactive Compounds Isolated from *Jatropha pelargoniifolia* Roots Native to Saudi Arabia. 66th annual meeting of the GA 2018, Shanghai, China, August 26-29, 2018, (accepted, id.294).

C. Peer reviewed articles

1- Hanan Y. Aati, Ali A. ElGamal, Oliver Kayser. A comparative study of the biological activities of *Jatropha pelargoniifolia* and *Jatropha glauca* native to Saudi Arabia, *Phytomedicine* (submitted).

2- Hanan Aati, Ali El-Gamal, Oliver Kayser. Chemical Composition and Biological Activity of the Essential Oil from the Root of *Jatropha pelargoniifolia* Native to Saudi Arabia, ***SPJ, 2019, 27 (1), 88-95*** (Published).

3- Hanan Y. Aati, Ali A. ElGamal, Oliver Kayser, Atallah F. Ahmad. The Phytochemical and Biological Investigation of *Jatropha pelargoniifolia* Root Native to the Kingdom of Saudi Arabia, ***Molecules, 2018, 23 (8)*** (Published).

4- Hanan Aati, Ali El-Gamal, Hamdy Shaheen, Oliver Kayser. Traditional Use of Ethnomedicinal Native Plants in Kingdom of Saudi Arabia, ***Journal of Ethnobiology and Ethnomedicine, 2019, 15 (2)*** (Published).

D. Poster publication

1- H Aati, K Oliver, A ElGamal. Phytochemical and Biological Investigation of *Jatropha pelargoniifolia* Root Indigenous to Saudia Arabia. Planta Medica, PMIO 2017; 4(S 01): S1-S202, (DOI: 10.1055/s-0037-1608189).

Appendix G: Curriculum vitae

Curriculum vitae

Name: Hanan Yahya Aati

Nationality: Saudi

Date and place of birth: 10-10-1982, KSA, Riyadh.

Academic qualifications

2015 PhD. student, EJSP (KSU and TU Dortmund).

2013 *Master's degree in Pharmacognostical Sciences,* King Saud University / College of Pharmacy - Riyadh, Saudi Arabia.

"The title of my thesis was "Study of Phytochemical and Biological Properties of *Buddleja polystachya* Growing in Saudi Arabia".

2006 *Bachelor degree in Pharmaceutical Sciences*, King Saud University / College of Pharmacy - Riyadh, Saudi Arabia.

Position and employment

2014 – Present Lecturer in the Pharmacognosy Department, King Saud University.

2009 - 2013 Teaching Assistant in the Pharmacognosy Department, King Saud University.

2006 - 2008 Pharmacist for 2 years (1-year experience in King Fahad Medical City and 1-year in Riyadh Military Hospital, Riyadh, KSA).

Research interests

- Natural product chemistry, isolation of plant bioactive constituents using advanced chromatographic techniques and elucidation of their structure using different chemical and spectroscopic methods
- Isolation of essential oils from plant origin and its chemical compositions analysis. Study of different biological activities for various active principles

isolated from plants such as anti-inflammatory, cytotoxicity, antimicrobial, antioxidant, and other substances.

Research accomplished

Master's thesis: "Study of Phytochemical and Biological Properties of *Buddleja polystachya* Growing in Saudi Arabia"

Awards

- King Saud University Award for Scientific Excellence, branch of graduated students, 2015.

Publications

- Chemical Composition of *Buddleja polystachya* Aerial Parts and its Bioactivity against Aedes aegypti, Ali El-Gamal, Shaza Al-Massarani, Ghada Fawzy, Hanan Ati, Adnan Al-Rehaily, Omer Basudan, Maged Abdel-Kader, Nurhayat Tabanca & James Becnel. Natural Product Research, https://doi.org/10.1080/14786419.2017.1378213, 2017.

- Antidiarrhoeal and Antispasmodic Effects of *Buddleja polystachya* is Mediated Through Dual Inhibition of ca^{++} Influx and Phosphodiestrase, Anwar Gilani, Najeeb-ur- Rehman, Aslam Khan, Maryam Nazneen, Ali A. El Gamal, Ghada A. Fawzy, Hanan Y. Al Ati and Majed S. Abdulkader. *Phytotherapy Research*, 29 (8) 2015 pp 1211-1218.

- Phytochemical and Biological Evaluation of *Buddleja polystachya* Growing in Saudi Arabia, Hanan Y. Al Ati, Ghada A. Fawzy, Ashraf Khalil, Kamal Eldin Eltahir Anwarul-Hassan Gilani and Ali A. El Gamal. *Pak. J. Pharm. Sci.*, 28, (4), 2015, pp.1533-1540.

- Chemical Composition, *In Vitro* Antimicrobial and Cytotoxic Activities of *Buddleja polystachya* Essential Oils, Hanan Yehya Al Ati, Ali Ali El Gamal, Ghada Ahmed Fawzy. *TEOP* 17 (6) 2014 pp 1112-1119.

- Antimicrobial and cytotoxic potentials of *Buddleja polystachya* extracts, Ghada Ahmed Fawzy, Ali Ali El Gamal and Hanan Yehya Al Ati. *Bangladesh Journal of Pharmaclogy*, 2013, 8 (2): 136-141.

- Chemical composition and biological evaluation of essential oils of *Pulicaria jaubertii*, Ghada A. Fawzy, Hanan Y. Al Ati and Ali A. El Gamal. *Pharmacognosy Magazine*, 2013, 9 (33): 28-32.