

Hexane Extract Analysis of *Pergularia Tomentosa* Plant from Southern Libya

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Abstract

The aim of the present study was to identify various bioactive constituents present in non-polar hexane extract of aerial part extract of *Pergularia Tomentosa*, of Chenopodiaceae family using GC-MS technique. The results showed presence of wide variety of constituents, mostly of them were nonpolar including long chain saturated and unsaturated hydrocarbons and their oxygenated derivatives in addition to many alkyl halides. Fatty acids were also found in the extract, in addition to some pentacyclic triterpenes, especially, Lupeol and Sitetosterol. The components existed exhibit reported diverse biological activity including antifungal, antibacterial, antioxidant, anticancer, anti-inflammatory and other activities.

Key words: *Pergularia Tomentosa*, Biological activity, antioxidant activity, anticancer activity.

1. Introduction

Plant derived phytochemicals became center of attention in pharmaceutical industry due to their wide applications in traditional, modern medicines for the treatment of a variety of diseases with very results [1]. Plants produce wide range of useful secondary metabolites such as terpenoids, phenolics, tannins, flavonoids, alkaloids, quinones, coumarins and glycosides which constitute a rich source of potent bioactive substances [2]. Also, Phytochemicals extracted from plants could work as very promising and effective green and ecofriendly therapeutical drugs with high degradability and low incidences of negative impacts in comparison with available synthetic drugs giving rise to residual toxicity, hormonal imbalance, and carcinogenicity [3]. *Pergularia Tomentosa* belongs to family Chenopodiaceae, it is native to Andean regions of North and South America, and distributed in Saharan and sub-Saharan countries of North Africa, including Algeria, Niger, and Egypt. The plant is a climbing to semi erect perennial herb of around 30 cm, the stem is pale green-white in color, highly branched and usually grow vertically (Figure 1) and milky latex is extracted from the plant [4].



Figure 1. *Pergularia Tomentosa* plant

Pergularia Tomentosa was administered in the case of diarrhea and the sap of leaves was used as ocular instillation and regarded as a sovereign remedy for the ills of the head [5]. In Egypt, the plant was used as a poultice, depilatory, laxative, antihelmintic, and abortifacient [6]. The latex of stems and leaves irritates the skin and eyes and can cause inflammation [7]. The plant was reported to contain cardiotoxic glycosides such as desglucouzarin, coroglaucigenin, and uzarigenin in the leaves [8], the plant also exhibits molluscicidal activity [9].

In Tunisia, *Pergularia tomentosa* was used in traditional medicine for treatment of skin diseases, asthma, and bronchitis. Analysis of stems, fruits and leaves extracts using liquid chromatography-mass spectrometry (LC-MS) revealed presence of five polyphenolic compounds, namely digitoxigenin, digitonin glycoside and calactina in the leaves, kaempferol in the fruit, and calotropagenin in the stems [10].

Recently, a Phytochemical investigation of the aerial parts and latex of *Pergularia tomentosa* from Jordan, utilizing column chromatography accompanied with NMR (1 & 2D), IR, UV, and HR-ESI-Mass spectroscopy have led to the isolation and characterization of two new compounds, pergularol and 3-O-acetyl-28-hydroxy taraxasterol for the first time along with other ten known compounds including linoleic acid, 3'-didehydroafroside, apigenin, β -sitosterol glucoside, luteolin, apigenin 7-(6''-crotonoyl)glucoside, 3'-O- β -glucopyranosyl calotropin, apigenin-7-O- β -D-glycoside, pergularine A, taraxasterol [11]. This research work aimed at determined the phytochemicals present in hexane extract using GC-

MS technique as a primarily step in following research work for this plant.

2. Materials and Methods

Plant Material

Pergularia Tomentosa plant (aerial part) was collected from South of Sebha city (South of Libya) in summer 2022. The plant was identified by plant taxonomist from Botany department, Science Faculty, Elmergib University. The whole material was cleaned and dried in the shade till complete dryness which allowed to cut the material to a fine powder using electrical blender, the powder was stored in sealed plastic bottle at room temperature until use in analysis.

GC-MS Method

About 10.0 ml of methanol was added to 2.0 g of a homogenized powder sample, the mixture was shaken vigorously for 60 min to transfer phytochemicals from the sample matrix into the organic layer. The extract was centrifuged and the supernatant was collected and filtered through 0.20 μm syringe to remove particulate matter. The filtered extract was concentrated using rotary evaporation. The dried concentrated extract was dissolved in 5.0 ml hexane, then, 1.0 μl of reconstituted sample was injected into the GC injection port using a microliter syringe [12]. The chemical composition of roots powder ethanolic extract was performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 μm film thickness). The column oven temperature was initially held at 50 $^{\circ}\text{C}$ and then increased by 5 $^{\circ}\text{C}/\text{min}$ to 230 $^{\circ}\text{C}$ hold for 2 min. increased to the final temperature 290 $^{\circ}\text{C}$ by 30 $^{\circ}\text{C}/\text{min}$ and hold for 2 min. The injector and MS transfer line temperatures were kept at 250, 260 $^{\circ}\text{C}$ respectively; Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min. and diluted samples of 1 μl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 $^{\circ}\text{C}$. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

3. Results and Discussion

GC-MS analysis

The analysis was performed on hexane extract of powder of *Pergularia Tomentosa* plant as mentioned in the experimental part, produced the chromatogram

shown in **Figure 2**. As expected, hexane extract components were mostly long chain saturated and unsaturated hydrocarbons and their oxygenated derivatives hydrocarbons in addition to many alkyl halides. Fatty acids were also found in the extract, in addition to some pentacyclic triterpenes, especially, Lupeol and Sitetosterol.

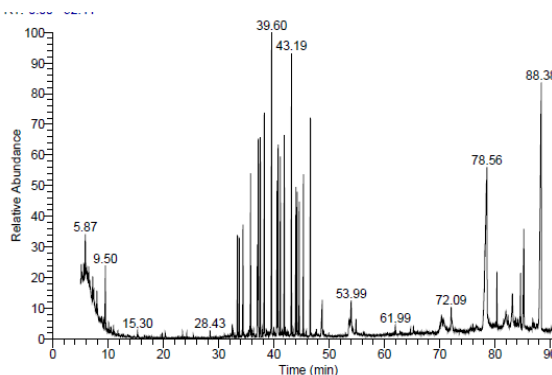


Figure 2. GC-MS chromatogram of ethanolic extract of *Pergularia Tomentosa* plant

Interpretation of mass spectrum from GC-MS was conducted using the database of National Institute Standard and Technology (NIST) library. The spectrum of the unknown component was linked with the spectrum of the known components stored in the NIST library. The retention times (RT), names, peak areas, molecular weights and molecular formulas (MF) of highest peak area and most important components are shown in Table 2.

The most abundant compounds were the esters; (3 β)-Lup-20(29)-en-3-ol acetate (Lupenyl acetate), 13,27-Cycloursan-3-ol acetate, which came out at 78.55 min. with 6.56% peak area, in addition to the alkane 1-methyl decyl benzene which eluted at 39.39 min. with 6.34% peak area. The pentacyclic triterpenes biological activities is well documented including Analgesic, Anti-inflammatory, Gastroprotective, Hepatoprotective and Insectifuge. [13].

Benfluorex, an interesting compound came out at 35.77 min. with peak area of 3.08 %, used as an anorectic and. The compound reported to have improve glycemic control and decrease insulin resistance in people with poorly controlled type-2 diabetes [14] The sesquiterpene Isochiapine B was eluted at 47.28 min. with 0.06% peak area, the compound is well known in folk Chinese medicine for treating arthritis, tonsillitis, and other ailments by Chinese medicine [15]. Hexadecanoic acid (also known as palmitic acid) is a long chain fatty came out at 48.74 min. with 0.76 %, the acid reported to exhibit anti oxidation, antimicrobial and anti-inflammatory and pesticidal properties [16].

Table 2. Major compounds from GC-MS spectrum of hexane extract of *Pergularia Tomentosa*

RT	Compound Name	Area	MF
5.13	3,5-Dimethyl- 1-Hexene	0.28	C8H16
5.13	4-Chlorooctane	0.28	C8H17Cl
5.27	7- Methylene Tridecane	0.16	C14H28
5.27	2- Ethyl-1,1-dimethylcyclopentane	0.16	C9H18
5.27	5-Methyl -1-Undecene	0.16	C12H24
5.60	1-Chlorododecane	0.27	C12H25Cl
5.60	1-Chloroundecane	0.27	C11H23Cl
5.60	1-Chlorodecane	0.27	C10H21Cl
6.11	2,7-Dimethyl-1-Octanol	0.10	C10H22O
6.48	1-Chlorononane	0.27	C9H19Cl
6.66	9-Methyl-1-Decene	0.17	C11H22
9.49	Bromocyclohexane	1.31	C6H11Br
9.49	Bromo-2,2,3-Trimethylcyclopropane	1.31	C6H11Br
9.49	3,3,6-Trimethyl-1,5-Heptadien-4-one	1.31	C10H16O
9.49	3-Ethyl-3-methyl-1-Pentene	1.31	C8H16
9.49	2,3,3-Trimethyl-1-Butene	1.31	C7H14
32.47	Octadecane	0.24	C18H38
32.47	Hexadecane	0.24	C16H34
32.47	2,6,10,15-Tetramethyl -1-Heptadecane	0.24	C21H44
33.40	1-Butylhexylbenzene	1.81	C16H26
33.73	1-Pr 1-Propylbutylbenzene	1.75	C13H20
33.73	1-Propylheptylbenzene	1.75	C16H26
34.40	1-Ethyldecylbenzene	2.00	C18H30
35.77	Benfluorex	3.08	C19H20F3NO2
35.77	1-Methylnonylbenzene	3.08	C16H26
37.02	1-Pentylheptylbenzene	1.99	C17H28
37.17	1-Hexylheptylbenzene	4.00	C19H32
39.39	1-Methyldecylbenzene	6.34	C17H28
47.28	Isochiapin B	0.06	C19H22O6
48.74	Hexadecanoic acid	0.76	C16H32O2
53.63	10(E),12(Z)-Conjugated linoleic acid	0.45	C18H32O2
53.63	9(E),11(E)-Conjugated linoleic acid	0.45	C18H32O2
53.63	(Z,Z)-9,12-Octadecadienoic acid	0.45	C18H32O2
53.98	Cis-13-Octadecenoic acid	0.53	C18H34O2
53.98	Oleic Acid	0.53	C18H34O2
53.98	Trans-13-Octadecenoic acid	0.53	C18H34O2
53.98	Cis-Vaccenic acid	0.53	C18H34O2
70.33	(3 β)-LUP-20(29)-ene-3,28-Diol	0.41	C30H50O2
70.33	Lupeol	0.41	C30H50O
78.55	13,27-Cycloursan-3-ol acetate	6.56	C32H52O2
78.55	(3 β)-Lup-20(29)-en-3-ol acetate	6.56	C32H52O2
80.36	Hextricontane	1.01	C36H74
80.36	Tetrateracotane	1.01	C44H90
83.17	(3 β)-9,19-Cyclolanost-24-en-3-ol acetate	0.82	C32H52O2
83.17	1-Heptatriacotanol	0.82	C37H76O
83.17	(3 β ,24S)-Stigmast-5-en-3-ol (PhytoSetrol)	0.16	C29H50O
83.17	α -Sitosterol	0.16	C29H50O
87.59	Rhodopin	0.19	C40H58O

10(*E*),12(*Z*)-Conjugated linoleic acid (Fatty acid) also found in the spectrum with 0.45 % peak area, the acid reported to have health benefits, such as reduced adiposity, while simultaneously promoting deleterious effects, such as systemic inflammation, insulin resistance, and dyslipidemia [17].

(*Z,Z*)-9,12-Octadecadienoic acid (Fatty acid) eluted at 53.63 min with 0.45 peak area, reported to exhibit anti-oxidation properties [18]. *Trans*-13-Octadecenoic acid is an unsaturated long chain fatty acid with a trans-double bond at position C-13, appeared at 53.98 min. with 0.53 % peak area, well known for anti-inflammatory, antiandrogenic, dermatitogenic, anaemiagenic, insecticides properties and used also as a flavor, its isomer, *Cis*-13-Octadecenoic acid, which came out at same retention time and peak area, has therapeutic uses in medicine and some applications in surgery [19]. With same retention time and peak area, Oleic acid, (the systematic name is *cis*-9-Octadecenoic acid) is an unsaturated fatty acid with 18 carbon atom chain, the acid found in many animal and vegetable fats and oils, and reported with antibacterial activity, particularly in inhibiting the growth of several Gram-positive bacterial species [20], in addition to antifungal, anti-inflammatory, antioxidation properties [21]. Also, *Cis*-vaccenic acid was identified in the spectrum, the acid is well known as a member of trans-fatty acid family (omega-7 fatty acid), some studies documented antibacterial and hypolipidemic properties in rats [22]. The pentacyclic triterpene, Lupeol was eluted at 70.33 min. with 0.41% peak area, lupeol is an essential component of grape, hazelnut and olive oils, cocoa butter, mango pulp, white cabbage, and a variety of medicinal plants. Lupeol exhibits a broad spectrum of biological activities including; Anti-melanoma, anticancer, anti-inflammatory and Cardioprotective properties [23]. Long chain wax, hexatriacontane with 36 carbon atoms, was present in the hexane extract with peak

6. References

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area of 1.01 %, the alkane was reported to have antioxidant activity, and have a significant impact on the resistance of the Gram-negative and Gram-positive pathogens of the plant species, in addition to reported antimicrobial activity [24].

Tetratriacontane, is a long-chain alkane consisting of an unbranched chain of 44 carbon atoms, came out at 80.36 min. with 1.01 % peak area, the alkane believed to have Antioxidant and cytoprotective activities [25]. Heptatriacontanol is a long chain primary alcohol, eluted at 83.17 min. with peak area of 0.82%, has anti-hypercholesterolemic effects [26]. Sitosterol, one of the well-known phytosterols, came out at 83.17 min. with peak area of 0.16 %, found in many plants, the sterol is reported to have antimicrobial anticancer anti-inflammatory anti-asthma, diuretic and antiarthritic [27]. Rhodopin, is a tertiary alcohol carotenoid showed up 87.59 min. with 0.19 peak area, the carotenoid work in prevention of blood vessel growth by preventing transcription factor activation and nuclear translocation [28].

4. Conclusion

Hexane extract of aerial part of *Pergularia Tomentosa* showed very useful results in terms of chemical content of beneficial organic compounds. GC-MS analysis illustrated to the presence of valuable biologically active compounds which have wide range of documented biological activity such as antibacterial, antioxidation, anticancer, anti-inflammatory and cardioprotective effects. The discoveries are very encouraging for more chemical studies of different solvents extracts, especially isolation of active compounds in pure state.

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