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CHEMICAL PROFILE AND MEMBRANE STABILIZATION ACTIVITY: UNVEILING THE ANTI-INFLAMMATORY MECHANISM OF *MYRRH*

Eman Ahmed El-Wakil, Heba Abdel-Hady*, Eman Abdalla Morsi

Medicinal Chemistry Department, Theodor Bilharz Research Institute. Kornish El-Nile St., Warrak El-Hadder, Imbaba, Mail Box 30, P.O. 12411, Giza, Egypt

Commiphora myrrha has been extensively used in traditional medicine. The biochemical characters of Commiphora myrrha hydro-methanolic and petroleum ether extracts were studied. The phenolic and flavonoid contents, DPPH assay, in vitro anti-inflammation activity of them by determining the membrane stabilizing activity and NO assay as well as chemical profile by GC-MS and HPLC were investigated. The results showed that phenolic and flavonoid content of hydro-methanolic extract (158.26 ± 3.44 mg Gallic acid equivalent (GAE)/g of extract and 91.43 ± 3.33 Rutin /g extract, respectively was higher than those of petroleum ether (126.16 ± 0.42 mg GAE/g of extract and 61.97 ± 2.5 mg Rutin /g extract, respectively). Also, hydro-methanolic has potent antioxidant activity than petroleum ether (IC_{50} = 46.56 ± 1.06 and 66.13 ± 1.64 µg/mL, respectively). Moreover, hydro-methanolic extract exhibited strong stabilizing effects on human red blood cell membrane as well as strong NO production inhibition. HPLC for hydro-methanolic extract showed 13 phenolic compounds and 8 flavonoid one. While, GC-MS analysis exhibited 49 compounds in the petroleum ether extract and 45 ones in the hydro-hydro-methanolicic extract. In general, the anti-inflammatory effect is correlated to antioxidant activity of the plant depends on the phenolic and flavonoid content

Keywords: *DPPH, Nitric oxide, HPLC, GC-MS, Petroleum ether, Hydro-methanolic*

INTRODUCTION

During the previous few a long time in drug discovery and drug improvement a secondary position turned into performed via way of means of herbal products. In affluent nations, the use of complementary medicine to improve health conditions is on the rise. Globally, particularly in the West, there has been a surge in research into innovative medicinal plants from various regions of the world and their botanical use[1].

The "Burseraceae" family includes the genus *Commiphora*. There are more than 200 species in it. It grows in arid tropical regions like India, Arabia, and Africa[2]. It yields oleo-gum resin, or myrrh. These resins are yellow-colored, and they are frequently mixed with a lighter-colored, dusty powder. Nonetheless, *Commiphora myrrha* wounding is used to create real myrrh[3]. It has a long history of use in the treatment of a number of illnesses, including fungal infections, ulcers, abscesses, and wounds, headaches, backaches, cramps, muscle aches, and spasms, as well as snake bites[4]. Numerous earlier studies have shown that a wide range of phytochemicals, including lignans, flavonoids, terpenoids, carbohydrates, and steroids, have the biological functions listed[5].

Researchers are looking into natural antioxidants to find substances that can guard against some of the illnesses linked to oxidative damage. Free radicals are widely recognized as the primary culprits behind a number of chronic illnesses, including diabetes, cancer, neurological diseases, and inflammatory illnesses. Antioxidants can prevent free radicals from doing their destructive damage. Antioxidants can postpone, decrease, or even stop oxidative stress by scavenging free radicals[6].

Since free radicals harm cells, oxidation and inflammation are intimately associated[7]. In addition to initiating the healing process, inflammation is an organism's protective response to harmful stimuli. When a chemical is used in conjunction with a treatment to lessen

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*Corresponding author: Heba Abdel-Hady, E-mail: h.abdelhady@tbri.gov.eg

inflammation, it is referred to as anti-inflammatory. Approximately half of analgesics are anti-inflammatory medications that reduce inflammation to relieve pain. However, analgesics with anti-inflammatory properties can present a number of special opportunities for disorders associated with inflammation. To prevent the gastrointestinal issues or liver cirrhosis linked to the use of synthetic anti-inflammatory medications, natural compounds made from rectified plants may provide excellent substitutes for innovative anti-inflammatory drugs that are less toxic[8,9].

The objectives of this work were to ascertain the antioxidant activity of *Commiphora myrrha* petroleum ether and hydro-methanolic extracts and explore in-vitro anti-inflammatory action of them; as we notice that is the first study for petroleum ether against nitric oxide assay; and further chemical analysis by GC-MS and HPLC for the bioactive compounds of both extracts.

EXPERIMENTAL

MATERIALS

Commiphora myrrha oleo-gum-resin was acquired from Harraz Medicinal Plant Co., an Egyptian herbalist located in Cairo. The oleo-gum-resin was subsequently pulverized into a fine powder, and a voucher specimen was stored in the Theodor Bilharz Research Institute's Department of Medicinal Chemistry.

Preparation of myrrh extracts

In order to obtain a crude hydro-methanolic extract and a petroleum ether extract, roughly 500 g of dried powder of myrrh was divided into two equal portions (250 g each) and extracted with two different solvents for one week: 85 % methyl alcohol (MeOH) and petroleum ether. The extracts were then filtered and concentrated using a Rotatory Evaporator Buchi at 40 °C.

Total phenolic content

Myrrh hydro-methanolic extract's phenolic content was assessed using spectrophotometric analysis, as reported by Abdel-Hady et al.[10]. 250 µg/mL of the extract, 2.5 ml of dissolved Folin-Ciocalteus reagent (10 %), and 2.5 ml of $NaHCO_3$ (7.5%) were used. 2.5 ml of dissolved Folin-Ciocalteus reagent (10%) in water, 2.5 ml of MeOH, and 2.5 ml of 7.5% $NaHCO_3$ make up the blank sample. The standard used was 2.5 ml of 200 µg/ml gallic acid, 2.5 ml of 10% Folin-Ciocalteus reagent, and 2.5 ml of 7.5% $NaHCO_3$. After shaking each combination, it was incubated for 45 minutes at 45°C. In comparison to a blank sample, the absorbance at 765 nm was measured. Three duplicates of the experiment were conducted. The amount of gallic acid equivalent (GAE) in milligrams per gram dry weight of extract.

Total Flavonoid content

Using a colorimetric technique, the amount of flavonoids in the hydro-methanolic extract of myrrh was ascertained[11,12]. A mixture of 0.5 ml of the extract, 2 ml of distilled water, and 150 µl of $NaNO_2$ (5 %) was combined for 6 minutes. After that, 150 µl of $AlCl_3$ (10 %) was added and left to stand for 5 minutes. Next, 2 ml of NaOH (4 %) was added, and the volume was adjusted to 5 ml using 200µl of distilled water. Rutin served as the standard and 0.5 ml of MeOH was used as the blank. The mixture was incubated at room temperature for fifteen minutes. At 510 nm, the absorbance was measured in relation to a blank sample. Three duplicates of the experiment were conducted. We calculated the total flavonoid content in milligrams of rutin equivalents per gram of extract.

Antioxidant activity estimation by DPPH assay

Myrrh hydro-methanolic extract and petroleum ether were tested for their antioxidant capacity using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging technique[13]. A range of extract concentrations, from 5 to 500 µg/ml, had been created. A mixture containing two milliliters of DPPH (0.1 mM/l) and two milliliters of each extract concentration dissolved in hydro-methanolic was prepared. Hydro-methanolic and DPPH were present in the control without extract. The mixes were well shaken and then allowed to sit at 37˚C in the dark for 30 minutes. At 517 nm, the absorbance was measured. The standard was ascorbic acid. Three duplicates of the experiment were conducted. This equation:

$$\text{Scavenging activity \%} = [(A_{control} - A_{sample}) / (A_{control})] \times 100$$

was used to determine the plant extract's DPPH scavenging percentage.

Where $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of sample. Data were expressed as IC_{50}. The lower IC_{50} value is an indication of more powerful antioxidant activity.

Evaluation of the anti-inflammatory activity

Membrane stabilization assay

154 mM NaCl in 10 mM sodium phosphate buffer (pH 7.4) was the isotonic buffered solution that was used three times to wash the blood in order to prepare the erythrocyte suspension. The blood was centrifuged at 3000 rpm for ten minutes.

Erythrocyte hemolysis caused by a hypotonic solution: Using erythrocyte hemolysis caused by a hypotonic solution, the samples' membrane stabilizing ability was evaluated[14]. The test sample was a 0.50 ml suspension of stock erythrocytes (RBCs) combined with 5 ml of hypotonic solution containing indomethacin or the extract "1000-7.81 µg/ml". A 0.5 ml mixture of RBC and hypotonic-buffered saline solution was used as the control sample. The mixtures were centrifuged for ten minutes at 3000 rpm after being incubated for ten minutes at room temperature. The absorbance of the supernatant in 96-well plates was measured at 540 nm. The methodology established by Shinde *et al.*[14] was followed in the calculation of the percent inhibition of hemolysis or membrane stabilization.

$$\text{Inhibition of heamolysis (membrane stabilization\%)} = \{OD_1 - OD_2/OD_1\} \times 100$$

Where:

OD_1 = Optical density of hypotonic-buffered saline solution, OD_2 = Optical density of test sample in hypotonic solution. The IC_{50} value was defined as the concentration of the sample to inhibit 50 % RBCs hemolysis under the assay conditions.

Nitric oxide (NO) assay

An animal macrophage cell line known as RAW 264.7 was procured from American Type Cell Culture (ATCC no. TIB-71; Rockville, MD, USA). Cultured cells were kept at 37 °C and 5% CO_2 in DMEM supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin-streptomycin. Following seeding in 96-well plates, RAW 264.7 cells (4.0 × 105 cells/ml) were co-treated with LPS (500 mg/ml), with samples varying in concentration from 12.5 to 100 µg/ml. After 18 hours, the culture supernatant was mixed with 50 µl of Griess reagent to measure the amount of NO produced and the absorbance was measured in an ELISA reader at 530 nm. Anti-inflammatory activity was expressed as a percentage of suppression of NO generation. RAW 264.7 cells' viability was determined by the MTT assay[15].

High-Performance Liquid Chromatography (HPLC)

Myrrh hydro-methanolic extract was subjected to HPLC analysis utilizing an Agilent 1260 series instrument. An Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm) was used for the separation. Water (A) and 0.05 % trifluoroacetic acid in acetonitrile (B) were combined to form the mobile phase, which was flowing at a rate of 0.9 ml/min. The following was the sequential linear gradient programming for the mobile phase: 8–12 min (60% A), 0–5 min (80 % A), 12–15 min (82 % A), 15–16 min (82 % A), and 16–20 min (82 % A). At 280 nm, the multi-wavelength detector was detected. 5 µl of each sample solution were injected, and the temperature of the column was set at 40 °C.

GC-MS analysis

Helium was used as a carrier gas on a TG-SQC column and the plant's crude hydro-methanolic extract was submitted to GC-MS analysis using a Thermo Scientific TRACE 1310 Series Gas Chromatograph[16,17]. The sample was analyzed using the following temperature program: 50 °C for one minute was the starting temperature; after that, it rose to 250 °C for five minutes, and then to 290 °C for two minutes. 1.5 ml/min split mode regular flow injection of the sample was used. The mass spectrum was set to 40–1000 Hz, the mass transfer line temperature was set to 300 °C and ion source temperatures was set to 300 °C. Computer searches in data libraries were used to identify the component parts.

RESULTS AND DISCUSSION

Results

Total phenolic and Flavonoid contents

Phenolic and flavonoid compounds are the major secondary metabolites of the plant[10]. Flavonoids and phenols are very powerful free radical scavengers[18]. According to our findings, the total phenolic content of the myrrh hydro-methanolic and Petroleum ether extracts was (158.26 ± 3.44 and 126.16 ± 0.42 mg GAE/g of extract, respectively). According to **Fig. 1**, the total flavonoid concentration of myrrh in hydro-methanolic and petroleum ether was (91.43 ±

3.33 and 61.94 ± 2.5 mg Rutin/g extract, respectively). Many diseases that are primarily linked to free radicals are prevented and treated using phenolic and flavonoid. Furthermore, a broad range of biological activities, including as anti-inflammatory, antibacterial, antiviral, and anti-allergic properties, have been linked to flavonoids[19]. Actually, our findings support recent investigations that found that the total phenolic and flavonoid contents for a number of *Commiphora sp*. were authorized[4,20].

DPPH Antioxidant assay

Certain free radicals, such as hydroxyl groups, single oxygen, and peroxyl radicals, can damage cells and cause a variety of diseases, which is why they are harmful. Antioxidants, thus, are crucial in limiting the effects of free radicals[12]. When assessing the antioxidant activity of plant extracts, substances' capacity to behave as hydrogen donors or free-radical scavengers is examined using the stable radical DPPH[10,21,22]. The study's findings, which are shown in **Fig. 2**, demonstrated the antioxidant qualities of myrrh. When compared to ascorbic acid (IC_{50} = 7.90 ± 0.21 µg/mL), the hydro-methanolic extract (IC_{50} = 46.56 ± 1.06 µg/mL) of it was greater than the petroleum ether extract (IC_{50} = 66.13 ± 1.64 µg/mL). These findings demonstrated a relationship between the antioxidant and phenolic and flavonoid levels of the studied extracts. Additionally, these findings are in good agreement with earlier research on several myrrh studies that shown the plant's antioxidant activity and capacity to scavenge and block DPPH free radicals[23,4].

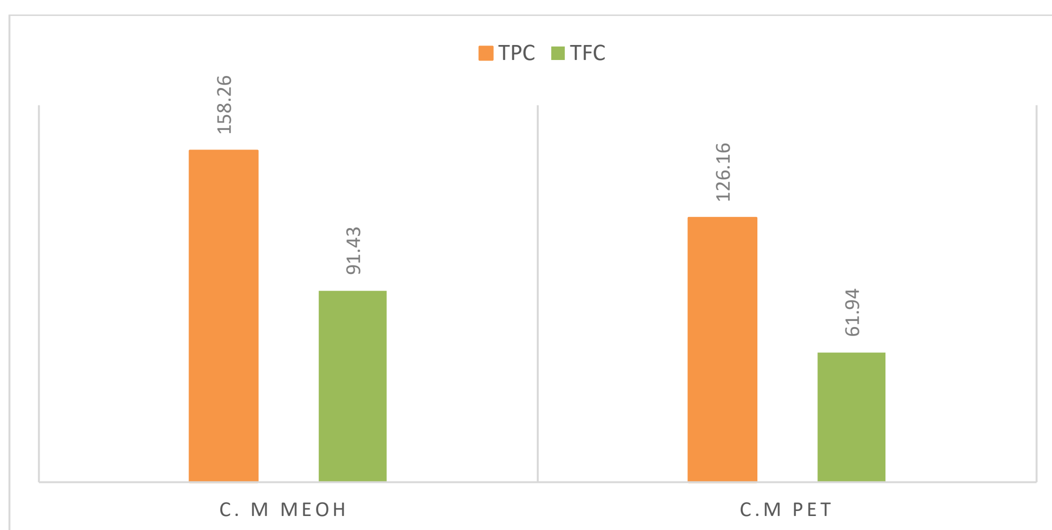

Fig. 1: Total phenolic and flavonoid content for MeOH and Pet ether of *myrrh.*

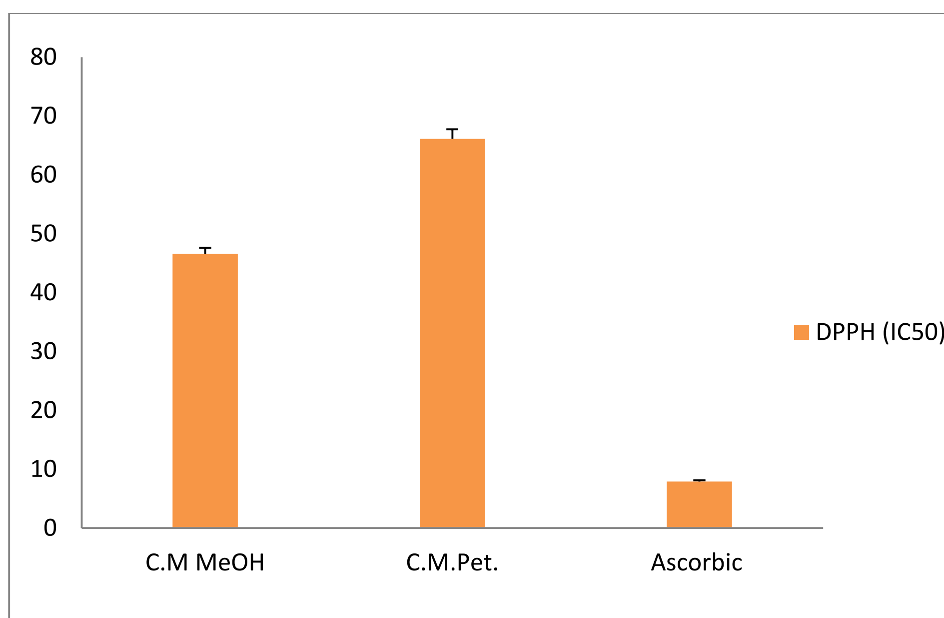

Fig.2: DPPH radical scavenging activity of myrrh extracts.

Evaluation of anti-inflammatory activity
Membrane stabilization assay

Anti-inflammatory effect of the extract was tested through Membrane stabilization mechanism. **Table 1** demonstrates that the human red blood cell (RBC) membrane was significantly stabilized by the myrrh hydro-methanolic extract. The concentration-dependent membrane stabilizing impact of myrrh hydro-methanolic extract on RBC hemolysis varied from 8.34 % to 81.34 %, with an IC_{50} of 40.40 µg/ml. However, because the IC_{50} was more than 1000 µg/ml, the petroleum ether extract's ability to prevent RBC hemolysis did not exhibit a discernible inhibition. In contrast, indomethacin's membrane stabilizing impact revealed an IC_{50} of 17.02 µg/ml and a greater inhibition percentage ranging from 32.18 % to 96.35 %. By stabilizing the membrane, serum fluid leakage into the tissue can be avoided. This procedure continues by inflammatory intermediators in which the membrane's permeability is increased[24]. These findings suggest that the myrrh hydro-methanolic extract possesses biological membrane stabilization qualities and inhibits the plasma membrane's deterioration caused by stress. Moreover, prior findings revealed that petroleum ether extract lowered the levels of inflammatory factor PGE2 in the edema paw tissue in experimental mice[25]. The plant's flavonoids and other bioactive substances may be the cause of this effect. Nevertheless, other earlier research demonstrated that flavonoids and other plant-based chemicals have an anti-inflammatory effect because they can stabilize membranes in a variety of experimental settings[26,1].

NO assay

In pro-inflammatory treatments, NO assay was utilized in LPS-induced RAW 264.7. Nitric oxide (NO) possesses the ability to eliminate bacteria and viruses, owing to its role in promoting vasodilation. When RAW 264.7 cells are stimulated with LPS, they can overexpress NO as well as the regulating proteins COX-2 and iNOS. Thus, we employed this tactic. **Fig. 3** presented the results, which indicated that myrrh hydro-methanolic extract had a stronger NO inhibition in LPS-induced RAW 264.7 cells than myrrh Petroleum Ether extract. The extracts' respective IC_{50} values were 59.8 and 79.7 µM, and their inhibition percentages were 46 % and 16 %, respectively[15].

Table 1: Anti-inflammatory activity (membrane stabilization %) of myrrh extracts.

Tested Concentration (µg/ml)	% Inhibiton of membrane stabilization		
	Hydro-methanolic myrrh extract	Petroleum ether myrrh extract	Indomethacin
1000	81.34	27.19 ± 1.50	96.35 ± 0.63
500	75.89	11.32 ± 2.10	85.35 ± 1.70
250	67.49	0	78.34 ± 2.10
125	60.41	0	72.35 ± 0.58
62.5	58.93	0	68.35 ± 1.50
31.25	46.32	0	56.38 ± 1.30
15.63	31.08	0	49.38 ± 0.72
7.81	8.34	0	32.18 ± 1.30
IC_{50}	40.40	> 1000	17.02

All determinations were carried out in triplicate manner and values are expressed as the mean ± SD. The IC_{50} value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions.

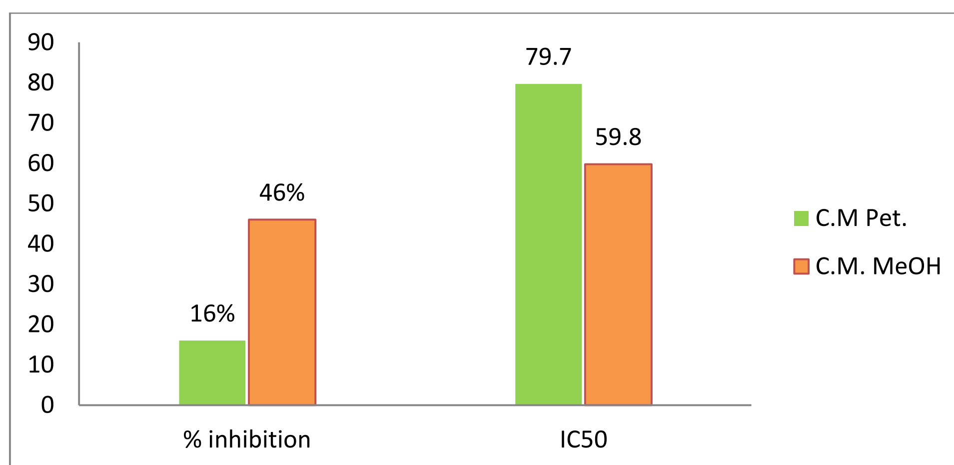

Fig.3: Inhibition% and IC_{50} of inhibitory activity on LPS-induced NO production in RAW 264.7 cells by myrrh.

High Performance Liquid Chromatography analysis

Plant phytochemicals can be detected and quantified using the most widely utilized separation technology, High-Performance Liquid Chromatography (HPLC)[27]. A class of phytochemical components known as phenolic compounds is present in several plants as secondary metabolites. Their broad ranges of bioactivity and assured impact on human health have drawn attention from the general public. Because of their anti-inflammatory, neuroprotective, anti-mutagenic, and antioxidant properties, among other things, natural polyphenols are regarded as significant substances[28,29,30,31]. According to evaluations in the scientific literature, they can function as pro-oxidant harmful macromolecules that cause cellular death or as antioxidants protective against oxidative deterioration[32,33].

In this study, the results of the phytochemicals screen, total phenolic and flavonoid content assay proved that the hydro-methanolic of myrrh has been compared by thirteen standards phenolic acids (chlorogenic acid, catechol, syringenic acid, p-coumaric acid, cinnamic acid, caffeic acid, pyrogallol, gallic acid, protocatechuic acid, ferulic acid, salicylic acid, ellagic acid and benzoic acid) and also, eight standards flavonoids (7-OH flavone, naringin, rutin, quercetin, kaempferol, luteolin, hesperidin and catechin) as shown in **Tables (2&3) and Fig. (4&5)**

Table 2: Concentrations of phenolic acids of MeOH extract of myrrh against thirteen standards phenolic acids.

NO	RT	Compound	Concentration	Compound	Concentration
1	3.0	Chlorogenic	7.88	-	_-
2	4.0	Catechol	3.45	-	-
3	5.0	Syringenic	3.56	Syringenic	1.89
4	6.0	p-coumaric	10.14	-	-
5	7.0	Cinnamic	9.79	Cinnamic	3.66
6	8.0	Caffeic	3.69	Caffeic	12.47
7	9.0	Pyrogallol	9.77	-	-
8	10.0	Gallic	2.56	Gallic	2.56
9	10.5	Protocatechulic	2.31	-	-
10	11.0	Ferulic	11.09	-	-
11	12.0	Salicylic	2.17	Salicylic	1.14
12	13.5	Ellagic	3.09	Ellagic	9.75
13	15.0	Benzoic acid	4.19	-	-

Table 3: Concentrations of flavonoids of MeOH extract of myrrh against eight standards flavonoids compounds.

NO	RT	Compound ST.	Concentration µg/ml	Compound Methanol ext.	Concentration µg/ml
1	3.0	7-OH flavone	6.11	7-OH flavone	0.77
2	4.0	Naringin	9.14	Naringin	6.12
3	5.0	Rutin	7.02	Rutin	5.22
4	7.0	Quersestin	6.88	Quersestin	11.47
5	8.0	Kampferol	3.88	Kampferol	0.23
6	9.0	Luteolin	10.22	Luteolin	4.19
7	10.0	Hisperdin	2.33	Hisperdin	12/39
8	12.0	Catechin	1.98	Catechin	1.02

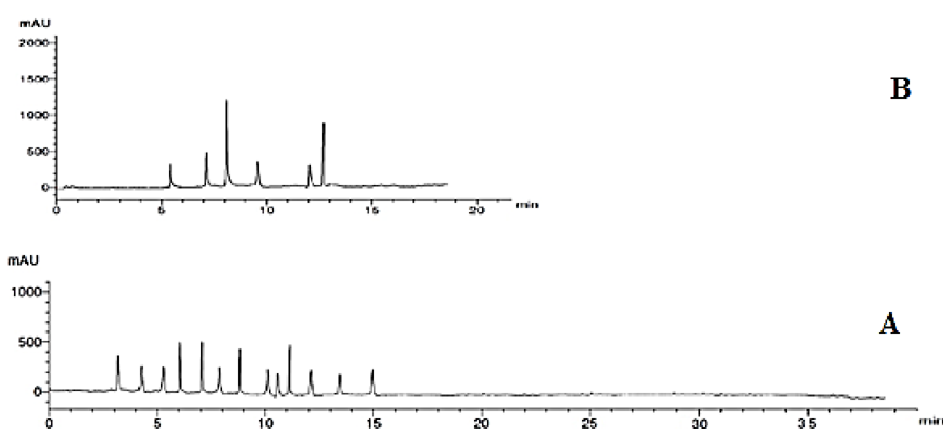

Fig. 4: HPLC fingerprint chromatogram of (A): thirteen standard phenolic acid compounds (B): Hydro-methanolic myrrh extract.

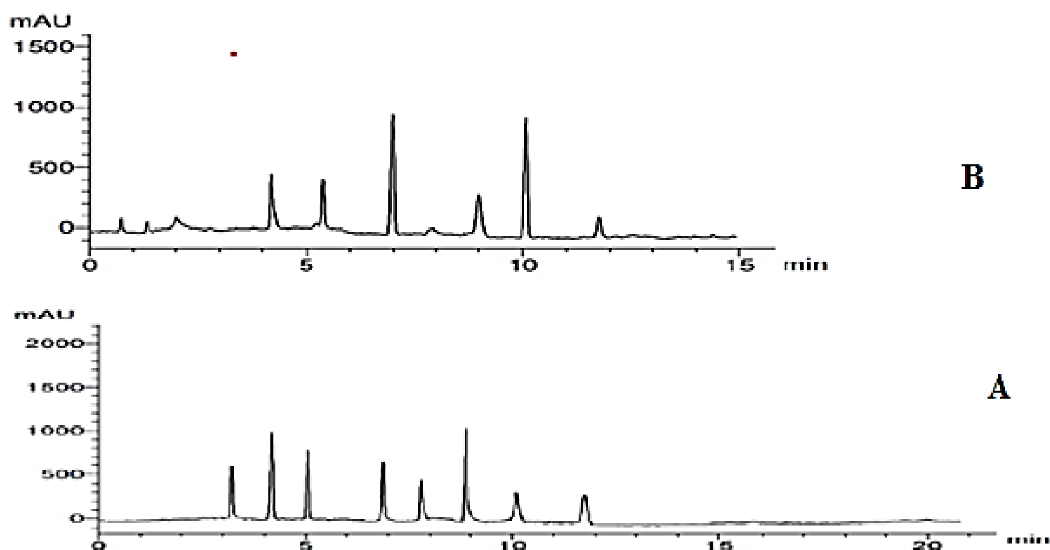

Fig. 5: High performance liquid chromatography -fingerprint chromatogram of (A): eight standard flavonoid compounds (B): MeOH extract of *Commiphora myrrha.*

Table 4 : Results of GC-MS analysis of methanol extract of myrrh**.**

Peak no.	Rt	Components	Molecular Formula	Molecular Weight	MeOH Area %
1.	5.51	caryophyllene oxide	$C_{15}H_{24}O$	220	0.32
2.	7.24	trans-3-Methyl-2-hexenoic acid	$C_7H_{12}O_2$	128	0.46
3.	7.37	3-[(E)-2-phenyl-1-propenyl] cyclohexanone	$C_{15}H_{18}O$	214	0.34
4.	8.10	5-Hydroxy-3,4,4-trimethyl-2-hexenoic acid	$C_9H_{17}NO_3$	187	0.83
5.	11.85	1-Dodecanamine, N, N-dimethyl	$C_{14}H_{31}N$	213	0.97
6.	14.46	10,12-Octadecadiynoic acid	$C_{18}H_{28}O_2$	276	0.21
7.	17.54	2,5-octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	0.25
8.	19.87	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	238	0.99
9.	20.02	Phytol	$C_{20}H_{40}O$	296	0.41
10.	20.16	5,7-Dodecadiyn-1,12-diol	$C_{12}H_{18}O_2$	194	0.48
11.	20.57	Methyl 5,7-hexadecadiynoate	$C_{17}H_{26}O_2$	262	1.97
12.	21.32	Methyl 4,6-tetradecadiynoate	$C_{15}H_{22}O_2$	234	0.31
13.	21.59	Retinol	$C_{20}H_{30}O$	286	1.23
14.	22.73	Docosahexaenoic acid methyl ester	$C_{23}H_{34}O_2$	342	0.٨٩
15.	25.99	6-[1-(Hydroxymethyl) vinyl]-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-2(1H)-naphthalenone	$C_{15}H_{24}O_2$	236	1.12

Table 4 : Continued.

16.	26.21	Lutein	$C_{40}H_{56}O_2$	568	2.01
17.	26.63	γ-Elemene	$C_{15}H_{24}$	204	0.19
18.	26.87	14-methyl palmitic acid	$C_{17}H_{34}O_2$	270	4.88
19.	27.34	3-ethyl-3-hydroxy-androstan-17-one	$C_{21}H_{34}O_2$	318	0.55
20.	28.01	5,8,11-heptadecatriynoic acid, methyl ester	$C_{18}H_{24}O_2$	278	0.80
21.	28.31	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280	0.36
22.	28.95	Monopalmitin	$C_{19}H_{38}O_4$	330	14.90
23.	29.31	5,8,11-Eicosatriynoic acid, methyl ester	$C_{21}H_{30}O_2$	314	2.09
24.	29.71	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	$C_{18}H_{30}O_2$	278	0.45
25.	30.02	glyceryl 1-linolenate	$C_{21}H_{36}O_4$	352	1.24
26.	30.20	Curzerene	$C_{15}H_{20}O$	216	15.25
27.	30.63	Methyl-9,9,10,10-d4-octadecanoate	$C_{19}H_{34}D_4O_2$	302	1.67
28.	30.98	6,9,12-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292	0.58
29.	31.73	1-Heptatriacotanol	$C_{37}H_{76}O$	537	4.63
30.	32.20	cis-11-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	٢٩٦	21.96
31.	32.44	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	2.21
32.	32.74	2-(7-Heptadecynyloxy) tetrahydro-2H-pyran	$C_{22}H_{40}O_2$	336	1.17
33.	33.28	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	0.93
34.	33.78	Methoxyfuranodiene	$C_{16}H_{22}O_2$	246	6.98
35.	34.26	α -Selinene	$C_{15}H_{24}$	204	0.19
36.	34.50	methyl octadeca-10,13-dienoate	$C_{19}H_{34}O_2$	294	0.28
37.	34.58	α-Guaiene	$C_{15}H_{24}$	204	0.11
38.	34.85	Cycloisolongifolene,8,9-dehydro-9-vinyl-	C17H24	337	1.01
39.	35.25	6β- Hydroxytestosterone	$C_{19}H_{28}O_3$	304	0.28
40.	35.56	1,8,15,22-Tricosatetrayne	$C_{23}H_{32}$	308	0.52
41.	35.96	2,9-Heptadecadiene-4,6-diyn-8-ol, (Z, E)-	$C_{17}H_{24}O$	244	0.63
42.	36.19	Cyclodecacyclotetradecene,14,15-didehydro-1, 4, 5, 8, 9, 10, 11, 12, 13, 16,17,18,19,20-tetradecahydro-	$C_{22}H_{32}$	296	0.20
43.	36.39	Isovelleral	$C_{15}H_{20}O_2$	232	0.30
44.	37.52	methylricinelaidate	$C_{19}H_{36}O_3$	312	1.25
45.	39.42	Eremophila-1(10),11-diene	$C_{15}H_{24}$	204	0.83
					98.4

Table 5: Results of GC-MS analysis of the petroleum ether extract of myrrh.

Peak no.	Rt	Components	Molecular Formula	Molecular Weight	Area %
46.	9.12	1-Ethyl-2-methylcyclohexane	C_9H_{18}	126	0.23
47.	9.64	2,4,6-Trimethylheptane	$C_{10}H_{22}$	142	0.41
48.	10.95	2-Butyl-1-octanol	$C_{12}H_{26}O$	186	2.04
49.	11.15	2-Hexyl-1-decanol	$C_{16}H_{34}O$	242	3.11
50.	11.35	5-Eicosene	$C_{20}H_{40}$	280	0.90
51.	14.95	1-Docosanol	$C_{21}H_{44}O$	312	1.71
52.	15.30	1-Nonadecanol	$C_{19}H_{40}O$	284	0.49
53.	16.25	17-Octadecynoic acid	$C_{18}H_{36}O$	268	2.42
54.	17.63	2-Hexyl-1-octanol	$C_{14}H_{30}O$	214	13.48
55.	18.42	1-Acetoxy-p-menth-3-one	$C_{12}H_{20}O_3$	212	1.07
56.	18.83	Oleyl alcohol	$C_{18}H_{36}O$	268	1.10
57.	18.91	1,16-Hexadecanediol	$C_{16}H_{34}O_2$	258	0.92
58.	20.02	Phytol	$C_{20}H_{40}O$	296	3.07
59.	20.81	13-Octadecenal	$C_{18}H_{34}O$	268	2.30
60.	21.66	α-ylangene	$C_{15}H_{24}$	204	1.33
61.	22.19	2-hexyl-l-decanol	C18H38O	270	0.7-
62.	24.22	Cyclohexene,4-ethenyl-4-methyl-3-(1-methyl ethenyl)-1-(1 methylethyl)-, (3R-trans)-	$C_{15}H_{24}$	204	0.67
63.	25.27	α-Copaene	$C_{15}H_{24}$	204	0.23
64.	25.51	α-Bourbonene	$C_{15}H_{24}$	204	0.17
65.	25.78	α- Elemene	$C_{15}H_{24}$	204	8.97
66.	27.53	Nonylbenzene	$C_{15}H_{24}$	204	0.50
67.	28.03	α -Muurolene	$C_{15}H_{24}$	204	0.۸۸
68.	28.39	α-Selinene	$C_{15}H_{24}$	204	0.47
69.	29.53	Valencene	$C_{15}H_{24}$	204	2.68

Table 5: Continued.

70.	30.30	Benzene, (1-ethyloctyl)-	$C_{16}H_{26}$	218	2.65
71.	30.48	Isovelleral	$C_{15}H_{20}O_2$	232	2.08
72.	31.53	Dodecylbenzene	$C_{18}H_{30}$	232	1.03
73.	32.07	(1-Pentylhexyl) benzene	$C_{17}H_{28}$	232	6.36
74.	32.95	Benzene, (1-ethylnonyl)-	$C_{17}H_{28}$	232	4.66
75.	33.06	Eremophilene	$C_{15}H_{24}$	204	1.38
76.	33.41	10,13-Octadecadiynoic acid,methyl ester	$C_{19}H_{30}O_2$	290	0.37
77.	33.84	Benzene, (1-propylnonyl)-	$C_{18}H_{30}$	246	6.30
78.	34.30	Benzene, (1-pentylheptyl)-	$C_{18}H_{30}$	246	3.33
79.	34.40	Benzene, (1-butyloctyl)-	$C_{18}H_{30}$	246	1.97
80.	34.66	Benzene, (1-methyldecyl)-	$C_{17}H_{28}$	232	4.35
81.	34.83	4,7-Methanofuro[3,2-c] oxacycloundecin-6(4H)-one,	$C_{15}H_{18}O_3$	246	0.09
82.	35.15	Benzene, (1-ethyldecyl)-	$C_{18}H_{30}$	246	2.87
83.	35.24	α-Guaiene	$C_{15}H_{24}$	204	0.13
84.	35.35	(3E)-5-Isopropyliden-6-methyl-3,6,9-decatrien-2-one	$C_{14}H_{20}O$	204	0.16
85.	35.57	8-epi-. γ.-eudesmol	$C_{15}H_{26}O$	222	0.41
86.	36.01	Benzene, (1-methylundecyl)-	$C_{18}H_{30}$	246	3.62
87.	36.29	Benzene, (1-pentyloctyl)-	$C_{19}H_{32}$	260	2.23
88.	36.48	Benzene, (1-butylnonyl)-	$C_{19}H_{32}$	260	1.29
89.	36.74	9-Methoxybenzo[b]fluorene-11-one	$C_{18}H_{12}O_2$	260	1.28
90.	37.22	8,9-Dihydrocyclohepta[a]phenalen-7,10-dione	$C_{18}H_{12}O_2$	260	1.60
91.	37.38	Vitamin A alcohol	$C_{20}H_{30}O$	286	0.06
92.	37.78	Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane],	$C_{15}H_{24}O_3$	252	0.18
93.	38.05	1-Heptatriacotanol	$C_{19}H_{32}$	260	0.91
94.	38.61	Androstan-17-one,3-ethyl-3-hydroxy-, (5à)-	$C_{21}H_{34}O_2$	318	0.23
					99.3

A devised HPLC technique was used to determine six phenolic compounds and eight flavonoid compounds. Caffeic and ellagic were found as major phenolic acid (12.47and 9.75

µg/ml, respectively). Cinnamic, gallic, syringenic and salicylic were detected as minor components (3.66, 2.56, 1.89 and 1.14 µg/ml, respectively). While chlorogenic acid, catechol, p-coumaric acid, pyrogallol, protocatechulic, ferulic and benzoic acid were not detected in the extract. On the other hand, the hydro-methanolicic extract of myrrh has hesperidin and quersestin as major flavonoid (12.39 and 11.47 µg/ml respectively). While, naringin, rutin, luteolin and catechin have been found in moderate components (6.12, 5.22, 419 and 1.02 µg/ml, respectively). But, 7-OH flavone and kampferol were found in little compounds (0.77 and 0.23 µg/ml, respectively).

Due to their antioxidant qualities, quercetin and hesperdin help with renal and hepatic dysfunctions[34]. Because of their structure and modes of action, the phenolic compounds in this extract have the capacity to scavenge free radicals, which gives them antioxidant and anti-inflammatory properties[35,36]. All these results suggest decreased inflammation and oxidative stress by the hydro-methanolic extract of myrrh that has free radical scavenging activities.

GC-MS Analysis

The bioactive components of the hydro-methanolic and the petroleum ether extracts of myrrh were evaluated by GC-MS analysis that was identified by comparing their mass spectral fragmentation patterns to those published in the Wiley and NIST libraries. The retention periods of these compounds are listed as shown in **Tables 4&5.**

The present data showed that the identified compounds in the hydro-methanolic extract are 45 compounds whereas in the petroleum ether extract are 49 compounds. The percent of total identified compounds in the hydro-methanolic extract is 98.4 % and in the petroleum ether extract is 99.3 %, respectively. Because different polarity solvents are employed to extract different types and quantities of phytochemical components from the plant, distinct substances from extracts of the same plant have been found [37]. The major compounds in the petroleum ether extract identified as 2-Hexyl-1-octanol (13.48 %), α- Elemene (8.97 %), (1-Pentylhexyl) benzene (6.36 %) and Benzene, (1-propylnonyl)- (6.3 %). 2-Hexyl-1-octanol is an aliphatic alcohol that has microbiological, antioxidant and anti-inflammatory properties[38]. α- Elemene is sesquiterpene and has anti-inflammatory and antitumor effects[39]. 1-Pentylhexyl) benzene and Benzene, (1-propylnonyl)- are alkylbenzene and having antimicrobial activity[40].

The major compounds in the hydro-methanolic extract identified as cis-Vaccenic acid methyl ester (21.96 %), Curzerene (15.25 %), Monopalmitin (14.90 %) and Methyl ricinoleate (6.98 %). cis-11-Octadecenoic acid methyl ester is a form of the monounsaturated fatty acid that exhibits various biological properties, such as antimicrobial, antioxidant, and anti-inflammatory activities[22]. Curzerene is a sesquiterpene which showed anticancer, anti-inflammation, and anti-leishmaniasis[41]. Monopalmitin is a glycerol-esterified fatty acid, with antibacterial and antifungal activities[42]. Methoxyfuranodiene is furanosesquiterpenoid that has principally analgesic, antipyretic, and anti-inflammatory actions[43,44]. So, myrrh has anti-inflammatory and antioxidant activities, these results are in agreement with previous studies on the plant[25,44].

Conclusion

According to the study, myrrh hydro-methanolic extract exhibits strong antioxidant activity. Furthermore, it might be useful as a treatment in conditions where human red blood cell membrane stability is a problem. It is believed that the herb has potent anti-inflammatory properties. One of the oldest Albkhurih trees, myrrh was utilized in religious ceremonies and Pharaoh Worship millions of years ago by the Greeks and Pharaohs. This makes it important to take advantage of the plant's benefits, including its ability to boost sales and produce scented extracts.

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نشرة العلوم الصيدليـــة
جامعة أسيوط

التوصيف الكيميائى لنبات كومفورا ميريا و دراسة قدرتة على ثبات نفاذية الغشاء كآلية مضادة للإلتهاب

إيمان أحمد الوكيل – هبة عبد الهادى* – إيمان عبد الله مرسى

قسم الكيمياء العلاجية، معهد تيودور بلهارس للأبحاث ، كورنيش النيل ، وراق الحضر، إمبابة ، جيزة ، مصر

يتم اســـتخدام نبات الكومفورا ميريا على نطاق واســـع في الطب التقليدي. حيث تمت دراســـة الـ صفات الكيموحيوية لمـ ستخلـ صي الميثانول و البترووليم إيثر للنبات. كما تمت درا سة محتويات الفينول والفلافونويد، و قياس قدرتة كمـ ضاد للأكسدة بواسطة DPPH ، والنـ شاط المـ ضاد للالتهاب في المختبر عن طريق تحديد نشاط تثبيت الغشاء ومقايسة NO وكذلك التحليل الكيميائي بواسطة GC-MS وHPLC. و قد أظهرت النتائج أن محتوى الفينول والفلافونويد في مـ ستخلص الميثانول (١٥٨,٢٦ ± ٣,٤٤ جاليك اسيد / جم من المستخلص و ٩١,٤٣ ± ٣,٣٣ روتين / جم من المستخلص، على التوالي) كان أعلى من محتوى البتروليم إيثير (١٢٦,١٦ ± ٠,٤٢ / GAE جم من المـ ستخلص) و(٦١,٩٧ ± ٢ روتين/جم من المستخلص، على التوالي). كما أن للميثانول نشاط مضاد للأكسدة قوي مقارنة بالبتروليوم إيثر ١,٠٦± ٤٦,٥٦ =IC50) و٦٦,١٣ ± ١,٦٤ ميكروجرام/مل، على التوالي). علاوة على ذلك، أظهر مـ ستخلص الميثانول تأثيرات قوية على استقرار غشاء خلايا الدم الحمراء البشرية بالإضافة إلى تثبيط قوي لإنتاج NOكما أظهر HPLC لمســـتخلص الميثانول ١٣ مركب فينولي و ٨ فلافونويد واحد، في حين أظهر تحليل GC-MS ٤٥مركب في مـ ستخلص البتروليوم ايثر و ٤٩ مركب في مـ ستخلص الميثانول. بـ شكل عام يرتبط التأثير المضاد للالتهابات بنشاط النبات كمضاد للأكسدة و على محتوى الفينول والفلافونويد.