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Original Article

Chemical Profile and Biological Activities of Essential oil of Aerial parts of *Artemisia monosperma* Del. Growing in Libya

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ABSTRACT

Background: From the bioactivity stand point *Artemisia monosperma* Del. is reputed to have antispasmodic and anthelmintic properties. Various types of secondary metabolites were reported in *A. monosperma* plants from different localities. **Objective:** The current study was planned aiming to investigate the influence of stage of development on the composition, antimicrobial, antiinflammatory and antioxidant activities of the essential oil derived from the aerial parts of the Libyan plants. **Material and Methods:** *A. monosperma* volatiles were hydro-distilled from aerial parts of Libyan plants, collected at different stages of growth: before flowering (A_1), and at beginning (A_2) and by the end (A_3) of flowering stage. Yields ranged from 0.16-0.26 ml/100g fresh material (A_3, highest). GC/FID and GC/MS analyses were performed. **Results:** Among 16-20 identified components (97.63-99.00% of total composition), 11 were common in all samples. A_1 and A_2 showed close amounts of hydrocarbons (63.56 and 66.55%), but lesser than A_3 (88.36%); monoterpenoids were mainly represented by sabinene (13.15-22.85%), β-pinene (9.00-24.03%) and β-*cis*-ocimene (3.73-12.92%); while sesquiterpenoids appeared absent. Among oxygenated components (11.29, 31.08 and 35.44 % in A_3, A_2 and A_1), bornyl acetate was the major monoterpenoid (8.00-31.00%, highest in A_1); and the sesquiterpenoid, β-eudesmol (8.01%) was detected in A_2 only. Moreover, A_2 demonstrated significant antifungal effect against *Aspergillus fumigatus* and *Geotricum candidum* (MIC 0.98 and 0.24 µg/ml). **Conclusion**: A_3 exerted the highest anti-inflammatory activity as compared to the other volatiles. A_1 restored the reduced blood GHS level in diabetic animals almost as efficiently as Vitamin E. The antioxidant activity of the volatiles is decreased during the flowering stage, being the highest before flowering (A_1); this could be associated to the decreasing bornyl acetate content of the samples.

Key words: *Artemisia monosperma*, Essential oil composition, GC-MS, Antimicrobial, Anti-inflammatory, Anti-oxidant, Libya.

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INTRODUCTION

Genus *Artemisia* (Asteraceae) comprises about 300 species of broad distribution all over the world.[1] Moreover, the essential oils derived from several members of this genus have acquired special importance due to their wide variety of applications in folk and modern medicine. [2,3] The genus is represented in Libya by five species *viz*., *A. arborescens*, *A. herba-alba*, *A. judaica*, *A. campestris* and *A. monosperma*.[4] The latter is a Saharo-Arabian element and has been reported from Gasar Gharian, Ghat area and Southern Tripolitania.[4] *A. monosperma* is a green glabrous shrublet, 50-70 cm high, with ascending or diffuse stems ending in many flowered panicles; capitula are small (1mm in diameter) with 10-12 tubular florets; the flowering season extends from September to December in most areas.[4] From the bioactivity stand point *A. monosperma* Del. is reputed to have antispasmodic, anthelmintic and anti-hypertensive properties;[5,6] its leaves are traditionally used in Jordan to induce abortion[7] and were found to possess antioxidant,[8] insecticidal,[9] anti-malarial[10] and anticancer activities.[11] Various types of secondary metabolites were reported in *A. monosperma* plants from different localities including: coumarins,[12,13] flavonoids,[14-16] acetylenes,[9,17,18] alkaloids,[19] sesquiterpenoids[20] and triterpenoids.[16] However, nothing could be traced in the available literature concerning either the constituents or biological activities of the plant growing in Libya.

MATERIAL AND METHODS

Plant material

Aerial parts of *A. monosperma* Del. 500 g fresh plant samples were collected from plants growing in Libya (Tobruk Desert) at 3 time intervals: before flowering (A_1), and at the beginning (A_2) and by the end (A_3) of the flowering stage in March, September and late November (2013). The plants were kindly authenticated by Dr. Mohamed El-Gebaly, Botany Specialist.

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Voucher specimen 1192013 is kept in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Oil samples

The essential oils were hydro-distilled from 500 g fresh plant samples in a Clavenger-type apparatus, isolated and dehydrated. Aliquots (5 µl, each) of the prepared oils were, separately, mixed with approximately 1 ml of CH_2Cl_2 in auto sampler vials and saved for chromatographic investigation. For biological evaluation solutions (1mg/ml) were prepared in DMSO.

Microorganisms, culture media and standard antimicrobial agents

A set of 12 representative fungal and Gram-positive and -negative bacterial strains were used. The tested microorganisms: *Aspergillus fumigatus* (RCMB 02564), *Candida albicans* (RCMB 05035), *Geotricum candidum* (RCMB 05096), *Syncephalastrum racemosum (RCMB 05922), Staphylococcus aureus* (RCMB 010027), *Staphylococcus epidermidis* (RCMB 010024) *Streptococcus pyogenes* (RCMB 010015) *Neisseria gonorrhoeae* (RCMB 010076), *Proteus vulgaris* (RCMB 010085) *Klebsiella pneumoniae* (RCMB 010093) *Shigella flexneri* (RCMB 01005420) and *Escherichia coli* (RCMB 010056) were maintained in the Regional Center for Mycology and Biotechnology (RCMB), Cairo, Egypt. The tested bacteria were sub-cultured on nutrient agar medium (Oxoid laboratories, UK) slopes. Sabouraud's dextrose agar (Oxoid laboratories, UK) was used as culture medium for fungi.

Chemicals and kits

Penicillin G (Oxoid, UK) and Streptomycin (Oxoid, UK) were used as standard antibacterial agents, meanwhile Amphotericin B (Sigma Chemical Co. St. Louis, Mo.) was used as standard antifungal. Carrageenan: Sigma Co.(0.1 ml of 1% solution, to induce inflammation), Indomethacin: Epico, A.R.E.(20 mg/kg body weight [b. wt.], as standard anti inflammatory). Alloxan: Sigma Co., Vitamin E (7.5mgkg^{-1}b. wt.) (dlα-tocopheryl acetate): Pharco Pharmaceutical Co. Biochemical kits: Biodiagnostic glutathione kit.

Experimental animals

Adult Sprague-Dawley rats weighing 160 ± 10 g were provided from the animal-breeding unit of National Research Center, El-Dokki, Giza, Egypt. They were kept under standard conditions with temperature at 23 ± 2 °C and a 12/12 hours light/dark cycle and allowed free access to food and water throughout the experiment. This study was conducted in accordance with the standard guidelines used in handling of the experimental animals and approved by the Institutional Animal Care and Use Committee (IACUC) (No. 9-031), College of Pharmacy, Cairo University.

GC/MS Analysis

Instrument

Shimadzu capillary gas chromatograph (GC 17A ver.3) directly coupled to mass spectrometer-MS QP5050A and equipped with a capillary column SLB-5ms (30 m×0.25 mm, film thickness 0.25 µm).

Operating conditions

Injection volume, 1 µl of CH_2Cl_2 solution of tested samples; oven temperature programming: initial temperature, 40 °C (isothermal for 3 min), increased (12°C/min) to 180 °C and hold for 5 min, then further increased to final temperature 240 °C (40°C/min) and maintained for 5 min; injector temperature, 240 °C and split ratio, set at 1:54; carrier gas, He at flow rate 0.9 ml/min; mass spectrometer, electronic ionization (EI) mode 70eV, scan range 40-500 and transfer line temperature, 230 °C.

GC/FID Analysis

Instrument and operating conditions

A Shimadzu GC gas chromatograph (GC-17ver.3) coupled with FID detector was used, and detector temperature set at 240°C. To obtain the same elution order as with GC/MS, simultaneous auto injection was carried out on a duplicate of the same operational conditions.

Identification of Components

This was performed *via* comparison of the retention times (Rt) and retention indices (RI, Kovat's indices) of the detected components with those of authentic samples. RIs were computed relative to those of a mixture of a continuous series of *n*-alkane hydrocarbons (C4-C28) analyzed under the same operating conditions as for the samples. Individual components were identified through matching their MS fragmentation patterns with those reported in computerized MS-data bank spectral libraries[21-23] or reported in the literature.[24-25] Relative percentages of the identified components were calculated based on GC/FID peak areas without the use of correction factors. Results are compiled in Table 2, Figure 1 & 2.

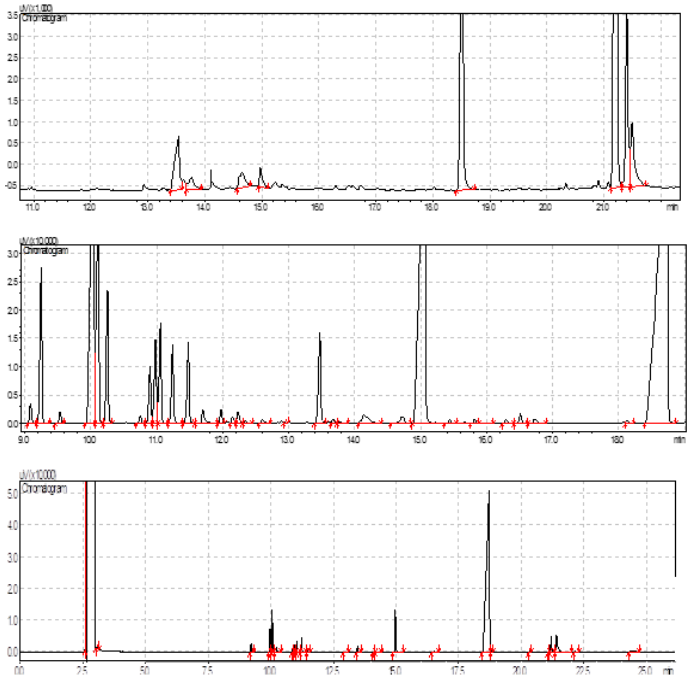

Figure 1: Chromatograms 1, 2 and 3 representing the GC-FID of the essential oils (A1, A2 and A3) samples collected before flowering, and at the beginning and end of the flowering stage of *A. monosperma,* respectively.

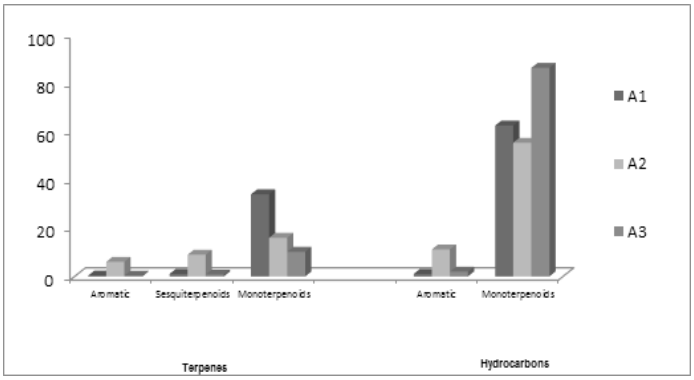

Figure 2: Histogram representing the relative percentages of the different classes of constituents in the essential oils (A1, A2 and A3) samples collected before flowering, and at the beginning and end of the flowering stage of *A. monosperma,* respectively.

Table 1: Yield and physical characters of the essential oil samples of the aerial parts of *Artemisia monosperma*

Characteristics	Oil samples		
	A1	A2	A3
% Yield v/ fresh wt	0.16	0.20	0.26
Color	Greenish yellow		
Odor	Aromatic		
Optical rotation	+ 4.7	+ 4.5	+ 3.9
Specific gravity	0.914	0.911	0.901

A1, A2 & A3: samples collected before flowering, and at the beginning and end of the flowering stage.

Table 2: Constituents identified by GC/FID and GC/MS analyses in the essential oil samples of the aerial parts of *A. monosperma*.

SN	Identified Constituents	Rt	RI*	RI**	Relative percentages			Mwt	Formula
					A1	A2	A3		
1	α-Thujene	7.52	929	930	-	-	0.76	136	$C_{10}H_{16}$
2	α-Pinene	7.67	936	935	6.20	1.10	5.93	136	$C_{10}H_{16}$
3	Camphene	7.98	953	946	0.74	-	-	136	$C_{10}H_{16}$
4	Sabinene	8.38	976	972	22.85	13.15	17.10	136	$C_{10}H_{16}$
5	β-Pinene	8.48	981	977	9.00	16.91	24.03	136	$C_{10}H_{16}$
6	Myrcene	8.62	989	987	7.02	4.57	5.60	136	$C_{10}H_{16}$
7	α-Phellandrene	8.85	999	1002	0.99	-	-	136	$C_{10}H_{16}$
8	(+)-4-Carene	8.90	1009	1008	-	1.26	1.44	136	$C_{10}H_{16}$
9	α-Terpinene	9.12	1020	1014	0.54	-	-	136	$C_9H_{12}O$
10	p-Cymene	9.24	1027	1020	1.06	11.20	1.98	134	$C_{10}H_{14}$
11	Limonene	9.34	1033	1024	3.00	4.50	5.27	136	$C_{10}H_{16}$
12	β-Trans-ocimene	9.38	1036	1031	5.32	5.47	10.98	136	$C_{10}H_{16}$
13	β-Cis-ocimene	9.56	1047	1044	3.73	4.09	12.92	136	$C_{10}H_{16}$
14	δ-Terpinene	9.78	1062	1054	3.11	4.30	2.62	136	$C_{10}H_{16}$
15	Cis-sabinene hydrate	10.02	1076	1065	0.47	-	-	154	$C_{10}H_{18}O$
16	Trans-sabinene hydrate	10.03	1090	1098	-	-	0.86	154	$C_{10}H_{18}O$
17	Norbilan [1-(p-Tolyl ethanol)]	10.13	1099	1104	-	-	0.31	136	$C_{10}H_{16}$
18	(-)Terpinen-4-ol	11.68	1190	1177	1.49	1.94	1.34	224	$C_{14}H_{24}O_2$
19	Citronellyl formate	12.79	1274	1271	0.81	-	-	154	$C_{10}H_{18}O$
20	Bornyl acetate	13.06	1293	1284	31.00	14.05	8.00	184	$C_{11}H_{20}O_2$
21	Eugenol methyl ether	14.4	1402	1401	0.24	-		196	$C_{12}H_{20}O_2$
22	Linalyl butyrate	13.06	1400	1420	0.25	-	-	178	$C_{11}H_{14}O_2$
23	Methyl iso-eugenol	14.40	1402	1461	--	1.65	-	178	$C_{11}H_{14}O_2$
24	Guaiol	16.49	1560	1600	1.06	-	-	240	$C_{16}H_{16}O_2$
25	7-Methyl-1-naphthol	17.08	1599	1620	-	0.63	-	222	$C_{15}H_{26}O$
26	Dihydro-cis-α-copaene-8-ol	18.12	1643	1633	-	1.01	0.78	158	$C_{11}H_{10}O$
27	Capillin [2,4-hexadyinophenone]	18.14	1648	1637	-	3.16	-	222	$C_{15}H_{26}O$
28	β-Eudesmol	18.51	1667	1649	-	8.01	-	168	$C_{12}H_8O$
29	p-Toluoin [4,4'-dimethylbenzoin]	15.432	1686	1790	0.12	0.63	-	222	$C_{15}H_{26}O$
	Total number of identified constituents				20	18	16		
	Total % of identified constituents				99.00	97.63	99.9		

RI*: Retention indices relative to n-alkanes on SLB-5ms column; RI**: Retention indices reported on non polar column; A1, A2 & A3: samples collected before flowering, and at the beginning and end of the flowering stage.

Table 3: Antimicrobial activity of essential oil samples of the aerial parts of *A. monosperma*, expressed as diameter of zone of inhibition in mm.

Tested microorganisms	Tested Samples			Standard
Fungi	A1	A2	A3	Amphotericin B
Aspergillus fumigatus (RCMB 02564)	18.3±0.11	21.3±0.20	15.6±0.10	23.7±0.10
Candida albicans (RCMB 05035)	15.4±0.23	17.8±0.15	14.1±0.20	21.9±0.12
Geotricum candidum (RCMB 05096)	21.9±0.27	23.4±0.18	16.4±0.12	26.4±0.20
Syncephalastrum racemosum (RCMB 05922)	NA	NA	NA	25.4+0.16
Gram-positive bacteria				Ampicillin
Staphylococcus aureus (RCMB 010027)	16.2±0.28	18.4±0.44	12.6±0.18	28.9±0.14
Staphylococcus epidermidis (RCMB 010024)	17.4±0.37	20.8±0.25	16.3±0.35	25.4±0.18
Streptococcus pyogenes (RCMB 010015)	NA	NA	NA	26.4±0.34
Gram-negative bacteria				Gentamycin
Neisseria gonorrhoeae (RCMB 010076)	14.4±0.20	15.2±0.58	12.3±0.18	19.9±0.18
Proteus vulgaris (RCMB 010085)	NA	NA	NA	23.4±0.3
Klebsiella pneumoniae (RCMB 010093)	16.8±0.24	18.3±0.44	17.1±0.15	26.3±0.15
Shigella flexneri (RCMB 01005420)	15.2±0.35	17.4±0.58	14.1±0.24	24.8±0.24
Escherichia coli (RCMB 010056)	18.6±0.19	20.1±0.25	14.9±0.15	25.3±0.18

A1, A2 & A3: samples collected before flowering, and at the beginning and end of the flowering stage; NA: no action

Evaluation of Antimicrobial Activity

Antimicrobial assay

The antibacterial activity of the essential oils was screened by the agar well-diffusion method, zone of inhibitions being measured in mm, as described by Holder and Boyce (1994).[26] The essential oils were, separately, tested against the selected strains at concentration of 1mg/ml. DMSO was used as a negative control; meanwhile Penicillin G and Streptomycin (10 mg/ml) were used as a positive controls for bacterial strains and Amphotericin B as positive control for fungi. Experiments were carried out in triplicates. Bacterial cultures were incubated at 37 °C, for 24 h and the fungal ones at 25-30°C, for 3-7 days. Results are recorded (Table 3) as mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (1 mg/ml) concentration of tested samples. [27] Potencies relative to the appropriate antibiotic are represented by the histograms in (Figures 4-6).

Determination of minimal inhibitory concentrations (MIC)

Broth dilution method[28] was used for determination of minimum inhibitory concentrations (MIC) of the most active sample. Serial dilutions of each essential oil sample were prepared in DMSO and mixed with broth media in a 96-wells microtiter plate to achieve a final concentration range from 0.003-4 % v/v. The plates were then inoculated with standardized suspension containing 5×10^5 bacterial count per well. Optical density was measured at 600 nm after a specified incubation period by means of a JENWAY 6051 colorimeter (U.K.) and was used as a measure of bacterial growth.[29]

Determination of Median Lethal dose (LD_{50})

The acute toxicity resulting from oral administration of the volatile oils of the aerial parts of *A. monosperma* Del. was investigated. In this respect, its median lethal dose (LD_{50}) was determined according to Karber (1931)[30] as follows: Preliminary experiments were carried out to determine the minimal dose that kills all animals (LD_{100}) and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were selected in between these two doses, and each was orally

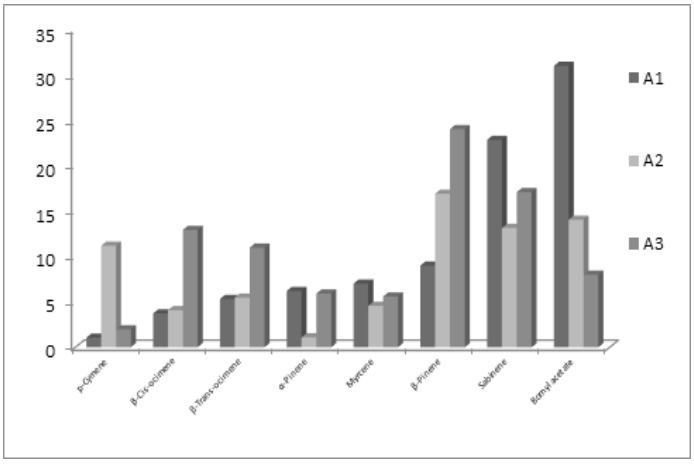

Figure 3: Histogram representing the relative percentages of the major components detected in the essential oils (A1, A2 and A3) samples collected before flowering, and at the beginning and end of the flowering stage of *A. monosperma,* respectively.

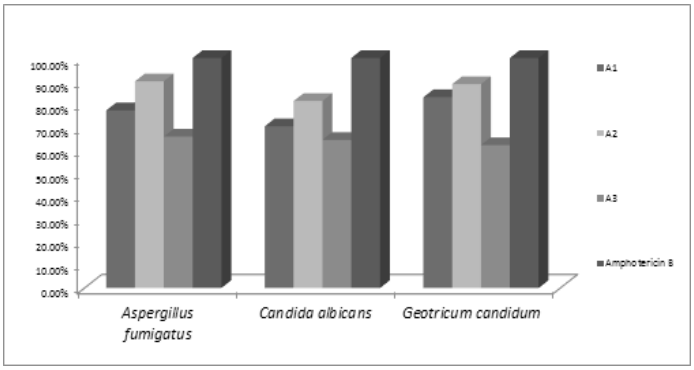

Figure 4: Histogram representing the antifungal activity of essential oils (A1, A2 and A3) samples collected before flowering, and at the beginning and end of the flowering stage of *A. monosperma,* respectively; expressed as potencies as compared to standard Amphotericin B.

Table 4: Antimicrobial Activity of A2 oil sample* expressed as MICs (µg/ml)

Tested microorganisms	MIC	Standard
Fungi		Amphotericin B
Aspergillus fumigatus (RCMB 02564)	0.98	0.12
Candida albicans (RCMB 05035)	7.81	0.24
Geotricum candidum (RCMB 05096)	0.24	0.03
Syncephalastrum racemosum (RCMB 05922)	NA	0.06
Gram-positive bacteria		Ampicillin
Staphylococcus aureus (RCMB 010027)	7.81	0.007
Staphylococcus epidermidis (RCMB 010024)	0.98	0.06
Streptococcus pyogenes (RCMB 010015)	NA	0.03
Gram-negative bacteria		Gentamycin
Neisseria gonorrhoeae (RCMB 010076)	31.25	3.9
Proteus vulgaris (RCMB 010085)	NA	0.12
Klebsiella pneumonia (RCMB 010093)	7.81	0.03
Shigella flexneri (RCMB 01005420)	15.63	0.06
Escherichia coli (RCMB 010056)	1.95	0.06

A2 sample of aerial parts collected at the beginning of the flowering stage; NA: no action.

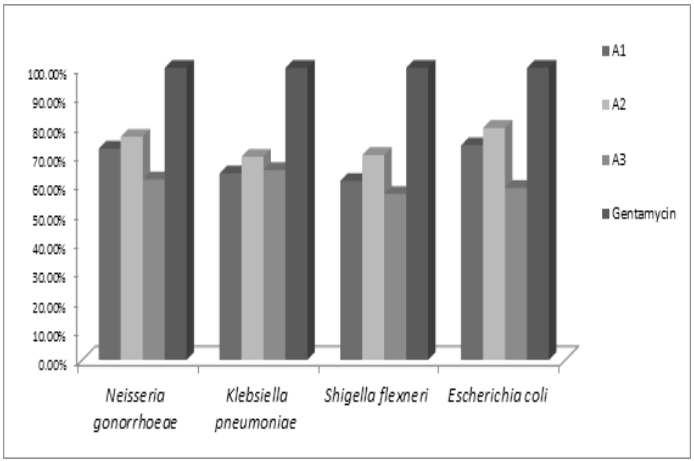

Figure 5: Histogram representing the antibacterial activity of essential oils (A1, A2 and A3) samples collected before flowering, and at the beginning and end of the flowering stage respectively of aerial parts of *A .monosperma*, expressed as potencies as compared to standard Gentamycin.

administered to a group of experimental animals (n=6). The mice were observed for 24 h and the symptoms of toxicity as well as mortality rates in each group were recorded.

Evaluation of Anti-inflammatory activity

The acute anti-inflammatory activity was assessed *in-vivo* by means of the paw swelling, or foot edema method, which is convenient for assessing inflammatory responses to antigenic challenges and irritants. This was performed by examining the ability of the tested samples to reduce or prevent the development of carrageenan-induced paw swelling. This model is commonly used to assess the efficiency of nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit prostaglandin production.[31]

Experimental design

The method of Winter *et al* (1962)[32] was adopted. Carrageenan suspension (1% in sterile saline) was prepared, and placed in a refrigerator, at 4ºC for an overnight, to allow complete hydration of the polysaccharide; then injected (100 µl) into the sub-plantar tissue of the right hind paw of Sprague-Dawley male albino rats (120-150 g) to induce edema. Meanwhile, the left hind paw was injected with 100µl saline.

The animals were randomized into 5 groups (n=6); one hour before carrageenan injection, they received (by oral administration, *p.o.*) appropriate doses of the vehicle, tested samples and standard drug, as follows:

Group 1 (negative control): received the vehicle (saline, 1 ml /Kg b. wt.).

Groups 2, 3 and 4: received the volatile oil samples (A_1-A_3 0.01 mL/kg b.wt., each).

Group 5 (positive control): received the standard anti-inflammatory drug, Indomethacin (20 mg/kg b. wt.)

The hind paw diameter was measured by means of Vernier caliper. Measurements were carried out immediately after carrageenan injection, and at selected time intervals (1 h, 2 h, 3 h and 4 h) after drug or samples administration. Experiments were carried out in triplicates. The percentage edema was recorded and percentage edema inhibition calculated as follows:

$$\% \text{ Edema inhibition} = (M_0 - M_t)/ M_0 \times 100,$$

where: M_0 = Mean paw diameter of control group at a given time;

M_t = Mean paw diameter of treated (extract or standard) group at the same time.

Evaluation of the antioxidant activity

Glutathione (GSH) is an important antioxidant in plants, animals, fungi, and some bacteria, preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides and heavy metals.[33] In healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the disulfide form (GSSG); and an increased GSSG-to-GSH ratio is indicative of oxidative stress.[34]

Various methods have been devised for the determination of glutathione in biological samples, including spectrophotometric- and high-performance liquid chromatography (HPLC)-based techniques. The most popular is the 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) spectrophotometric method.[35] Blood glutathione was determined in Alloxan-diabetic rats by adopting the Beutler *et al* (1963).[36] The restoration of GSH level reduced due to oxidative stress induced by diabetes was taken as a measure of antioxidant activity.

The principle of the assay depends on that protein and non-protein SH-groups react with Ellman's reagent [5, 5-dithiobis-(2-nitrobezoic acid)] to form the stable yellow colored 5-thio-nitrobenzoic acid, which can be measured at 412 nm.

Experimental design

Thirty-six adult male albino rats of the Sprague-Dawley strain (120-150 g) were used as experimental animals. *Diabetes mellitus* was induced by a single intra-peritoneal injection of Alloxan (150 mg/kg b. wt.) followed by an overnight fast.[37] Hyperglycemia was assessed, after 72 h of diabetes induction, by measuring the blood glucose levels according to Trinder(1969).[38]

The rats were divided into 6 groups, each of 6 animals (n=6).

The 1st group received 1ml saline and was kept as a negative control (normal, non-diabetic). Diabetes was induced to the other 6 groups as previously indicated.

Group 2: (Diabetic control) was kept untreated.

Group 3: (Diabetic + Vitamin E, positive control) received the reference drug, Vitamin E (7.5mg/ kg b. wt.).

Groups 4, 5 and 6: (Diabetic + Volatile oils) received A_1, A_2 and A_3 (0.01 ml/kg b. wt.), respectively. Experiments were carried out in triplicates.

Table 5: Results of evaluation of the acute anti-inflammatory activity of the volatile oil samples (A1-A3) of the aerial parts of A. monosperma Del., as compared to Indomethacin

Group	1h		2h		3h		4h	
	% Oedema[a] Mean ± S.E	% of Oedema Inhibition[b]	% Oedema[a] Mean ± S.E	% of Oedema Inhibition[b]	% Oedema[a] Mean ± S.E	% of Oedema Inhibition[b]	% Oedema[a] Mean ± S.E	% of Oedema Inhibition[b]
Control	4.41±0.1*	-	4.71±0.13*	-	4.81±0.12*	-	4.85±0.07*	-
A1	4.26±0.08*	21.36	4.19±0.10*	19.37	4.15±0.05*	18.23	3.90±0.07*	11.68
A2	4.37±09*	21.72	4.30±0.06*	19.77	4.24±0.07*	18.10	4.13±0.06*	15.04
A3	4.31±0.03*	27.45	4.26±0.02*	24.56	4.22±0.03*	24.27	4.18±0.01*	20.80
Indomethacin (20mg/kg)	4.16±0.09*	33.63	3.99±0.06*	35.07	3.92±0.01*	30.00	3.84±0.01*	28.54

A1, A2 and A3: volatile oils of aerial parts before, during and at the end of the flowering stage (0.01mL/kg); S.E. = standard error; *Statistically significant from the control normal inflamed group at the corresponding time: P< 0.05. Statistical analysis was carried out using repeated measures one way ANOVA followed by Least significant test for multiple comparison. a % Oedema =wt of right paw-wt.of left paw x 100/wt of left paw; b %oedema inhibition (% of change)=(Dc-Dt)x100/ Dc ; Dc is the mean oedema in control rats; Mt is the mean Oedema in drug-treated animals.

Table 6: Effect of volatile oil samples (A1-A3) of the aerial parts of *A. monosperma* on GHS blood level in Alloxan-induced diabetic rats, as compared to Vitamin E.

Group (n=6)	Dose / (Kg b.wt.)	Blood GHS (mg %) Mean ± SE	[a]% Change	[b]Relative potency %
Control	-	36.2±1.4	-	-
Diabetic	-	21.4±0.5*	-	-
Diabetic + Vitiamin E	7.5mg	35.8±1.3*	67.89	100
Diabetic + A1	0.01ml	35.1±1.3*	64.01	94.28
Diabetic +A2	0.01ml	34.3±1.2*	60.28	88.79
Diabetic +A3	0.01ml	31.2±1.1*	45.79	67.44

GHS: reduced glutathione; A1, A2 and A3: volatile oil of aerial parts before, during and at the end of the flowering stage; * Statistically significant for control group at P< 0.01; Statistical analysis was carried out using repeated measures one way ANOVA followed by Least significant test for multiple comparison. a % Change from control=(Mc-Mt)x100/ Mc ; Mc is the mean change in control rats; Mt is the mean change in drug-treated animals. b %Relative potency calculated as regard to standard drug.

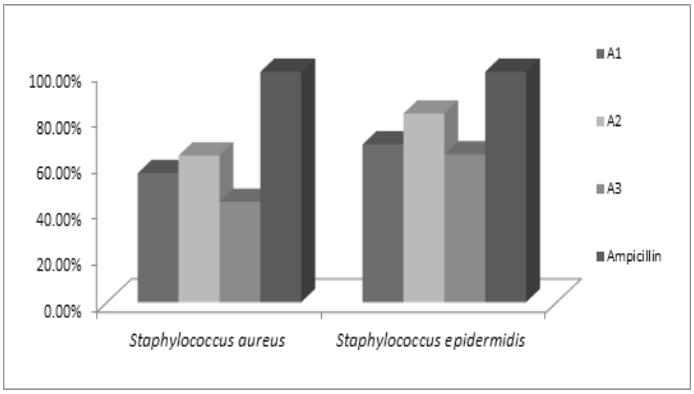

Figure 6: Histogram representing the antibacterial activity of essential oils (A1, A2 and A3) samples collected before flowering, and at the beginning and end of the flowering stage of *A. monosperma,* respectively; expressed as potencies as compared to standard Ampicillin.

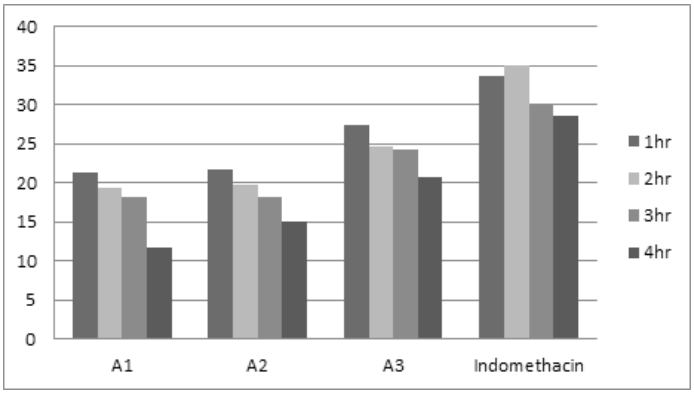

Figure 7: Histogram representing the antiinflamatory activity of essential oils (A1, A2 and A3) samples collected before flowering, and at the beginning and end of the flowering stage of *A. monosperma,* respectively; expressed as potencies as compared to standard indomethacin.

The blood GSH level was recorded, and percentage change from control computed as follows:

% Change = $(G\text{-}G_0) \times 100/G_0$; where:$G_0$ is the GHS level in diabetic animals prior sample administration and G that measured after.

RESULTS AND DISCUSSION

Yield and Composition of the Volatiles

The hydro-distilled volatiles of the aerial parts of *A. monosperma*, collected before flowering (A_1), and at the beginning (A_2) and end (A_3) flowering stage, were obtained as greenish yellow dextro-rotatory liquids with characteristic odour. Oil yields ranged from 0.16 to 0.26% v/w (on fresh weight basis), the highest being recorded for A_3 (Table 1).

Comparative GC profiling revealed a qualitative and quantitative variability among the samples (Tables 2; Figures 1-3). A total of 16-20 components were identified; 11, including 9 non-oxygenated, were common in all the samples. Identified constituents represented 97.63-99.00% of the total composition of the analyzed oils.

Samples were noticeably enriched in hydrocarbons; A_1 and A_2 showed appreciable and close amounts of these constituents (63.56 and 66.55%) although distinctly lower than in A_3 (88.36%). Monoterpenoids dominated the group, while sesquiterpenoids were missing and aromatics represented by *p*-cymene only. Sabinene (13.15-22.85%) and *β*-pinene (9.00-24.03%) were detected in appreciable amounts in all samples and *p*-cymene mainly in A_2 (11.20%).

Oxygenated components were detected in lesser amounts (11.29, 31.08 and 35.44 % in A_3, A_2 and A_1, respectively) being gradually decreased during flowering (highest % in A_1, before flowering). They include esters, alcohols, ketones and phenols. They are mostly terpenoids with few aromatics *viz*., eugenol methyl ether, methyl iso-eugenol, 7-methyl-1-naphthol and capillin. The bicylic monoterpenoid ester, bornyl acetate was the major identified oxygenated compound in all the samples (8.00-31.00%) with highest amount in A_1 decreasing to about the quarter by time (least in A_3). Whereas, the sesquiterpenoid alcohol, *β*-eudesmol was detected in appreciable amounts (8.01%) in A_2 only. The latter was, in addition, characterized by the presence of capillin (2,4-hexadiynophenone), a phenyl ketonic polyacetylene typically detected in *Artemisia* species.[39]

Moreover, the chemical profiles of the analyzed Libyan samples were different from those reported for their analogues obtained from plants growing in other localities of close climatic conditions. Among samples collected from Egypt various compounds were detected as major including: 3-methyl-3-phenyl-1,4-pentadiyne, capillene;[17] dibenzofuran, phenylbicyclo (3.3.1), non-2-en-9-ol benzoate,[40] 2-pinene-4-one and 3-phenylethyl propenoate.[41] Meanwhile, β-pinene and α-terpinolene prevailed among components of Saudi leaf and stem essential oils.[42]

Antimicrobial Activity

The antimicrobial activity of the different samples was evaluated against a set of microorganisms, including 4 fungal and 8 bacterial strains (Table 3 and 4) (Figures 4-6).The tested samples exhibited moderate to remarkable growth inhibitory potential against most of the tested strains as compared to the appropriate standard antibiotics. Yet, all were inactive against the filamentous fungus *Syncephalastrum racemosum*, the Gram-positive bacterium *Streptococcus pyogenes* and the Gram-negative *Proteus vulgaris*. Sample A_2 demonstrated the highest activity against all affected microorganisms with best efficiency as antifungal, especially on *Aspergillus fumigatus* (potency, 89.87% relative to Amphotericin B). In all cases A_3 was the least active, except on *Klebsiella pneumoniae* on which it revealed a slightly higher effect than A_2. The potentiality of the oil sample derived from aerial parts collected at the beginning of the flowering stage (sample A_2) may be attributed to its bornyl acetate content (31%).[43]

Median lethal dose LD_{50}

The 24 h LD_{50} was approximately more than 0.05 ml /kg b. wt., of the essential oils of the aerial parts. These results showed that the essential oils are safe and non-toxic.

Anti-inflammatory activity

Results presented in Table 5 and Figure 7 revealed that the A_3 volatile sample (hydro-distilled from the aerial parts of the plant collected at the end of the flowering stage) exerted the highest anti-inflammatory activity as compared to the other volatiles obtained from earlier gathered plant material. The sample exhibited a significantly high potency ranging from (70.03-81.62%) relative to the reference NSAID Indomethacin; and its efficiency appeared the highest after 1 h of treatment. This remarkable effect could be correlated to its relatively high monoterpenoid content *viz*., *β*-Pinene (24.03%), Sabinene (17.10%), *β-trans*-Ocimene (10.98%) and *β-cis*-Ocimene (12.92 %) with reported anti-inflammatory activity.[44]

Anti-oxidant activity

The results represented in Table 6 and Figure 8 revealed that the A_1 (volatiles hydro-distilled from plants collected before flowering) restored the reduced blood GHS level in diabetic animals almost as efficiently as Vitamin E (relative potencies 97.03 and 94.28 %, respectively) followed by A_2 and A_3 (88.79 and 67.44%). The antioxidant activity of the volatiles is thus decreased during the flowering stage, being the highest before flowering (A_1); this could be associated to the decreasing bornyl acetate content of the samples (31.00, 14.05 and 8.00% in A_1, A_2 and A_3, respectively). In fact, both borneol and bornyl acetate are considered as major contributors in the antioxidant activity of essential oils.[45,46]

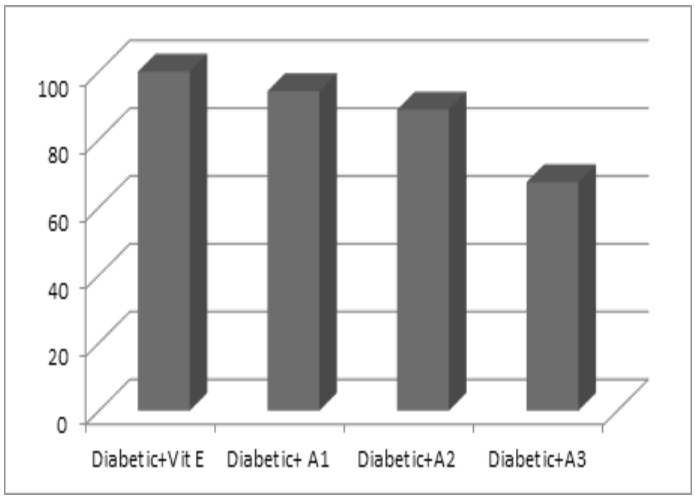

Figure 8: Histogram representing the antioxidant activity of essential oils (A1, A2 and A3) samples collected before flowering, and at the beginning and end of the flowering stage of *A. monosperma* respectively, as potencies as compared to standard vitamin E.

CONCLUSION

The genotype, ontogenic development and environmental growth conditions of any plant species are known to have a great impact on the qualitative and quantitative composition of its constituents, thus resulting in different chemotypes. In this respect, it was here evident that the stage of development influenced the yield and composition of the hydro-distilled volatiles of the aerial parts of *Artemisia monosperma* and consequently affected its antimicrobial, anti-inflammatory and antioxidant potencies. The latter could not be exclusively correlated to the efficiency of a

specific constituent but rather to a synergistic effect of all components. Meanwhile, the difference in composition observed in comparison with samples obtained from other localities may be referred to a number of extrinsic factors which affect growth conditions and consequently the production of secondary metabolites.

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CONFLICT OF INTEREST

The authors report no declarations of interest.

ABBREVIATION USED

A_1: Essential oil sample collected before flowering stage; **A_2:** Essential oil sample collected at beginning of flowering stage; **A_3:** Essential oil sample collected at end of flowering stage; **GC/MS:** Gas chromatography / mass spectrometry; **Rt:** retention times; **RI:** retention indices; **GC/FID:** Gas chromatography coupled with flame ionization detector; **DMSO:** Dimethylsulfoxide; **p.o.:** per oral; **b.wt:** body weight.

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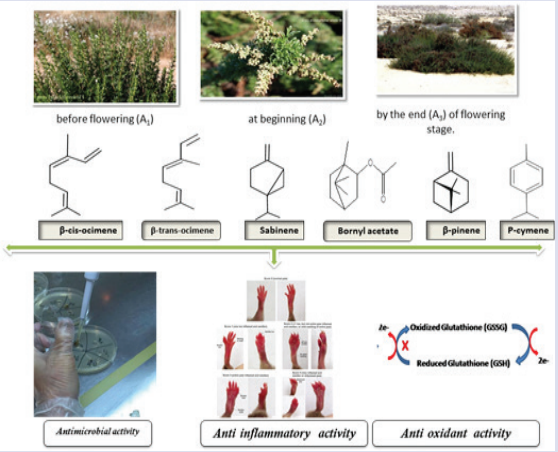
GRAPHICAL ABSTRACT



SUMMARY

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