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CHEMOTAXONAMY OF *AVICENNIA MARINA* (FORSK.) VIERH

A. M. Abdel-Twab[a], M. A. Gouda[a], M. I. Nassar[b] and M. Abdel-Mogib[a]
[a]Chemistry Department, Faculty of Sciences, Mansoura University
[b]Natural Compounds Chemistry Department, National Research Centre, 12622, Dokki, Cairo, Egypt.

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ABSTRACT

Chemical investigations include phytochemical screening and essential oil analysis by GC/MS as well as the bioassay screening of the antioxidant activity and bleomycin-dependent DNA damage of *Avicennia marina* compared with *Lippia nodiflora*, a Verbenaceae species, has supported that *Avicennia marina* belongs taxonomically to the family Avicenniaceae.

Keywords: *Avicennia marina, Lippia nodiflora*, Avicenniaceae, phytochemical screening, essential oils, antioxidant activity, Bleomycin-dependent DNA damage

INTRODUCTION

Avicennia marina is used as abortive in tropical Africa, aphrodisiac, for treatment of snakebite, and for sores **[Lewis & Elvin-Lewis (1977)]**. In addition, it possesses antifungal **[Marim *et al.*, (2006)]**, antibacterial and cancer chemopreventive activities **[Masataka *et al.*, (2001)]**. Also the plant extract has a significant increase in leucocytes in rates **[Ali & Bashir (1998)]**. *A. marina* contains different classes of natural products including steroids, triterpenoids **[Wannigama *et al.*, (1981)]**, iridoid **glycosides [Kőnig & Rimpler (1985)]**, naphthoquinones **[Li Han *et al.*, (2007)]**, flavonoids [M.Sharaf *et al.*, (2000)], shikimates and phenolic compounds **[Jia. Rui *et al.*, (2004)]**.

The genus *Avicennia* is considered as a member of the family Verbenaceae by some authors **[Briquette (1887); Mabberly (1987)]**, while others put it in the family Avicenniaceae **[Moldenke (1960); Tomlinson (1986)]**.

L. nodiflora, has been reported as vermifuge, anti-septic, antipyretic and anti-inflammatory agent. It has been used in the treatment of osteoarticular pains and bronchitis respiratory diseases **[Hooker (1885); Kartikar (1918); Chopra *et al.*, (1956)]**. Different natural products' classes were identified from *L. nodiflora* such as triterpenoids, steroids **[Farid, (1993)]**, iridoid glucosides **[Horstrimpler & Herbert.., (1986)]**, flavonoids **[Nair *et al.*, (1973)]** and volatile constituents **[Stellad (1985)]**.

In this article, the essential oils of the flowers, aerial parts, and fruits of *A. marina* as well as the essential oils of the flowers and aerial parts of *Lippia nodiflora*, a

Verbenaceae species, were extracted and investigated by GC/MS analysis. In addition, the antioxidant activity of the isolated essential oils of the two species was studied, and phytochemical screening of the two plant species was done for alkaloids, saponins, flavonoids, carbohydrates, triterpenoids and/or sterols and volatile oil, in order to support the taxonomic situation of *A. marina*.

EXPERIMENTAL

General

GC/MS analysis of the volatile fractions were performed on a Varian GC interfaced to Finnegan SSQ 7000 Mass Selective Detector (SMD) with ICIS V2.0 data system for MS identification of the GC components. The column used was DB-5 (J&W Scientific, Folosm, CA) cross-linked fused silica capillary column (30 m. long, 0.25 mm. internal diameter) coated with poloydimethyl-siloxane (0.5μm. film thickness). The oven temperature was programmed from 50°C for 3 min., at isothermal, then heating by 7°C/min. to 250°C and isothermally for 10 min., at 250°C. Injector temperature was 200°C and the volume injected was 0.5μl. Transition-line and ion source temperature were 250°C and 150°C, respectively. The mass spectrometer had a delay of 3 min. to avoid the solvent plead and then scanned from m/z 50 to m/z 300. Ionization energy was set at 70 eV.

DNA (calf thymus type 1), bleomycin sulfate, butylated hydroxyanisole (BHA), thiobarbituric acid (TBA), ethylene diamine tetraacetic acid (EDTA) and L-ascorbic acid were obtained from Sigma (USA). 2,2'-azo-bis-(2-amidinopropane) dihydrochloride (AAPH), 2,2'-azino-bis-3-ethylbenzthiazoline 6-sulfonic acid (ABTS) were purchased from Wako Co. (USA).

Ehrlich ascites carcinoma, (EAC) was derived from ascetic fluid from diseased mice (the cells were purchased from the National Cancer institute, Cairo, Egypt).

Plant materials

Avicennia marina was collected from the sub tidal water of Nabq gulf, Sharm Elsheik, Egypt in May 2009 by Prof. Mamdouh Abdel-Mogib. *Lippia nodiflora* was collected from Mansoura University Campus, Egypt in May 2009. The two plant species were identified by Prof. Ibrahim Mashaly, Botany Dept., Faculty of Science, Mansoura University.

Isolation of the essential oil

Samples of essential oils were isolated from fresh leaves, flowers and fruits by hydro-distillation, then extracted with CH_2Cl_2.

Preliminary phytochemical screening of *A. marina* and *L. nodiflora*

According to Elshamy (2009), the dried powdered samples of the aerial parts of *A. marina* and *L. nodiflora* were separately screened for phytochemical constituents(carbohydrates and/or glycoside, flavonoids, saponins, unsaturated sterols and/or terpenoid alcohols, tannins, alkaloids and/or nitrogenous bases and volatile oils).

Biological screening

Antioxidant activity screening *via* Superoxide Dismutase

Sodium pyrophosphate buffer (0.1M, PH 8.3): 4.46 gm of sodium pyrophosphate (Sigma, USA) were dissolved in 80 mL distilled water and PH was adjusted to 8.3 using 0.1M sodium hydroxide then the solution was completed to 100 mL with distilled water. Nitroblue tetrazolium (NBT, 0.3 mM): exactly 0.0042 gm of NBT (Sigma, USA) was dissolved in 5 mL distilled water. One volume of this solution was diluted with two volumes of distilled water prior to use. Phenazine methosulphate (PMS, 0.093 mM): a weight of 0.0032 gm of PMS (Sigma, USA) was dissolved in 10 mL distilled water. One volume of this solution was diluted with 9 volume of distilled water prior to use.

Superoxide dismutase (SOD) activity was assayed by the procedure of Niskikimi *et al.*, (1972). This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye.

The SOD reaction mixture was contained in a final volume of 3.5 mL . It was composed of 0.5 mL of 0.3 mM NBT, 0.5 mL of 0.47 mM NADH, 1.8 mL of 0.1M sodium pyrophosphate buffer, 0.1 mL of oil sample, 0.1 mL of 0.093 mM PMS and 0.5 mL absolute ethanol. The reaction was initiated by the addition of PMS and the increase in absorbance at 560 nm was followed with a recording spectrophotometer for 3.5 minutes.

Calculations:

Inhibition percent (I %) = (A blank - A sample / A blank) x 100

Where:

A blank = The change in absorbance at 560 nm over 3.5 minutes following the addition of PMS to the reaction mixture in the absence of enzyme or oils.

A sample = The change in absorbance at 560 nm over 3.5 minutes following the addition of PMS to the reaction mixture in the presence of enzyme or oils. The results are reported and tabulated as in Results and Discussion Section.

Antioxidant activity screening *via* ABTS method

For each of the investigated compounds, 2 mL of ABTS solution (60 mmol L^{-1}) was added to 3 mL MnO_2 solution (g = 25 mg mL^{-1}), all prepared in aqueous phosphate buffer (pH 7, 0.1 mol L^{-1}). The mixture was shaken, centrifuged, filtered and the absorbance of the resulting green-blue solution (ABTS radical solution) at 734 nm was adjusted to approx. 0.5. Then, 50 mL of 2 mmol L^{-1}) solution of the test compound in spectroscopic grade MeOH/phosphate buffer (1:1) was added.

The absorbance was measured and the reduction in color intensity was expressed as inhibition percentage. L-ascorbic acid was used as standard antioxidant (positive control). Blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of a sample. Negative control was run with ABTS and MeOH/phosphate buffer (1:1) instead of test essential oils.

Bleomycin-dependent DNA damage

The assay was done according to a method originated by Aeschlach *et al.*, (1994) and modified by Abu-Hashem *et al.*, (2010). The reaction mixture (0.5 mL) contained calf thymus DNA (0.5 mg mL^{-1}), bleomycin sulfate (0.05 mg mL^{-1}), $MgCl_2$ (5 mmol

L^{-1}), $FeCl_3$ (50 mmol L^{-1}) and the sample to be tested (50 mL of 2 mmol L^{-1} solution). L-ascorbic acid was used as a positive control. The mixture was incubated at 37°C for 1 h. The reaction was terminated by addition 0.05 mL EDTA (0.1 mol L^{-1}). The color was developed by adding 0.5 mL TBA (1 %, m/V) and 0.5 mL HCl (25 %, V/V) followed by heating at 37°C for 15 min. After centrifugation, the extent of DNA damage was measured by the increase in absorbance at 532 nm.

Damage to DNA in the presence of a bleomycin–Fe complex has been adopted as a sensitive and specific method to examine potential pro-oxidant agents. If the samples to be tested are able to reduce the bleomycin–Fe^{3+} to bleomycin–Fe^{2+}, DNA degradation in this system will be stimulated, resulting in a positive test for pro-oxidant activity. DNA degradation is accompanied by the formation of a product similar to malondialdehyde (MDA). L-ascorbic acid as a reducing agent can reduce Fe to Fe^{2+}.

RESULTS AND DISCUSSION

The phytochemical screening results of aerial parts of *A. marina* and *L. nodiflora* were illustrated in table 1.

Table 1: Phytochemical screening of aerial parts of *A. marina* and *L. nodiflora*

Chemical constituents	Aerial part of *A. marina*	Aerial part of *L. nodiflora*
1. carbohydrates and/or glycoside	+++	++
2. flavonoids	+++	+++
3. saponins	+	-
4. unsaturated sterols and/or terpenoid alcohols	+++	++
5. tannins	++	+++
6. alkaloids and/or nitrogenous bases	+	-
7. volatile oils	++	+

(- : absence, + : less presence , ++: moderate presence , +++ : high presence)

Table 1 indicated that *A. marina* and *L. nodiflora* may differ in the content of volatile oils, alkaloids and/or nitrogenous bases and saponins.

Investigation results of the essential oils composition of both plant species by the GC/MS technique were reported in table 2.

Table 2: Major constituents of volatile fractions of *A. marina* and *L. nodiflora* parts.

Compound name[a]	R_t (min)	*A. marina*			*L. nodiflora*	
		A[b]	Fl	Fr	A	Fl
tetrahydro-2-methyl-furan	3.06	0.73		0.27		
3-penten-2-ol	3.18				6.73	
3-hydroxy-2-butanone	3.55	0.54				
methylglyoxal	3.60		0.09			
toluene	4.14				0.97	
2,2-dimethyl-3-propyl-oxirane	4.14	1.23				
propylene glycol	4.19			5.31		
(2-methylpropyl)-cyclopentane	4.20		2.46			
3,5,5-trimethyl-1-Hexene	4.73				1.95	
2-methyloct-2-ene	4.77		5.81	5.20		
(R)-2-hexanol	5.06	1.73				
(E)-7-methyloct-3-ene	5.06		18.78	18.95		
3-nonene	5.21					7.15
2-methylheptan-4-ol	5.24				5.30	
2,5,5-trimethyl-1-hexene	5.54					19.22
furfural	5.78	1.09	1.99			
2,3-dimethyloct-2-ene	6.33			2.14		
cis-2,3-epoxyoctane	6.39	1.75				
4-decene	6.48					0.23
ethylbenzene	6.69		2.76		1.69	0.15
2-(1-methylpropyl)-cyclopentanone	6.87			0.34		
dimethylbenzene	6.93					2.75
1-decene	7.30	2.62				
3-methoxy-3,7-dimethylocta-1,6-diene	7.36		13.26	12.26		
2-methyl-non-2-ene	7.59					0.84
2,3-dimethyloct-2-ene	8.10			24.91		
3,7-dimethyl- (Z)-2-octene	8.18		29.11			
3-methyl-3-buten-2-ol	8.20				3.22	
(Z)-hex-3-enyl acetate	8.50				0.26	
6,7-dimethyl-oct-3-ene	8.88					30.09
benzaldehyde	9.17	0.40				
2-ethyl-2-hexen-1-ol	9.23				0.08	
1-octen-3-ol	9.47		2.87			
(E)-2-nonen-1-ol	9.51			2.33		
3-octanol	9.97	1.35				
(E)-2,7-dimethylocta-2,6-dien-1-ol	10.02		1.80			
(E)-3-undecene	10.15			2.33		
(Z)-oct-3-enyl acetate	10.21				0.32	

Compound name[a]	R_t (min)	*A. marina*			*L. nodiflora*	
		A[b]	Fl	Fr	A	Fl
1-octen-3-ol	10.31					2.09
(E)-undec-4-ene	10.36		0.66			
citronellol epoxide	10.48				0.15	
2,6-dimethyl-1,7-octadien-3-ol	10.68		0.49			
p-menth-8-en-2-ol	10.74				0.80	
2-methyl-Decane	11.05	0.51				
7-ethyl-3-nonene	11.22					2.69
limonene	11.53	1.45		1.18		2.68
(E)-2,6-dimethylocta-1,3,7-triene	11.54		5.68			
4-ethyl-2-methyl-thiazole	11.66	8.12				
methyl dec-2-ynoate	11.70				1.06	
benzyl alcohol	11.95				0.93	
5-ethyldihydro-2(3H)- furanone	12.05	1.18				
(E)-dec-3-en-1-ol	12.18			1.18		
2,6,6-trimethyl-bicyclo[3.1.1]heptan-3-ol	12.36	4.41				
phenylethyl Alcohol	13.77		1.40			
2-decyne	14.29	0.53				
2,6,6-trimethyl-2-cyclohexene-1,4-dione	14.65	0.75				
(Z)-2-undecene	15.32	0.08				
cis-p-menthan-3-one	15.65					1.17
1-(1,1-dimethylethyl)-4-methyl-cyclohexane	16.41			0.12		
estragole	16.65			0.46		
4,6,8-trimethyl-1-nonene	16. 94			0.29		
tetradec-7-ene	17.06					0.35
tridecane	17.15			0.82		
5-(2-propenyl)-1,3-benzodioxole	17.31		0.31			
edulan	17.48	2.49				
p-mentha-6,8-dien-2-one	17.61					0.34
10-undecyn-1-ol	18.51	5.21				
α-damascenone	18.56			0.32		
3-methyl-2-(2-pentenyl)- (Z)-2-cyclopenten-1-one	18.89		0.15			
cadinol	19.24					0.31
vanillin	19.27	4.23				
1-dodecene	19.48	3.74				
3-phenyl- ethyl ester, (E)-2-propenoic acid	19.86		0.39			
4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl)-3-	20.00			0.39		

Compound name[a]	R_t (min)	*A. marina*			*L. nodiflora*	
		A[b]	Fl	Fr	A	Fl
Buten-2-one						
caryophyllene	20.23					0.2
5,6,7,7a-tetrahydro-4,4,7a-trimethyl- (R)-2(4H)-benzofuranone	20.80	0.82	0.11			
n-cetane	21.00		0.24			
cis-Z-α-bisabolene epoxide	21.15	2.69				
2,6,10-trimethyl-dodecane	21.93			0.49		
5-methyl-1-[2,6,6-trimethyl-2,4-cyclohexadien-1-yl]-1,4-hexadien-3-one	22.34			0.26		
1,3-diphenyl-2-propanone	22.91		0.24			
3,7,11-trimethyl- (Z) -1,6,10-dodecatrien-3-ol	21.95	1.24				
octadecane	22.75	0.98				
hexadecanoic acid butyl ester	25.48	1.14				

[a] Compounds identification is based on NIST library; [b] area percentage of components (%); A= essential oil of aerial part; Fl= essential oil of flowers; Fr= essential oil of fruits.

The GC/MS results obtained from the volatile fraction of *L. nodiflora* aerial parts indicated the presence of five constituents, in major amounts, which were absent in the *A. marina* aerial parts volatile fraction. The constituents were 3-penten-2-ol, 3,5,5-trimethyl-1-hexene, 2-methylheptan-4-ol, ethylbenzene and 3-methyl-3-buten-2-ol. On the other hand, (R)-2-hexanol, furfural, cis-2,3-epoxyoctane, 1-decene, limonene, 2,6,6-trimethyl-bicyclo[3.1.1]heptan-3-ol, 10-undecyn-1-ol, 10-undecyn-1-ol, vanillin, 1-dodecene, cis-Z-α-bisabolene epoxide and 3,7,11-trimethyl- (Z) -1,6,10-dodecatrien-3-ol, were presented in major amounts in the volatile fraction of *A. marina* aerial parts and absent in the volatile fraction of *L. nodiflora* aerial parts.

Also, the results of volatile fraction of *L. nodiflora* flowers revealed the presence of eight major constituents; 3-nonene, 2,5,5-trimethyl-1-hexene, dimethylbenzene, 6,7-dimethyl-oct-3-ene, 1-octen-3-ol, 7-ethyl-3-nonene, limonene and cis-p-menthan-3-one, which were absent in the *A. marina* flowers part volatile fraction. While (2-methylpropyl)-cyclopentane, 2-methyloct-2-ene, (E)-7-methyloct-3-ene, ethylbenzene, 3,7-dimethyl- (Z)-2-octene, 1-octen-3-ol, (E)-2,7-dimethylocta-2,6-dien-1-ol, 3-methoxy-3,7-dimethylocta-1,6-diene, 3-octanol and (E)-2,6-dimethylocta-1,3,7-triene were the major volatile fraction constituents of the flowers of *A. marina* and were absent in the volatile fraction of *L. nodiflora* flowers.

The overall antioxidant activity was evaluated by the SOD antioxidant activity method described by Nishikimi *et al.* A moderate to high antioxidant effect was exhibited by the plant essential oils compared to L-ascorbic acid as a positive control. Interestingly, flowers of *L. nodiflo*ra showed high antioxidant activity while flowers of *A. marina* showed a moderate antioxidant activity compared with L-ascobic acid. On the other hand, *A. marina* (aerial parts) showed high antioxidant activity more than *L.*

*nodiflo*ra (aerial parts) compared with L-ascobic acid by the SOD antioxidant activity method.

Table 3: The antioxidant activity of plant essential oil parts by mean of SOD method

	Plant essential oil (part)	Antioxidant activity (%)
1	Ascorbic acid	39.3
2	*Avicennia marina* (Aerial parts)	68.6
3	*Avicennia marina* (Flowers)	32.8
4	*Avicennia marina* (Fruits)	21.4
5	*Lippia nodiflora* (Flowers)	68.6
6	*Lippia nodiflora* (Aerial parts)	57.8

The antioxidant activity of the two different plant essential oils of different parts was evaluated by the ABTS antioxidant activity method described by Lissi *et al.* All the investigate plant essential oils manifested a weak antioxidant activity compared to the L-ascorbic acid and almost all the antioxidant activity values were in the same range.

Table 4: The antioxidant activity of plant essential oil parts by mean of ABTS method

	Plant essential oil (part)	Absorbance of samples	% inhibition
1	Control of ABTS	0.486	0 %
2	Ascorbic acid	0.091	81.27%
3	*Avicennia marina* (Aerial parts)	0.373	23.25%
4	*Avicennia marina* (Flowers)	0.373	23.25%
5	*Avicennia marina* (Fruits)	0.386	20.57%
6	*Lippia nodiflora* (Flowers)	0.382	21.39 %
7	*Lippia nodiflo*ra (Aerial parts)	0.410	15.63%

The two different plant essential oils of different parts were tested for bleomycin-dependent DNA damage (Table 5). Damage to DNA in the presence of a bleomycin–Fe complex has been adopted as a sensitive and specific method to examine potential pro-oxidant agents Gutteridge *et al.*, (1981). If the samples to be tested are able to reduce the bleomycin–Fe^{3+} to bleomycin–Fe^{2+}, DNA degradation in this system will be stimulated, resulting in a positive test for pro-oxidant activity. DNA degradation is accompanied by the formation of a product similar to malondialdehyde (MDA). L-Ascorbic acid as a reducing agent can reduce Fe to Fe^{2+}.

Table 5 shows that *A. marina* (aerial parts) have an ability to protect DNA from the induced damage by bleomycin more than *L. nodiflo*ra (aerial parts) while *L. nodiflora* (flowers) showed high activity more than *A. marina* (flowers) and *A. marina* (fruits) compared with the control (L-ascorbic acid).

Table (5) : The effect of plant essential oil parts on DNA

	Plant essential oil (part)	Absorbance of samples
1	Ascorbic acid	0.098
2	*Avicennia marina* (Aerial parts)	0.108
3	*Avicennia marina* (Flowers)	0.131
4	*Avicennia marina* (Fruits)	0.150
5	*Lippia nodiflora* (Flowers)	0.119
6	*Lippia nodiflora* (Aerial parts)	0.128

GC/MS analysis of both essential oils parts as well as the antioxidant activity assessments, in addition to phytochemical screening, indicated that the essential oils of the two plant species were different in the chemical constituents, which supports the idea that *A. marina* belongs to the family Avicenniaceae.

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الملخص العربي

التصنيف الكيمائيي لنبات الافيسينيا مارينا

اسماء عبدالتواب، مصطفي جوده، ممدوح عبدالمجيب

قسم الكيمياء، كليه العلوم، جامعه المنصوره، مصر

لدراسه الوضع التصنيفي لنبات الافيسينيا مارينا علي انه تابع لعائله الافسينيسي وهذا من خلال مقارنه المكونات الكيميائيه للزيوت الطياره له وتلك لنبات ليبيا نوديفلورا وهو نوع من عائله الفربينسي.

نتج عن الفحص الكيميائي مشتملا التقييم الفيتو كيميائي وتحليل الزيوت الطياره من خلال تقنيه كروماتوجرافيا الغاز المتصله بمطياف الكتله بالاضافه للفحص البيولوجي لتلك الزيوت كمضادات للاكسده ان الافيسينا مارينا يتبع عائله الافيسينيسي وليس الفربينسي.