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COMPOSITION, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF THE ESSENTIAL OILS FROM *MELALEUCA ERICIFOLIA* AND *ACOKANTHERA SPECTABILIS*

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ABSTRACT

GC-MS investigation of the essential oils isolated from the leaves of *Melaleuca ericifolia* Smith (Myrtaceae) and flowers of *Acokanthera spectabilis* Hook F. (Apocynaceae) revealed that *M. ericifolia* leaves contain more than 28 major compounds four of them were investigated for the first time. Concerning *A. spectabilis*, it biosynthesized more than 32 compounds. It is the first time to investigate its essential oil. *In vitro*, assays using a variety of essential oils isolated from *M. ericifolia* and *A. spectabilis* revealed a particularly antibacterial effect. *M. ericifolia*, which was used as a component of a diverse range of pharmaceutical and cosmotic products is a potent antimicrobial agent affecting the viability of a broad spectrum of bacteria and yeast such as *Escherichia coli*, *Staphylococcus aureus*, *Asperagillus niger* and *Candida albicans*. The antibacterial effect of the essential oil of *A. spectabilis* Hook F. revealed less activity than *M. ericifolia* Smith as anti-candida. Concerning the antioxidant activity, the assay revealed that both of the *M. ericifolia* leaves and *A. spectabilis* flowers showed significant antioxidant effect.

Keywords: *Melaleuca ericifolia*, *Acokanthera spectabilis*, essential oils, antimicrobial, antioxidant.

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INTRODUCTION

Melaleuca ericifolia Smith (Myrtaceae) is an evergreen tree and from its leaves the volatile oil, which has antimicrobial and preservative properties, are widely applied for several pharmaceutical purposes. Also *Acokanthera spectabilis* Hook F. *(Apocynaceae)* is also evergreen and aromatic trees. This plant is attractive to bees, butterflies and/or birds and its flowers are fragrant.

Volatile oil of genus *Melaleuca (Myrtaceae)* is very bioactive oil hereby it was investigated by many authors (Abdel Bar *et al.*, 2008; Yoshimura *et al.*, 2008; Yoshida *et al.*, 2008; Hussein *et al*, 2007 and Youssef, 2003). Previous studies have demonstrated that, leaves and branches of the tea tree *(Melaleuca alternifolia)* are the most important organs which contain the bioactive components of the plant. Tea tree oil is widely available from pharmacies and health shops.

It was reported that the tea tree (*Melaleuca alternifolia)* oil has antifungal activity against *Trichophyton equinum* (Pisseri *et al.*, 2009), is active as anti-*Legionella pneumophila* (Mondello *et al.*, 2009), exerted a wide spectrum of antimycotic activity (Terzi *et al.*, 2007), has bactericidal activity against *Staphylococcus aureus* (Kwieciński *et al.*, 2009). In addition, the oil reported antiviral effect (Schnitzler *et al.*, 2001).

Hammer *et al.* (2004) and Mondello *et al.* (2006) investigated the anti-candida activity of the essential oil of *M. alternifolia.* The later found that the terpinen-4-ol of oil could control *C. albicans* vaginal infections. Millar and Moore (2008) have investigated the potential use of *M. alternifolia* oil in the treatment of common warts due to human papilloma virus. Also, it was reported the effective of tea tree oil against both lice and house dust mites (Williamson *et al.*, 2007).

Concerning *A. spectabilis,* it was used in southern Africa for tipping deadly arrows (bushman's poison). It contains the most powerful heart-stimulating cardiac glycosides or cardenolides (Karawya *et al.*, 1974 and Shanta *et al.*, 2008).

It is worthy to be note that the investigation of the essential oil of *A. spectabilis* has not been reported so far. So, in current study the composition of volatile oils produced in leaves of *M. ericifolia* and the flowers of *A. spectabilis* and their antimicrobial and antioxidant activities were investigated.

MATERIALS AND METHODS

Plant Material

Leaves of *M. ericifolia* and the flowers of *A. spectabilis* samples were collected during flowering stage from Experimental Farm, Faculty of Agriculture, Zagazig Univ., Egypt.

Preparation and Analysis of the Oil

The oil of *M. ericifolia* was prepared by hydrodistillation of the fresh leaves (600g) for 8 hours, while the oil of *A. spectabilis* was prepared by extracting the fresh flowers (400g) with hexane. The oils were dried separately over anhydrous sodium sulfate to give 0.045 and 0.02 ml/g *V/W*, respectively and kept at 4 °C in sealed vials for analysis.

Gas Chromatography-Mass Spectrometry

The GC/MS analysis was carried out on Gas Chromatography Mass Spectrometer (GC-17A Shimadzu, Auto Injector- AOC-201, Shimadzu, Japan), using capillary column of fused silica (30m x 0.25mm i.d.) coated with 5% phenyl methyl polysiloxane (DB-5). Helium was used as carrier gas at flow rate 25 ml/min. Oven temperature was programmed at 60–200 °C at 10 °C/min. split ratio: 1: 50. Injector temperature: 240 °C, detector temperature: 300 °C. The MS detection routine was from 9-60 min. Identification of the constituents was performed by aid of the computer library search (CAS No. 5989-27-5, Entry 8747, LIB #1).

Antimicrobial Screening

The antimicrobial screening (Clark *et al.*, 1981) and the determination of MIC (Harkenthal *et al.*, 1999) of the oils were performed using the following microorganisms: *Escherishia coli, Staphylococcus aureus* and *Candida albicans,* all of them are clinical isolates strains obtained from stock cultures of the Department of Microbiology, Faculty of Pharmacy, King of Saud University.

Antioxidant Activity

The electron donation ability of the obtained methanol extracts was measured by bleaching of the purple colored solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato *et al.* (1988). Oil extracts at different concentrations (0.1, 0.5, 1.0 and 2.0ml) from 10 mg /10 ml were added to 4ml of 0.1mM DPPH methanolic solution. The absorbance was determined against a blank at 517nm.

Percentage inhibition of free radical DPPH (PI%) was calculated as follow: PI% = [(A blank − A sample)/A blank] × 100. Where

A blank: is the absorbance of the control reaction and

A sample: is the absorbance in the presence of plant extract.

RESULTS AND DISCUSSION

Volatile Oil Content of *M. ericifolia* Leaves

GC/MS analysis resulted in the identification of twenty-eight compounds (Table 1). Among of these, anisyl acetone: 67.4 %, di-isooctylphythalate: 10.4 %, varatral-dehyde; 5.73%, benzothiazole: 2.74%, 3, 4-dimethoxy cinnamal-dehyde: 2.66%, and cis-isoelemicin: 1.96% are being the main components.

While the main minor ones were represented by cinnamic acid methyl ester: 0.62%, linalool: 0.53%, caryophyllene: 0.4%, trans-methyl isoeugenol: 0.32%, anisole: 0.21%, eugenol: 0.17%, spathulenol: 0.17%, aromadendrene: 0.17%, and α-terpineol: 0.16%.

Volatile Oil of *A. spectabilis* Flowers

GC/MS analysis resulted in identification of thirty-three compounds (Table 2).

The major components of oil were represented by Di-isooctyl-phthalate: 27.64 %, benzylacohol: 18.18 %, Benzoic acid: 3.5 %, epoxy linalol: 2.7 %, methyl salicylate: 2.5%, benzothiazole: 1.92%, cinnamic acid: 1.86 %, geranyl linalool: 1.78 %, and linalool; 1.27 %.

While the minor compounds (less than 1%) were represented by myrtenyl acetate: 0.79 %, neryl acetone 0.77 %, geranyl phenyl acetate: 0.6 %, linalool oxide: 0.5%, and 8-hydroxy linalool: 0.48%.

Antimicrobial Activity

Generally, essential oils are a complex mixture of different chemical structures natural products, so that the antimicrobial responsibility cannot attribute to certain compound of this mixture. It could say that the activity was due to the major compounds but may be the minor compounds have a synergistic effect to the mixture.

Data in Table 3 reveal that both oils showed antimicrobial activity against gram-positive (*Staphylococcus aureus)* and gram-negative (*Escherishia coli)* bacteria. Obviously, *M. ericifolia* oil provided greater activity against *S. aureus* and *E. coli* than oil of *A. spectabilis*, with MIC

Table 1. Identified constituents in the volatile oil of *M. ericifolia* by GC/MS

P. No	Compound name	RT	M^+	Base peak	Major peaks	Relative %
1	Octanediol	4.6	146	67	125,109,95,82,67,41,31	0.11
2	Cis-1-Formylbicyclo (3.3.0) oct-3-ene	5.7	136	79	136,122,94,79,63	0.13
3	Linalool	8.6	154	71	154,136,121,105,93,80,71,55,43	0.53
4	Alpha-terpineol	10.2	154	93	154,136,121,107,93,81,67,59,43	0.16
5	Anisole	10.3	148	148	148,133,121,117,105, 91, 77,65	0.21
6	Citronellol	10.7	156	69	156,138,123,109,95,81,69,55,41	0.06
7	Benzothiazole	10.8	135	135	135,108,91,82,69	2.74
8	2,6,11,15 tetramethyl-Hexadecane	11.6	282	71	282,197,183,169,155, 35,127,110,96,85,71, 57,43,41,27	0.09
9	Cinnamic acid methylester	12	162	131	162,144,131,117,103, 91, 77,63,51,39	0.62
10	2,6,-octadienoic acid,3,7-dimethyl	12.2	182	69	182,167,151,125,114, 107,83,69,67,41	0.26
11	Eugenol	12.7	164	164	164,149,137,131,107, 103,91,77,55	0.17
12	Copaene	13	204	119	204,189,147,133,119, 55, 41,39	0.08
13	Anisyl acetone	13.6	178	178	178,163,147,135,115, 107,91,77,65,51	67.4
14	Caryophyllene	13.8	204	93	204,189,175,161,148, 133,69,55,41,39	0.4
15	Aromadendrene	14.3	204	91	204,189,161,147,133, 119,105,91,79,69,55	0.17
16	Varatraldehyde	14.5	166	166	166,151,137,119,105, 95, 77,65,51,28	5.73
17	Trans-methyl isoeugenol	14.6	178	178	178,163,147,135,115, 107,91,77,65,39	0.32
18	3,4-dimethoxy benzene propanenitrile	14.8	191	191	191,180,163,151,135, 121,107,91,75,65	0.87
19	Cis-isoelemicin	15.4	208	208	208,177,165,150,133,118,105,91,77,65,53, 39	1.96
20	Spathulenol	15.9	220	91	220,205,187,177,159,147,131,119,105,91, 69	0.17
21	Caryophyllene oxide	16	220	79	220,204,189,177,163,147,135,189,177,13, 147,135,121,109,93,79,69	0.14
22	4,7- dimethoxy Indan-1-one	16.3	192	192	192,177,163,149,134, 121,106,91,77,63	0.73
23	3,4-Dimethoxy cinnamaldehyde	18.2	192	192	192,177,161,151,138, 121,103,91,77,65	2.66
24	Palmitic acid	20	256	73	256,227,213,199,185,171,157,143,129, 115, 97,83,73,60	0.89
25	Linoleic acid	21.8	280	69	280,264,235,221,207,193,180,166,151, 137, 123,110,97,83,69	0.99
26	Octadecanoic acid	22	284	73	284,255,241,227,213,199,185,171157,143, 129,111,97,73,60,43	0.76
27	Di-isooctylphythalate	25.5	390	149	390,279,167,149,113, 71	10.4
28	Octadecenoic acid-2-hydroxy-1-(hydroxymethyl) ethyl ester	26.3	356	67	356,264,151,137,123, 98, 83,69,55,41,29	1.2

Table 2. Identified constituents in the volatile oil of *A. spectabilis* flowers by GC/MS

P. No	Compound name	RT	M^+	Base peak	Major peaks	Relative %
1	Trimethylsilylmethanol	4	104	73	104,89,73,45,29,15	10.11
2	Benzalaldhyede	6.4	106	106	106,105,77,51	0.15
3	Pentachloroethane	6.6	200	116	200,167,130,117,95,83,60,47, 35,25	0.18
4	Benzylacohol	7.6	108	79	108,91,79,65,51,39	18.18
5	Hexachloroethane	8.3	234	116	234,201,199,166,131, 117, 94, 82,47,32,28	1.33
6	Lilac alcohol	8.4	170	94	170,155,137,119,111, 94, 83, 68	1.3
7	Linalool	8.6	154	71	154,136,121,107,93,80,71,55, 43	1.27
8	Epoxy linalol	9.9	170	68	155,137,122,105,94,77,68	2.7
9	Benzoic acid	10.1	122	105	122,105,94,77,57,51,28	3.5
10	Methyl salicylate	10.3	152	120	152,120,105,92,65,53, 39	2.5
11	Benzothiazle	10.8	135	134	135,108,91,82,69,63,45,38	1.92
12	Myrtenyl acetate	11.4	194	91	194,178,165,134,91,82,73	0.79
13	8-hydroxy linalool	12.7	170	71	170,137,127,111,95,85,71	0.48
14	Linalool oxide	13.1	170	68	170,155,137,127,111, 94,81,68	0.5
15	Cinnamic acid	13.8	148	147	148,147,131,119,103, 91,77, 63, 51,27	1.86
16	Neryl acetone	14.1	194	69	194,176,151,136,107, 93, 69, 43	0.77
17	2,4-bis(1,1-dimethyl-ethyl) phenol	14.8	206	94	206,94,91,175,163,88, 74, 57, 41,29	2.3
18	Nerolidol	15.4	222	96	222,189,161,148,136,123, 107, 93,81,69	0.28
19	Tetradecanoic acid	17.8	228	73	228,199,185,171,157,143, 129, 115, 97,85,73	0.38
20	1,2-diphenyl cyclobutane	18	208	104	208,104,78,56,43,28	0.35
21	Benzyl bezoate	18.1	212	105	212,194,167,105,91,77,51	0.65
22	13a,3a,- (epoxyethano)-1H-indolizion (8,1-cd) carbazol-7- ol, 6-acetyl-2	18.7	414	69	414,202,160,138,125, 11, 97, 83,69	0.68
23	Benzyl salicylate	19	228	91	228,91,65,39	3.37
24	Palmitic acid	20	256	172	256,213,185,171,157, 129, 115, 97, 83,73,60	2.53

Table 2. Cont.

P. No	Compound name	RT	M^+	Base peak	Major peaks	Relative %
25	Geranyl linalool	20.8	290	69	290,203,189,161,136, 109, 95,81,161,136,109,95,81,69,55,41	1.78
26	Geranyl phenyl acetate	21.2	272	91	272,144,137,123,111,91,81, 69	0.6
27	Linoleic acid	21.8	280	67	280,264,222,207,193,180,165 , 151,137,123, 110 ,97,81,67	1.14
28	5-hydroctadecanoic acid	23.9	282	99	282,264,220,151,114, 99, 83, 70,55,43,29	0.14
29	Behenic acid ME	25.2	354	74	354,311,255,199,143, 87, 74, 55	1.33
30	Diisooctylphthalate	25.5	390	149	390,179,167,149,71,57	27.64
31	1-propene,3-(2-cyclo pentenyl)-2-methyl-1,1-diphenyl	25.9	274	91	274,207,191,178,165, 129, 91, 67, 39	1.91
32	Trans-(2,3- Diphenyl cyclopropyl) methyl phenyl sulfoxide	26	232	91	332,207,129,91,77,69	3.27
33	Lupeol	35	426	95	426,218,207,189,175, 161, 147, 135,121,109, 95, 81,68, 43	0.95

Table 3. The antimicrobial effect of the essential oils isolated from *M. ericifolia* and *A. spectabilis*

Microorganism	Inhibition Zone (mm)		
	Staphylococcus aureus	*Escherichia coli*	*Candida albicans*
Ampicillin	27	18	-
Gentamicin	15	22	-
Amphotericin B	-	-	20
M. ericifolia volatile oil	12	11	17
A. spectabilis volatile oil	9	7	2

Values 0.52, 0.66 and 1.44, 2.3, respectively (Table 4).

Concerning the antifungal activities, oil of *M. ericifolia* has great efficient against *Candida albicans*. On the other hand, there was no antifungal activity attained with oil of *A. spectabilis* (Table 3).

Antioxidant Activity

It is of great interest to investigate the antioxidant activity of the volatile oils in order to evaluate their potential medicinal importance.

As shown in (Fig. 1) the anti-oxidant activity of volatile oils of *M. ericifolia* leaves and *A. spectabilis* flowers were assayed by using DPPH radical. The results revealed that increasing the concentrations of the extracts increased the antioxidant effect in a dose dependant manner. It is clear that the antioxidant effect of *M. ericifolia* leaves oil was more than *A. spectabilis* flowers oil.

To the best of our knowledge, no data have been published on antioxidant activity, using the DPPH method, on *M. ericifolia* leaves oil or *A. spectabilis* flowers oil.

Table 4. Minimum inhibitory concentration (MIC) of the essential oils isolated from *M. ericifolia a* and *A. spectabilis* by serial dilution method

Volatile oil	MIC		
	Staphylococcus aureus	*Escherichia coli*	*Candida albicans*
M. ericifolia	0.52	0.66	2.81
A. spectabilis	1.44	2.3	-

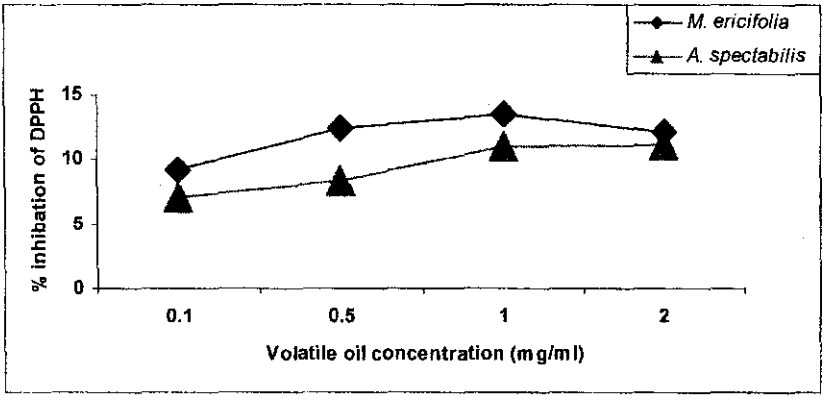

Fig.1. Antioxidant activity of volatile oil of *Acokanthera spectabilis* flowers and *Melaleuca ericifolia* leaves

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التركيب والتأثير المضاد للميكروبات والمضاد للاكسدة للزيوت الطيارة المستخلصة من نباتي الميلالوكا والأكوكانثيرا

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هذا العمل يهدف إلى فصل الزيت الطيار من أوراق نبات الميلالوكا وأزهار نبات الأكوكانثيرا لتحديد مكوناتة الكيميائية باستخدام كروماتوجرافيا الغاز المتصل بمطياف الكتلة، وكذلك لدراسة التأثيرالمضاد للميكروبات و التأثير المضاد للاكسدة للزيت المنفصل. و قد أشارت النتائج أنّ الزيت الطيار لأوراق نبات الميلالوكا يحتوى على حوالى ٢٨ مركباً وبالنسبة للزيت الطيار لأزهار نبات الأكوكانثيرا فإنة يحتوى على حوالى ٣٢ مركباً. وعند دراسة التأثيرالمضاد للميكروبات لهذه الزيوت وجد أن لها تأثيراً فعالا واسع المجال ضد حيوية الميكروبات الموجبه و السالبة ، و علاوة على ذلك فقد لوحظ أن الزيت الطيار لأوراق نبات الميلالوكا كان له تأثير فعال ضد فطر الكانديدا أكثر من زيت أزهار نبات الأكوكانثيرا. أما فيما يتعلق بالتأثير المضاد للأكسدة فان التحليلات أوضحت أن الزيت الطيار لأوراق نبات الميلالوكا و كذا الزيت الطيار لأزهار الاكوكانثيرا قد أظهرا تأثيراً معنوياً مضاداً للأكسدة.