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PHYTOCHEMISTRY AND ANTIMICROBIAL PROPERTIES OF METHANOL EXTRACTS OF SELECTED PLANT SPECIES

H. K. Abd-Elhady, G. E. Abo-Elghar, Zeinab A. Elbermawy and A. G. Yousef

Department of Pesticides, Faculty of Agriculture, Minufiya University, Egypt

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ABSTRACT: ***Methanolic extracts of three plant species, Custard apple, Annona squamosa L.; Madagascan periwinkle, Catharanthus roseus syn. Vinca rosus, and Conyza, Pluchea dioscoridis (L.) DC., were screened for their phytochmical and antimicrobial properties. Two Gram-positive bacteria (Bacillus subtilis and Streptomyces spp.) and three fungi strains (Fusarium oxysporum, Macrophomina phaseolina and Aspergillus niger) were used for evaluation of antimicrobial properties of selected plant extracts. The agar gel diffusion method was used to assay for the antimicrobial properties on the test isolate. The methanol extract from Conyza dioscoridis leaves was superior to other tested extracts showing an obvious inhibitory effect on the growth of bacterial isolates. The results of inhibitory activity of tested extracts against Streptomyces spp. indicated that C. dioscoridis extract showed the highest zone of growth inhibition occurred after 48 h of treatment with a zone diameter of 27.0 mm at a concentration of 1.0 % and of 20.0 mm at a concentration of 0.5%. However, the most antifungal activity was observed in methanol extract of Annona squamosa seeds, at concentration of 1.0%, against Fusarium oxysporum showing inhibition zone with a diameter of 44.0 mm, while at concentration of 0.5 %, the growth inhibition occurred with a zone of 19.0 mm. The extract from Catharanthus roseus leaves, at 1.0%, showed also an obvious inhibitory effect, where the zone diameter of growth inhibition was 22.0 mm. GC/MS analysis of tested plant extracts demonstrates the presence of some phytochemicals (phythalic acid esters, alkaloids, terpenes, and fatty acids) which may provide the antimicrobial properties of these extracts against tested organisms.***

Key words: ***Antimicrobial activity, Annona squamosa, Cathranthus roseus, Pluchea dioscoridis, Phytochemical compounds, GC/MS***

INTRODUCTION

Use of plants as a source of medicine has been inherited and an important component of the health care system. Plants naturally synthesize several carbon compounds, basically for physiologic functions or for use as chemical weapons against disease organisms, insects and predators (Fatope, 1995). There is a continuous and urgent need to discover new

antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use (Rojas *et al.*, 2003 and 2006). The investigation of plants for bioactive secondary metabolites is an area which most plant scientists have recently focused with an aim of discovering new clinically useful and commercially important plant products (Dewick, 1997). Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests (Suffredini *et al.,* 2004). The systemic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with the potential to act against multi- resistant pathogenic bacteria and fungi. A special feature of higher angiospermic plants is their capacity to produce a large number of organic chemicals of high structural diversity. The so-called secondary metabolites (Evans *et al.,* 1986), which are divided into different categories based on their mechanism of function like chemotherapeutic, bacteriostatic, bactericidal and antimicrobial (Purohit and Mathur, 1999). Biological and pharmacological activities of phytochemical compounds take into account different parameters and factors such as species, ecological factors and environmental conditions. Thus, each plant species will present a profile a which it will express differently among these factors. Phenological age of the plant, percent humidity of the harvested material and method of extraction are possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts (Rajakaruna *et al.*, 2002). There is a wide variation in the susceptibility of organisms to toxic compounds. It is probable that a large number of plants with biological activities remain untested.

Three plant species widely distributed in Egypt have been selected in this present study, i.e. Custard apple, *Annona squamosa* L.; Madagascan periwinkle, *Catharanthus rosus* syn. *Vinca rosus*, and *Conyza, Pluchea* (*Conyza*) *dioscoridis* (L.) DC. The medicinal properties of different parts e.g. of *Annona squamosa* (Annonaceae) are well documented in many research works (Atique *et al.*, 1985; Rathore, 1990; Patel and Kumar, 2008). Roots are employed internally in depression of spirits and spinal diseases. Bark is known to be a powerful astringent. Fruits are considered as a good tonic; enriches blood, used as expectorant, increases muscular strength, lessens burning sensation and tendency to biliousness. *Pluchea dioscoridis* (Asteraceae) is used in popular medicine for rheumatic pains (Boulos and El-Hadidi, 1989). *Conyza scarida* DC. (Asteraceae) is reported to be used to treat influenza, chest and stomach afflictions, fever, diarrhea, sores, and inflammation in the literature (Smith, 1966; Scot *et al.*, 2004). *Catharanthus roseus* L (apocyanaceae) also known as *Vinca Rosea*, has historically been used to treat a wide assortment of diseases. European herbalists used the plant for conditions as varied as headache to a folk remedy for diabetes. It

has more than 400 known alkaloids, some of which are approved as antineoplastic agents to treat leukemia, Hodgkin's disease, malignant lymphomas, neuroblastoma, rhabdomyos-arcoma, Wilms' tumor, and other cancers. Its vasodilating and memory-enhancing properties have been shown to alleviate vascular dementia and Alzheimer's disease (Fischhof *et al.*, 1996). The extracts of *Vinca* have demonstrated significant anticancer activity against numerous cell types (El-Sayed and Cordell, 1981). The two classes of active compounds in *Vinca* are alkaloids and tannins. The antimicrobial and wound healing activity of the flower extract of *Catharanthus* has been revealed by Nayak and Pinto Pereira (2006). The antibacterial properties of organic extracts from *C. roseus* have been demonstrated recently by Goyal *et al.* (2008).

The principle aim of the present work was to study the antimicrobial activity of methanol extracts of *Annona squamosa*; Madagascan periwinkle, *Catharanthus roseus* syn. *Vinca rosus*, and Conyza, *Pluchea dioscoridis* against two Gram-positive bacteria (*Bacillus subtilis* and *Streptomyces* spp.) and three fungi (*Fusarium oxysporum, Macrophomina phaseolina* and *Aspergillus niger*). To identify the phytochemicals presented in the selected plant extracts which are responsible for antimicrobial activity, GC/MS analysis of these extracts have been done.

MATERIALS AND METHODS

Collection and Extraction of Plant Materials

The selected three plants namely *Annona squamosa* (seeds and leaves), *Catharanthus rosus* syn. *Vinca roseus* (leaves) and *Pluchea* (*Conyza*) *dioscoridis* (leaves) were at the Experimental Research Farm of Faculty of Agriculture, Menoufiya University, in August and September 2006-2007. The plants were identified by botanists in the Department of Agriculture Botany at the same Faculty. Selected parts of collected plants were air dried naturally under laboratory conditions at room temperature (25- 30° C) and then dried in an oven at 45° C for 48 h and ground to powder with an electric blender. Plant samples were extracted with methanol by following similar procedure described by Doughari (2006). Each plant sample (50 g) was extracted with 200 ml methanol. Powdered material (50 g) was extracted with (200 ml) methanol by soaking in the solvent in a large flask (1 liter) at room temperature for 24 h. The flask was plugged and shaken for 4 h. The extracts were kept at 4° C for 1day, and filtered through four layers of gauze and then by delicate filtration through Whatman (No. 1) filter paper, and then the extract was concentrated by evaporating the solvent on water bath, at 40° C, until dryness to obtain the crude extract. For testing, the crude extracts obtained were weighted and re-dissolved in 5 ml methanol and then completed by distilled water to prepare stock solution (w/v). Two concentrations (0.5 and 1.0 %) of each plant extract were prepared by diluting

the stock extract to methanol: distilled water (1: 9). The control had the solvent alone without any extract to nullify the effect of the solvent on the test organisms. The yield of crude methanol extracts for each plant sample (50 g) was calculated on dry weight basis:

***Annona squamosa* (Seeds) = 2.5 g** ***Annona squamosa* (Leaves) = 4.0 g**

***Catharanthus roseus* (Leaves) = 4.3 g** ***Pluchea dioscoridis* (Leaves) = 3.7 g**

Test microorganisms

The selected microorganisms were obtained from culture collection of the Department of Agriculture Botany, Menoufiya University. Two Gram-positive bacteria (*Bacillus subtilis* and *Streptomyces* spp.) and three fungi strains (*Fusarium oxysporum, Macrophomina phaseolina* and *Aspergillus niger*) were used in this study.

Antimicrobial Activity Assay

Antimicrobial activity of the plant extracts was measured using the disc-diffusion inhibition on agar method. In the disc-diffusion test as described by Musyimi *et al.* (2008). Circular paper discs 6.0 mm diameter were cut out from Whatman No. 1 filter paper using a paper punch and each dipped in a known concentration of each plant extract for about 2 min, then were gently transferred to the centre of the inoculated agar media. Petri dishes inoculated with bacteria and fungi were kept for incubation for 24-48 h at 37 and 25º C, respectively. The diameters of growth inhibition zones were measured using a ruler and compared to the control disc to nullify the effect of the solvent on the growth of the test organisms.

Phytochemicals Identification by GC/MS Analysis

The isolation and GC/MS analysis of bioactive compounds presented in the hexane:methanol fractions of the crude methanolic extracts of tested plants were done according to Mitova *et al.* (2003). The fractions were isolated and purified after column chromatography. The identification was accomplished using computer searches by NIST98 Wiley MS Data library. In some cases where identical spectra were not found only the structural type of the component was proposed based on the MS fragmentation. When possible reference compounds were chromatographed to confirm GC retention times.

RESULTS

Antimicrobial Testing

The results of antibacterial screening tests of methanol extracts of tested plants against two Gram-positive bacteria (*Bacillus subtilis* and

***Streptomyces* spp.) are depicted in Table 1. It was clear that the methanol extract from *Conyza dioscoridis* leaves was superior to the other extracts showing an obvious inhibitory effect on the growth of bacterial isolates. The results of inhibitory activity of *C. dioscoridis* extracts against *Streptomyces* spp. showed that the highest zone of growth inhibition occurred after 48 h of treatment with a zone diameter of 27.0 and 20.0 mm at a concentration of 1.0 and 0.5 % respectively. Where, the higher zone of growth inhibition for *Bacillus subtilis* occurred by a concentration of 1.0 % of *C. dioscoridis* extract with a zone diameter of 17.0 mm, and the lower zone of growth inhibition occurred with 12.0 mm, at concentration of 0.5 %. The data of inhibitory effect of other plant extracts showed that the extract of *Catharanthus roseus* leaves, at 1 %, had slight inhibitory activity against *Streptomyces* spp. with a zone diameter of 8.0 mm (after 48 h). While the other plant extracts tested did not show any inhibitory activity against *Streptomyces* spp.**

Table 1: Antibacterial Activity of the Methanol Extracts of Selected Plants on Bacillus subtilis and *Streptomyces* spp.

Plant extract (parts used)	*Conc. (%)*	*Zone of inhibition (mm) against*			
		Bacillus subtilis		*Streptomyces spp.*	
		24 h	*48 h*	*24 h*	*48 h*
Annona squamosa (Seeds)	*0.5*	-- *	*7.0*	-- *	-- *
	1.0	*7.0*	*7.0*	-- *	-- *
Annona squamosa (Leaves)	*0.5*	-- *	-- *	-- *	-- *
	1.0	-- *	*7.0*	-- *	-- *
Catharanthus roseus(Leaves)	*0.5*	-- *	-- *	-- *	-- *
	1.0	-- *	-- *	*7.0*	*8.0*
Pluchea dioscoridis (Leaves)	*0.5*	*10.0*	*12.0*	*13.0*	*20.0*
	1.0	*14.0*	*17.0*	*16.0*	*27.0*

*** No inhibition zone observed**

Data in Table 2 show the antifungal effect of the methanol plant extracts against selected fungi strains. The results indicated that the highest zone of inhibition was recorded against *Fuseurium oxysporum* The *Annona squamosa* extract (seeds) had the most inhibitory effect against *F. oxysporum* causing inhibition zone with 44.0 and 19.0 mm diameter, at

concentrations of 1.0 and 0.5 %, respectively. Also, the extracts from *Catharanthus roseus* leaves and *A. squamosa* leaves, at concentration of 1.0%, showed an obvious inhibitory effect, where the zone diameter of growth inhibition was 22.0 and 20.0 mm, respectively. While the growth inhibition observed at the lower concentration of 0.5 % was occurred with a zone diameter of 14.0 and 13.0 mm, respectively. The results also show that the inhibitory effect of growth of the other two fungi strains: *Macrophomina phaseolina* and *Aspergillus niger* was not occurred by the tested extracts, except extract of *A. squamosa* seeds which had a slight inhibitory activity with a zone of growth inhibition 10.0 and 8.0 mm diameter, at concentration of 1.0 %, respectively.

Table 2: Antifungal Activity of the Methanol Extracts of Selected Plant Species on *Fusarium oxysporum, Macrophomina phaseolina* and *Aspergillus niger*

Plant extract (parts used)	Conc. (%)	Zone of inhibition (mm) against		
		Fusarium oxysporum	*Macrophomina phaseolina*	*Aspergillus niger*
Annona squamosa (Seeds)	0.5	19.0	8.0	-- *
	1.0	44.0	10.0	-- *
Annona squamosa (Leaves)	0.5	13.0	-- *	-- *
	1.0	20.0	-- *	-- *
Catharanthus roseus (Leaves)	0.5	14.0	-- *	-- *
	1.0	22.0	-- *	-- *
Pluchea dioscoridis (Leaves)	0.5	-- *	-- *	-- *
	1.0	-- *	-- *	-- *

*** No inhibition zone observed**

Phytochemical Analysis

Annona squamosa

The results of phytochemical analysis of tested plant extracts are presented in Tables 3-6. As a result of GC/MS analysis of the extract from *Annona squamosa* seeds, a complex mixture of 30 constituents were found. The composition of complex mixture of constituents and relative percentages of individual components are shown in Table 3. Phythalic acid ester, 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester was characterized as the major component with 46.62 %, followed by 9-

octadecanoic acid, methyl ester (11.79%), pentadecanoic acid, 14-methyl, methyl ester (9.08%), octadecanoic acid, methyl ester (6.82%), 9,12-octadecadienoic acid (Z,Z), methyl ester (6.69%), [(Z)-octadec-13-enyl] acetate (4.27%), N-(4-bromophenyl)-4-(4-methylphenyl)-1,3-thiazol-2-amine (3.72%), oxiraneoctanoic acid, 3-octyl-, methyl ester (1.29%) and 3-O-(trimethylsilyl)-5,7,3',4'-tetra-O-methyl quercetin (1.28%). The other identified compounds were existed in minor concentrations less than 1.0% of the total.

The GC-MS analysis data of the methanol extract of *A. squamosa* leaves are shown in Table 4. Also, 30 peaks representing a complex mixture of constituents were identified. Similarly, the phthalic acid ester, benzenedicarboxylic acid, bis(2-ethylhex-yl) ester seemed to be the major constituent in the eluted fraction from methanol extract of *A. squamosa* leaves, with 90.15 % abundance. The other identified components were mostly belonging to fatty acids and existed in minor concentrations less than 1.0 %, except two compounds: 2-Hexadecen-1-ol, 3,7,11,15- tetramethyl-,[R-[R*,R*-(E)]], 2.19 % , and 6-Octadecanoic acid, methyl ester, 1.23 %.

Cathranthus roseus

The results of GC/MS analysis of the methanol extract of *C. roseus* leaves are presented in Table 5. The phthalic acid ester, 1,2 benzenedicarboxylic acid, bis (2-ethylhex-yl) ester appered to be the most abundant component in this extract with 69.26 %, followed by an alkaloid, 6,7-dehydro-Vincadine (6.34 %), and 2-Bromo-4,4-di-N-propylcyclobutanone (4.43 %); benzoic acid, 4-methyl-, difluoromethyl ester (2.72 %); 2-Hexadecan-1-ol, 3,7, 11,15-tetramethyl-, [R-[R*,R*-(E)]] (2.07 %); dimethyl[3-(tert-butylace-toxy)-3-methyl-2-oxobutyl] phosphonate-(18)O (2.00 %); (S)-2,5,5-trimethyl-1,2,3,6-tetra-hydro-4 (5H) -azulenone (1.89 %) and O-Acetyl-N-2-butenylhydroxylamine (1.88 %). The other fractions were existed in minor concentrations less than 1 %.

Pluchea dioscoridis

The GC/MS analysis of the methanol extract of *P. dioscoridis* leaves revealed the presence of a complex mixture of twenty one components (Table 6). The major constituents identified in this fraction were: terpene, 1à,8à-dihydroxy-cyclocostunolide (25.82 %); citronellyl propionate (23.56 %); 2,3-L-oxobutyl-1à,3,3-trimethyl -7-oxabicyclo heptane (14.64 %) and sterol, trans-stigmasta-5,22-dien-3l-ol (11.70 %). The other components were existed in lower concentrations with < 4.5 % proportion.

Table 3 : Major identified constituents of the methanol:hexane fraction isolated from crude extract of *Annona squamosa* seeds analyzed by direct GC/MS

GC/MS Peak no.	Retention time (min)	Peak Area (%composition)	Compound	Formula
1	14.15	0.27	Thiophene, tetrahydro-, 1,1-dioxide	$C_4H_8O_2S$
2	31.09	9.08	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$
3	31.64	0.17	1-(Allenylsulfonyl)-2,5,5-trimethyl-3-vinyl-2-cyclohexene	$C_{14}H_{20}O_2S$
4	32.26	1.07	Hexadecanoic acid	$C_{16}H_{32}O_2$
5	32.38	0.64	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$
6	32.96	0.72	Methyl 2-(3-hydroxybutyl)-1-butyl diester of phthalic acid	$C_{17}H_{24}O_5$
7	34.32	6.69	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$
8	34.48	11.79	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
9	34.93	6.82	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$
10	35.61	4.27	[(Z)-octadec-13-enyl] acetate	$C_{20}H_{38}O_2$
11	35.96	0.47	(tetrahydroxy-cyclopentadienone) tricarbo-nyliron	$C_8H_4FeO_8$
12	36.09	0.60	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$
13	36.91	0.09	2-Hexenal	$C_6H_{10}O$
14	37.70	0.16	Nitric acid, nonyl ester	$C_9H_{19}NO_3$
15	37.99	1.29	Oxiraneoctanoic acid, 3-octyl-, methyl ester	$C_{19}H_{36}O_3$
16	38.38	0.43	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$
17	38.84	0.68	N-(trimethylsilyl)furan-2-carboxaldimine	$C_8H_{13}NOS$
18	39.89	0.33	Cyclohexane, 1,1'-dodecylidenebis[4-methyl	$C_{26}H_{50}$
19	40.79	0.21	methyl (E,Z)-3L-(tetrahydropyran-2'-yloxy)pregna-5,15,17 (20)-trien-21-oate	$C_{27}H_{38}O_4$
20	40.97	0.41	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$
21	41.11	0.10	2-3)-L-Oxobutyl)-1à,3,3-trimethyl-7-oxabicyclo [2.2.1]heptane	$C_{13}H_{22}O_2$
22	41.35	0.28	bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$
23	41.82	46.62	Phthalic acid ester, 1,2-Benzenedi-carboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$
24	43.48	0.28	Methyl N-(nitroacetyl)L-alaninate	$C_6H_{10}N_2O_5$
25	44.24	3.72	N-(4-bromophenyl)-4-(4-methyl-phenyl)-1,3-thiazol-2-amine	$C_{16}H_{13}BrN_2S$
26	44.60	0.15	Cyclopentaneundecanoic acid, methyl ester	$C_{17}H_{32}O_2$
27	48.51	0.10	Pentanamide	$C_5H_{11}NO$
28	51.28	0.35	2,3-bis[(Triisopropylsilyl)ethynyl]furan-2 (5H)- one	$C_{26}H_{44}O_2Si_2$
29	51.69	0.93	Stigmasta-5,23-dien-3-L-ol	$C_{29}H_{48}O$
30	52.68	1.28	3-O-(trimethylsilyl)-5,7,3',4'-tetra-O-methylquercetin	$C_{22}H_{26}O_7Si$

Table 4 : Major identified constituents of the methanol:hexane fraction isolated from crude extract of *Annona squamosa* leaves analyzed by direct GC/MS

GC/MS Peak no.	Retention time (min)	Peak Area (%composition)	Compound	Formula
1	21.77	0.21	1,2(trans),2,3(trans),3,4(trans),-2,4-bis (p-cyanophenyl)-1,3-diphenylcyclobutane	$C_30H_{22}N_2$
2	24.84	0.68	1-Hydroxy-1-methyl-7(methylethenyl) [1,2,3, 3a,4,5,6,7]octahydro azulene	$C_{14}H_{22}O$
3	27.21	0.11	2à-T-Butyl-1,2,3,4,4a,5,6,7,8,8,LaL- Deca-hydronaphthalen-2L-ol	$C_{14}H_{26}O$
4	28.15	0.04	Benzene, 1,2-dimethoxy-4-(1-methylene-pentyl)	$C_{14}H_{20}O_2$
5	29.23	0.30	2,2-Dimethyl-1-isopropenyl-cyclopentane	$C_{10}H_{18}$
6	31.04	0.80	Nonanoic acid, methyl ester	$C_{10}H_{20}O_2$
7	31.64	0.25	trans-1-(phenylthio)-6-oxo-4-oxahept-1-ene	$C_{12}H_{14}O_2S$
8	32.96	0.03	myrac aldehyde 1 and 2	$C_{13}H_{20}O$
9	34.23	0.34	1-Undecyne	$C_{11}H_{20}$
10	34.37	1.23	6-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
11	34.55	2.19	Cyclic terpenoid, 2-Hexadecen-1-ol, 3,7,11, 15-tetramethyl-, [R-[R*,R*-(E)]]	$C_{20}H_{40}O$
12	34.85	0.45	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$
13	35.57	0.05	3-Undecene, 5-Methyl-, Cis/Trans	$C_{12}H_{24}$
14	40.74	0.10	2-deutero-benzobicyclo[2.2.2]octen-2-ol	$C_{12}H_{13}DO$
15	41.71	90.15	Phthalic acid ester, Benzenedicarboxylic acid, bis (2-ethylhex-yl) ester	$C_{24}H_{38}O_4$
16	43.14	0.12	3-chloro-3-trifluoromethyl-2-thiabi-cyclo [2.2.1] hept-5-ene-2,2-dioxide	$C_7H_6ClF_3O_2S$
17	43.71	0.49	4-Methoxyphenyl 4-Butylcyclo-hexane-carboxylate	$C_{18}H_{26}O_3$
18	46.14	0.07	à-tocopherylquinone-5,6-oxide	$C_{29}H_{50}O_4$
19	46.42	0.06	2,5-Cyclohexadiene-1,4-dione, 2,5-dihydr-oxy-3-(2,6,10,14-tetramethylhexadecyl)	$C_{26}H_{44}O_4$
20	48.29	0.14	1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradeca-methylheptasiloxane	$C_{14}H_{44}O_6Si_7$
21	48.64	0.09	1-(2-trimethylsiloxy-1,1-dideuteriovinyl)-4-trimethylsiloxy-benzene	$C_{14}H_{22}D_2O_2Si_2$
22	49.81	0.04	1-Butanol, 2-nitro	$C_4H_9NO_3$
23	49.94	0.54	N,N-Diisopropyl-2',3,4,3',4'-pentamethoxy -2-biphenylcarboxamide	$C_{24}H_{33}NO_6$
24	50.09	0.05	3-tert-butyl-2-[tert-butyl(trimethylsilyl) amino]-4-(1-propenyl)-1,3,2-oxazaboretidin	$C_{15}H_{33}BN_2OSi$
25	50.57	0.06	2-methoxy-5-trimethylstannylcyclohepta-2,4,6-trien-1-one	$C_{11}H_{16}O_2Sn$
26	51.27	0.25	2-(4-hydroxy-2-butenyl)-2-nitrocyc-loctanone	$C_{12}H_{19}NO_4$
27	51.65	0.15	cis-2,6-dimethyl-2-nitrocyclohexanone	$C_8H_{13}NO_3$
28	51.99	0.08	2-(tert-butylamino)-5,5-diphenyl-4-(methylthio)-5-isoimidazole	$C_{20}H_{23}N_3S$
29	52.31	0.02	N-Allyloxymethylacrylamide	$C_7H_{11}NO_2$
30	52.65	0.90	Stigmast-5-en-3-ol, (3,24,LS)	$C_{29}H_{50}O$

Table 5 : Major identified constituents of the methanol:hexane fraction isolated from crude extract of *Catharanthus roseus* leaves analyzed by direct GC/MS

GC/MS Peak no.	Retention time (min)	Peak Area (%composition)	Compound	Formula
1	14.19	1.88	O-Acetyl-N-2-butenylhydroxylamine	$C_6H_{11}NO_2$
2	15.03	2.72	Benzoic acid 4-methyl, difluoromethyl ester	$C_9H_8F_2O_2$
3	15.27	4.43	Anthraquinone, 2-Bromo-4,4-Di-N-propylcyclo-butanone	$C_{10}H_{17}BrO$
4	17.06	0.24	2-Methoxy-5-vinylphenol	$C_9H_{10}O_2$
5	23.64	0.40	4,4-Dimethyl-2-Methylidene-3-(3'-Oxobut-ylidene) Cyclohexyl Acetate	$C_{15}H_{22}O_3$
6	24.71	1.89	Anthraquinone, (S)-2,5,5-trimethyl-1,2,3,6-tetrahydro-4(5H)-azulenone	$C_{13}H_{18}O$
7	27.47	0.12	6-(2-Iodoethyl)-5,7-dimethylphthalide	$C_{12}H_{13}IO_2$
8	28.24	0.59	Terpene lactone, Loliolide (calendin)	$C_{11}H_{16}O_3$
9	28.60	2.00	dimethyl [3-(tert-butylacetoxy)-3-methyl-2-oxo-butyl]phosphonate-(18)O	$C_{13}H_{25}O_6P$
10	31.04	0.94	Nonanoic acid, methyl ester	$C_{10}H_{20}O_2$
11	31.64	0.13	Isobutyl 2-Chloro-3-Phenylpropionate	$C_{13}H_{17}ClO_2$
12	32.01	0.86	Hexadecanoic acid	$C_{16}H_{32}O_2$
13	32.36	0.69	Hexadecanoic acid, 2-Methyl-, Methyl Ester	$C_{18}H_{36}O_2$
14	34.35	0.80	9-Octadecenoic acid, methyl ester, (E)- (CAS)	$C_{19}H_{36}O_2$
15	34.56	2.07	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]	$C_{20}H_{40}O$
16	34.85	0.22	Nonanoic acid, methyl ester	$C_{10}H_{20}O_2$
17	35.33	0.95	12-Chlorododecanal Dimethyl Acetal	$C_{14}H_{29}ClO_2$
18	35.57	0.86	2-Nitro-2-(3-oxobutyl) cycloheptanone	$C_{11}H_{17}NO_4$
19	36.05	0.15	methyl (2R*,4S*)-2,4-dimethyl-5-hexenoate	$C_9H_{16}O_2$
20	41.64	69.26	Phthalic acid ester, 1,2-Benzenedi-carboxylic Acid, Bis(2-Ethylhexyl) Ester	$C_{24}H_{38}O_4$
21	42.42	0.22	5,12-Dimethyl-9-Methylene-8-(1-Hydroxy-3-Oxobuten-2-Yl)-6,7,8,9,10,11-Hexa-hydro-6,10-Imino-5H-Cyclooct[B] indolle	$C_{21}H_{24}N_2O_2$
22	44.17	6.34	Alkaloid, 6,7-Dehydro-Vincadine	$C_{21}H_{26}N_2O_2$
23	47.81	0.31	Phenyl-Tert-Butyl-Acetylene	$C_{12}H_{14}$
24	49.50	0.38	Dimethyl 6-Amino-7-Nitro-2,3-Naphthalene- dicarboxylate	$C_{14}H_{12}N_2O_6$
25	49.94	0.20	[3-Deuterium)-Ç-Tocopheryl Methyl Ether	$C_{29}H_{49}DO_2$
26	50.79	0.66	Alkaloid, vindoline (racemic)	$C_{25}H_{32}N_2O_6$
27	51.26	0.17	N-(4',6'-Dimethoxy-2',3'-Diphenylindol-7'-Ylmethylene)Methylamine N-Oxide	$C_{24}H_{22}N_2O_3$
28	51.63	0.21	(2'-Nitro-2'-Propenyl) cyclohexane	$C_9H_{15}NO_2$
29	52.30	0.17	di-T-Butyl [3',3',4'-Trimethyl-2'-Furanyl-	$C_{16}H_{31}O_4$

			idene) methyl] phosphonate	P
30	52.62	0.13	8,9:14,15-dibenzo-2,4,6,16,18,20-Docosa-hexaene-10,12-diynedial	$C_{30}H_{22}O_2$

Table 6 : Major identified constituents of the methanol:hexane fraction isolated from crude extract of *Pluchea. dioscoridis* leaves analyzed by direct GC/MS

GC/MS Peak no.	Retention time (min)	Peak Area (%composition)	Compound	Formula
1	21.76	1.76	1,2(trans),2,3(trans),3,4(trans),-2,4-bis(p-cyanophenyl)-1,3- diphenyl-cyclobutane	$C_{30}H_{22}N_2$
2	29.22	23.56	Citronellyl propionate	$C_{13}H_{24}O_2$
3	29.73	0.94	Citronellyl 3-Methylbutanoate	$C_{15}H_{28}O_2$
4	30.10	4.44	Citronellyl acetate	$C_{12}H_{22}O_2$
5	31.03	1.10	Octanoic acid, Methyl Ester	$C_9H_{18}O_2$
6	34.34	0.25	1,4-bis-(2-hydroxymethyl) cyclo-hexane	C8H16O2
7	34.54	1.89	4-[(4S)-[(1S)-1-(Chloromercurio)((2R)-tetra-hydrofuran-2-yl)methyl]-2,2-dimethyl-1,3-dioxolane	$C_{10}H_{17}ClHgO_3$
8	41.05	0.18	Bis-(3,5,5-trimethylhexyl) ether	$C_{18}H_{38}O$
9	41.61	14.64	2,3)-L-Oxobutyl)-1à,3,3-trimethyl-7-oxabi-cyclo[2.2.1]heptane	$C_{13}H_{22}O_2$
10	42.58	0.76	meso-2,2'-dichloro-3,3,3',3'-tetramethyl-2,2'-azobutane	$C_{12}H_{24}Cl_2N_2$
11	43.09	25.82	Terpene, 1à,8à-dihydroxycyclocastunolide	$C_{15}H_{20}O_4$
12	43.37	2.27	(E)-2-Methylbut-2-enoic Anhydride	$C_{10}H_{14}O_3$
13	43.93	3.39	Terpene, 9-(Ethoxycarbonyl)-10- propyli-dene-bicyclo[3.2.2]nona-3,6-dien-2-one isomer	$C_{15}H_{18}O_3$
14	44.88	0.35	(Bis-trifluoromethylamino-oxy) cyclohexane	$C_8H_{11}F_6NO$
15	45.73	2.81	2-Propenoic acid, 2-methyl-, 2-propenyl ester	$C_7H_{10}O_2$
16	48.22	1.09	Terpene, 9,10,11,12-Tetrahydrocycloocta chroman -6-one	$C_{15}H_{14}O_2$
17	49.93	1.83	ethyl 4,5-diaza-12,12--dimethoxy-8,9,10,11-tetrachlorotricyclo [5.5.0.1 (1,9)] dodeca-2,5,9-trien-4-carboxylate	$C_{15}H_{16}Cl_4N_2O_4$
18	51.26	0.24	Ethyl (4S)-(E)-4-(N-benzyl-p-toluenesulfon-amido) -2-methyl-5-phenylpent-2-enoate	$C_{28}H_{31}NO_4S$
19	51.66	11.70	Sterol, trans-Stigmasta-5,22-dien-3l-ol	$C_{29}H_{48}O$
20	52.60	0.72	di-Lauryl Thio-di-Propionate	$C_{30}H_{58}O_4S$

21	52.94	0.24	N-benzoyl-1,2,2-trimethoxyethyl amine	$C_{12}H_{17}NO_4$

DISCUSSION

The extracts of higher plants can be very good source of antibiotics (Fridous *et al.*, 1990) against various fungal and bacterial pathogens. Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Higher plants have also made important contributions in the areas such as cancer therapies. Early examples include the antileukaemic alkaloids, vinblastine and vincristine, which were both obtained from Madagascan periwinkle (*Catharanthus roseus* syn. *Vinca rosea,* Apocynaceae). The inhibitory effect of the methanolic extract from *C. roseus* leaves against *Fusarium oxysporum* and *Streptomyces* spp. have been demonstrated in this present work. The preliminary analysis of phytochemical composition in *C. roseus* extract showed that phythalic acid esters, terpenes, alkaloids, and anthraquinones were the major constituents. Magnotta *et al*. (2006) indicated that *C. roseus* is the only source of the monoterpenoid indole alkaloids. Also, *C. roseus* was found to contain a very large number of alkaloids, about 100 of which have been isolated so far (Verpoorte *et al*. 1997, Samuelsson 1999). Also, triterpenoids, tannins and alkaloids extracted from *C. roseus* served as bioactive agents (Elujoba *et al.* 2005, Nayak and Pereira 2006).

Our study revealed also a remarkable inhibitory effect of methanol extract of *Annona squamosa* seeds against *Fusarium oxysporum,* and slight inhibitory effect against *Bacillus subtilis*, at tested concentrations, 0.5 and 1.0 %. Present phytochemical screening of the selected fraction from *A. squamosa* extract based on GC/MS showed different type of compounds. Phythalic acid ester seemed to be the major compound in this fraction. Phthalic acid esters are presently being used in amounts and products that can easily, although inadvertently, contribute to environmental pollution. Phthalic acid esters are the most widely used plasticizers, particularly poly (vinyl chloride) plastics. However, a little work has been done to examine the inhibitory activity of phthalic acid esters against bacteria and fungi. Di-2-ethylhexyl phthalates (DEHP) and other phthalate esters have been tested for acute toxicity primarily in mice and rats and tissue culture cells. An earlier indication about the biological activity properties of phthalic acid esters as insect repellent and acaricides had been reported by Farm Chemicals (1971). The low degree of toxicology and the high excretion rate of di-*n*-butyl and di-2-ethylhexyl phthalates might suggest that these compounds would be relatively safe as far as aquatic organisms are concerned (Mayer Jr. and Sanders, 1973). However, these compounds can be detrimental to the reproduction of aquatic organisms at low chronic concentrations. Octadecenoic acid, methyl ester was found also in the *A. squamosa* extract

in considerable amounts. The fatty acids are well known active metabolites. They serve as an important energetic substrate for the cells. Linoleic acid is essential for maintenance of growth and α-linolenic acid for natural functions. Both acids were shown to be potent cycloxygenase-2-(COX-2) catalyzed prostaglandin biosyntesis inhibitors (Ringbom *et al.*, 2001).

Terpene, citronellyl propionate, and sterol, trans-stigmasta were identified in the eluted fraction from the methanol extract of *Pluchea dioscoridis* leaves. Mahmoud (1997) re-examined the chemical constituents of the leaves of this plant and reported seven new sesquiterpene derivatives (two 7-epi-eudesmanes, two eudesmanoic acids, eudesmanolide, guaiane and xanthane epoxide). Grace (2002) identified 36 components in the volatile oil of *Pluchea dioscoridis,* where farnesol was the major component (16.5%) accompanied by a high percentage of sesquiterpene alcohols. Oxygenated sesquiterpenes (26.4%) and sesquit-erpene hydrocarbons (39.4%) represented the main constituents in the oil. El-Hamouly and Ibraheim (2003) reported that the leaves of *Pluchea dioscoridis* containing 3-5% volatile oil, where 112 compounds were detected consisting mainly of sesquiterpene hydrocarbons (mainly β-maaliene and α-elemene), oxygenated sesquiterpenes (mainly α-cadinol, muurolol and caryophyllene oxide isomer). The plant also containing triterpenoid as hexacosanol, octacosanol, tetracosanol, cholesterol and campesterol. The presence of terpenes observed in the phytochemical screening may be responsible for the enhanced effect of the antimicrobial properties of the methanol:hexane fraction, from *P. dioscoridis* methanol extract. All terpene hydrocarbons are antiseptic, anti-inflammatory and antibacterial. Terpenes retard the retention of toxins in human organisms, they increase the abstraction of aggregated toxic material from the veins and liver, and act as antispasmodicagents (Damnjanovic, 2000). Sterol was present in a considerable amount in the *Pluchea dioscoridis* extract. Sitosterol posses antihyperlipop-roteinammic, antibacterial and antimicotic activity and has been shown to act as inhibitor of tumor promotion *in* vivo (Yasukawa *et al.,* 1991). Sigmasterol was found to markedly inhibit tumor promotion in two-stage carcinogenesis in mice (Kasahara *et al.*, 1994). Therefore, the presence of sterols in *P. dioscoridis* is of practical importance.

In conclusion, the methanol extract of *Pluchea dioscoridis* has an obvious antibacterial activity against *Bacillus subtilis and Streptomyces* spp. strains, whereas, the extracts from *Annona squamosa* (seeds, leaves) and *Catharanthus roseus* leaves had a strong antifungal activity against *Fusarium oxysporum*. The antimicrobial properties reported in this study can be attributed to the presence of a mixture of bioactive constituents, e.g. terpenes, alkaloids, and steroids, in the eluted fractions isolated from methanol extracts from these plants. This has an important practical implication in the strategy adopted in the search for an use of plants and their phytochemicals for using as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens.. However, further

research is required to establish the *in vivo* activities as well as the therapeutic index of such plants in respect to the management and possible cure of infectious diseases.

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دراسة التركيب الكيماوى والخصائص الإبادية للمستخلص الميثانولى الناتج مِن أنواعِ نباتِيه منتخبة على الميكروبات

هانى كمال عبد الهادى ، جمال السيد أبو الغار ، زينب عبد الغنى البرماوى ، عادل غطاس يوسف

قسم مبيدات الآفات ، كلية الزراعة ، جامعة المنوفية

الملخص العربي

لقد تم دراسة نشاط المستخلص الميثانولى لثلاثة من الأنواع النباتية وهى: القشطة – الونكا – البرنوف بغرض التعرف على تركيبها الكيماوى وكذلك خصائصها الإبادية على بعض أنواع الميكروبات. فلتقييم الخصائص الإبادية للمستخلصات النباتية المنتخبة على الميكروبات تم إستعمال نوعين من البكتيريا الموجبة لجرام وهما: باسيلس سبتيلس ، ستربتوميسيس وكذلك ثلاثة أنواع من الفطريات وهم: فيوزاريوم اوكسيسبوريم ، ماكروفومينا فاصولينا ، اسبرجلس نيجر. حيث تم تقييم الخصائص الإبادية للمستخلص الميثانولى على عزلات الميكروبات من خلال قياس قطر منطقة التثبيط فى طبقة الآجار. فكان المستخلص الميثانولى الخام لأوراق البرنوف أكثر المستخلصات فاعلية فى تثبيط نمو العزلات البكتيرية. وعند قياس نشاط المستخلصات النباتية المنتخبة ضد بكتيريا ستربتوميسيس قد أشارت النتائج إلى أن مستخلص أوراق البرنوف بتركيز ١.٠% بعد ٤٨ ساعة من بداية المعاملة قد أدى إلى إحداث منطقة تثبيط مقدارها ٢٧.٠ مم وعند تركيز ٠.٥% كان قطر منطقة التثبيط ٢٠.٠ مم. على أية حال فإن المستخلص الميثانولى الخام لبذور القشطة كان أكثرالمستخلصات النباتية المنتخبة فاعلية ضد فطر الفيوزاريوم اوكسيسبوريم عند تركيز ١.٠% كان قطر منطقة التثبيط ٤٤.٠ مم بينما بتركيز

٠.٥% كان قطر منطقة التثبيط ١٩.٠ مم . وأظهر مستخلص أوراق الونكا بتركيز ١.٠% تأثيرا مثبطا واضحا حيث كان قطر منطقةَ تثبيط النمو ٢٢.٠ مم. ولقد تم إستعمال جهاز الكروماتوجرافى الغازى المرتبط بمطياف الكتلة GC/MS للتعرف على المكونات الكيماوية للمستخلصات النباتية المنتخبة والتى أظهرت وجود بعض المكونات الكيماوية مثل (إسترات حامض الفثاليك – قلويدات – تربينات – أحماض دهنية) والتى ربما تبرهن إمتلاك تلك المستخلصات النباتية خصائص إبادية على الميكروبات تحت الإختبار.