INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–4–638–642
http://www.ijab.org

Antifungal Activity of Extracts and Sesquiterpene Lactones from *Magnolia grandiflora* L. (Magnoliaceae)

SOAD M. AHMED AND SAMIR A.M. ABDELGALEIL[1]
Pesticide Chemistry Department, Faculty of Agriculture (Elshatby), Alexandria University, Alexandria, Egypt
[1]Corresponding author's e-mail: samir1969us@yahoo.com

ABSTRACT

The antifungal activity of dichloromethane leaves and stem bark extracts and methanol extract of the stem bark of *Magnolia grandiflora* (L.) was assessed using mycelial radial growth inhibition technique against six plant pathogenic fungi (*Alternaria alternata*, *Helminthosporium* spp, *Nigrospora* spp, *Fusarium oxysporum*, *Fusarium culmorium* and *Rhizocotonia solani*). Dichloromethane extracts of leaves and the stem bark exhibited a pronounced antifungal activity against four of the six tested fungi, while the stem bark methanol extract displayed a very weak or no antifungal activity against the tested fungi. Repeated column chromatography on silica gel followed by recrystallization led to isolate two sesquiterpene lactones, costunolide and parthenolide. The structure of these compounds and a parthenolide derivative, 1,10-epoxyparthenolide, was determined by spectroscopic methods. These sesquiterpene lactones were tested for their antifungal activity. Costunolide showed the strongest antifungal activity among the tested sesquiterpene compounds against three fungi, *Nigrospora* spp, *R. solani* and *Helminthosporium* spp, with EC_{50} values of 0.48, 2.92 and 2.96 μg mL^{-1}, respectively, while parthenolide exhibited the highest antifungal activity against *A. alternata* and *F. culmorium* (EC_{50} = 4.07 & 50.27 μg mL^{-1}, respectively). The three sesquiterpene lactones showed higher antifungal activity than a reference fungicide, thiophanate-methyl, against *Helminthosporium* spp. This is the first report on the antifungal activity of these sesquiterpene lactones against plant pathogenic fungi. The results of this study indicate that these sesquiterpene lactones may provide a useful starting point for the development of a new class of fungicides.

Key Words: *Magnolia grandiflora;* Sesquiterpene lactones; Antifungal activity; Plant pathogenic fungi

INRODUCTION

Chemicals largely used as pesticides in crop protection could be environmental pollutants and have undesirable biological effects on animals and human beings. Therefore, the development of biopesticides has been focused as a viable pest control strategy in recent years. One source of potential new pesticides is natural products produced by plants. Not only might certain natural products be source of new pesticides, but botanical derivatives may be more environmentally benign than synthetic chemicals (Parka *et al*., 2002; Hashim & Devi, 2003).

Sesquiterpene lactones are the most distinctive secondary metabolites of the members of the Compositae (Asteraceae). However, they have been reported from several plant families, such as Acanthaceae, Amaranthaceae, Apiaceae and Magnoliaceae. They have a diversity chemical structures and a wide range of biological activities, including antitumougenic, insect antifeedant, plant growth regulating, antibacterial, antifungal and cytotoxic properties (Picman, 1986; Baruah *et al*., 1994; Goren *et al*., 1996; Mansilla & Palenzuela, 1999; Neerman, 2003).

Magnolia grandiflora (L.) (Magnoliaceae) is large evergreen ornamented tree. This plant has been listed in the United States Pharmacopoeia and pharmacognosy texts as bitter tonic, antimalarial and diaphoretic. In Chinese traditional medicine, the plant is also used for treatment of cold, headache and stomachache (Rao & Davis, 1982; Wu *et al*., 1988). Several natural products, including sesquiterpenes (El-Feraly & Chan, 1978; El-Feraly *et al*., 1979; El-Feraly, 1984; Luo *et al*., 2001), biphenyls (El-Feraly & Li, 1978), lignan glycosides (Rao & Wu, 1978) and alkaloids (Nakano, 1954) have been isolated from different parts of this plant.

To the best of our knowledge, the antifungal activity of *M. grandiflora* different extracts has not been investigated. In this paper, we describe the antifungal activity of dichloromethane and methanol extracts of leaves and the stem bark of this plant against six plant pathogenic fungi. Sesquiterpene lactones (costunolide (1), parthenolide (2) have been isolated from the plant extracts. These two compounds and parthenolide derivative, 1,10-epoxyparthenolide (3), were also evaluated for their antifungal activity.

MATERIALS AND METHODS

General experimental procedures. Silica gel (70-230 mesh, Merck) was used on open column chromatography. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ on a JEOL FX-400 spectrometer. Ultraviolet (UV)

spectra were measured in MeOH on a Shimadzu UV-160A spectrophotometer. Optical rotation was measured in $CHCl_3$ at 22°C using JASCO P-1030 spectropolarimeter. Infrared (IR) spectra (KBr) were performed on a JASCO FT/IR 5300. Mass spectra (HR-FAB-MS) were run on JMS-102A mass spectrometer. Melting points were determined using a Mitamura melting point apparatus and were uncorrected.

Plant collection and preparation. The stem bark and leaves of *Magnolia grandiflora* (L.) were collected in April 2004 from Antonyades Public Garden, Alexandria, Egypt and identified by Dr. Ahmed Moharib of Alexandria University. A voucher specimen is deposited in the herbarium at Antonyades Public Garden, Alexandria. The stem bark and leaves were cut into small pieces and were shade-dried for three weeks.

Fungi. The six fungi species used, *Alternaria alternata*, *Helminthosporium* spp, *Nigrospora* spp, *Fusarium oxysporum*, *Fusarium culmorium*, *Rhizocotonia solani*, were obtained from the Fungicide Bioassay Laboratory, Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University. The fungi were maintained during the course the experiments on Czapek-Dox Agar (CDA: sucrose 30 g, sodium nitrate 2 g, potassium monohydrogen phosphate 1 g, potassium chloride 0.5 g, magnesium sulphate 0.5 g, ferrous sulphate 0.01 g and 15 g agar in 1000 mL of distilled water) medium at 25ºC.

Extraction and isolation of costunolide (1) and parthenolide (2). *Magnolia grandiflora* leaves (1.5 kg) were soaked in dichloromethane (10 l) at room temperature for one week providing 43 g of crude extract which was suspended in 10% aqueous methanol (600 mL) and extracted with hexane (400 mL × 3). Evaporation of the aqueous methanolic fraction afforded 22 g of residue which was subjected to silica gel (1 kg) column chromatography using mixtures of hexane-ethyl acetate of increasing polarity (80, 65, 50, 25 & 0%) to give 63 fractions (each fraction 200 mL). Fractions 9~12 were rechromatographed on silica gel column eluted with 5% acetone/dichloromethane and recrystallized from hexane to provide 754.0 mg of pure costunolide (1). Fractions 19~22 were subjected to silica gel column eluted with 2% and 5% methanol/chloroform, respectively, to offer parthenolide (2), which was recrystallized from ether to give 4.9 g of pure crystals.

Successive extraction of the air-dried stem bark (1.9 Kg) of *M. grandiflora* with dichloromethane and methanol (10 l of each) at room temperature for one week in each solvent gave 32 and 164 g of dichloromethane extract and methanol extract, respectively. Dichloromethane extract was suspended in 10% aqueous methanol (500 mL) and extracted with hexane (300 mL × 3). The aqueous methanolic (10 g) was subjected to silica gel column (500 g) eluting with 1, 2 and 10% acetone/dichloromethane. Thirty-five fractions of 200 mL were collected and pooled into two main fractions, fr 1 (Fr nos 1~5, 2.6 g) and fr 2 (Fr nos 14~24, 3.8 g), on the basis of the similar TLC profiles. The first fraction was recrystallized from hexane to offer 1169.0 mg of costunolide (1). The second fraction was recrystallized from ether to give 2.7 g of parthenolide (2). The stem bark methanol extract (155 g) was dissolved in water-methanol (1:1) and extracted with dichloromethane (500 mL × 3). Dichloromethane phase (13 g) was fractionated on silica gel column chromatography (600 g) using 1, 2.5, 5 and 10% acetone/dichloromethane solvent system. Forty-three fractions of 200 mL have been collected. Recrystallization of fractions 11~16 from hexane provided 175 mg of costunolide (1). Similarly, fractions 24~28 were recrystallized from ether to give 247 mg of parthenolide (2).

Costunolide (1) Colourless needles from hexane, mp 105-106ºC; $[\alpha]_D$ 127º (c 0.50, $CHCl_3$); UV (MeOH) λ_{max} nm: 220 (ε 9000); IR (KBr) ν_{max} cm^{-1}: 2926, 1766, 1660, 1439, 1138, 966, 814 and 706; ^1H NMR ($CDCl_3$): δ 1.43 (3H, s, Me-14), 1.70 (3H, s, Me-15), 4.58 (1H, t, J = 9.9 Hz, H-6), 4.75 (1H, d, J = 9.9 Hz, H-5), 4.86 (1H, dd, J = 10.3 & 3.3 Hz, H-1), 5.55 (1H, d, J = 2.9 Hz, H-13), 6.22 (1H, d, J = 3.7 Hz, H-13); FAB *m/z* 233 $[M + H]^+$.

Parthenolide (2) Colourless prisms from ether, mp 115-116ºC; $[\alpha]_D$-94.8º (c 0.50, $CHCl_3$); UV (MeOH) λ_{max} nm: 217 (ε 4000); IR (KBr) ν_{max} cm^{-1}: 2932, 1765, 1444, 1290, 1249, 1141, 985 and 715; ^1H NMR ($CDCl_3$): δ 1.30 (3H, s, Me-15), 1.71 (3H, s, Me-14), 2.80 (1H, d, J = 8.8 Hz, H-5), 3.87 (1H, t, J = 8.8 Hz, H-6), 5.22 (1H, dd, J = 9.9 & 2.5 Hz, H-1), 5.64 (1H, d, J = 3.3 Hz, H-13), 6.29 (1H, d, J = 3.7 Hz, H-13); FAB *m/z* 249 $[M + H]^+$.

Preparation of epoxy derivative of parthenolide (2). To a solution of parthenolide (2, 500 mg) in chloroform (30 mL), *m*-chloroperbenzoic acid (575 mg, 75%) was added and the mixture was stirred at 5ºC for 3 hr. After addition of 100 mL dichloromethane, the reaction product was washed with 5% Na_2SO_3 (50 mL), 5% $NaHCO_3$ (50 mL) and water (50 mL), respectively. The organic phase (CH_2Cl_2 layer) was dried through anhydrous sodium sulphate and concentrated to give 609 mg of the white residue. Purification of the product was achieved using silica gel column chromatography with hexane/ethyl acetate (3:2) to yield 408 mg of pure 1,10-expoxyparthenolide (3).

1,10-epoxyparthenolide (3) Colourless prisms from ethanol, mp 166-168ºC; $[\alpha]_D$ -40.8º (c 0.50, ethanol); UV (MeOH) λ_{max} nm: 220 (ε 3500); IR (KBr) ν_{max} cm^{-1}: 2941, 1763, 1392, 1296, 1251, 1147, 949, 765; ^1H NMR ($CDCl_3$): δ 1.29 (3H, s, Me-15), 1.33 (3H, s, Me-14), 2.42 (1H, dd, J = 14.1 & 7.1 Hz, H-7), 2.81 (1H, dd, J = 10.6 & 2.4 Hz, H-1), 2.86 (1H, d, J = 9.4 Hz, H-5), 3.88 (1H, t, J = 9.9 Hz, H-6), 5.57 (1H, d, J = 3.3 Hz, H-13), 5.64, 6.27 (1H, d, J = 3.9 Hz, H-13); FAB *m/z* 265 $[M + H]^+$.

Antifungal assay. The antifungal activity of *M. grandiflora* extracts and the isolated sesquiterpene lactones was tested using the radial growth technique method (Zambonelli *et al*., 1996). Appropriate volumes of the stock solutions of the plant extracts and sesquiterpenes in dimethyl sulfoxide (DMSO) were added to molten nutrient agar (Czapek-Dox Agar; CDA) to obtain a range of concentrations (0.05, 0.1,

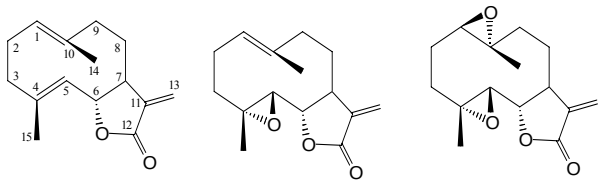
1, 10, 50, 100, 200, 400, 600, 800, 1000, µg mL^{-1}) immediately before pouring into the Petri dishes (9.0 cm in diameter) at 40-45ºC. Each concentration was tested in triplicate. Parallel controls were maintained with DMSO mixed with CDA. The discs of mycelial felt (0.5 cm diameter) of the plant pathogenic fungi, taken from 8-day-old cultures on CDA plates, were transferred aseptically to the centre of Petri dishes. Thiophanate-methyl (99.9%; Nippon Soda Co., Japan) was used as reference fungicide. The treatments were incubated at 25ºC in the dark. Colony growth diameter was measured after the fungal growth in the control treatments had completely covered the Petri dishes. Percentage of mycelial growth inhibition was calculated from the formula: Mycelial growth inhibition = [(DC-DT) /DC] × 100 (Pandy *et al*., 1982), where DC and DT are average diameters of fungal colony of control and treatment, respectively. The concentration of extract or/and compound that inhibiting the fungi mycelial growth by 50% (EC_{50}) was determined by a linear regression method (Finney, 1971).

RESULTS

Antifungal effect of *M. grandiflora* extracts. The antifungal activities of dichloromethane and methanol extracts of *M. grandiflora* in terms of radial growth inhibition are summarized in Table I. Dichloromethane extract of the stem bark showed the highest antifungal activity against *Helminthosporium* spp. and *R. solani* with EC_{50} values of 21.72 and 24.37 µg mL^{-1}, respectively. Dichloromethane leaves extract exhibited the strongest antifungal effect against *A. alternata* and *Helminthosporium* spp. with EC_{50} values of 21.50 and 23.97 µg mL^{-1}, respectively. *F. oxysporum* and *F. culmorium* were less sensitive than the other tested fungi to both dichloromethane extracts. Methanol extract of the stem bark showed weaker antifungal activity than the dichloromethane extracts.

Isolation and structure determination of sesquiterpene constituents of *M. grandiflora*. Combination use of solvent partitioning, chromatographic fractionation on silica gel columns and recrystallization of *M. grandiflora* different extracts led to isolate two sesquiterpene lactones, costunolide (1) and parthenolide (2). The three extracts of this plant contained these two compounds but with different concentrations; costunolide (1) represented 1.88, 4.33 and 0.11% of crude extracts in dichloromethane extracts of leaves, the stem bark and methanol extract of the stem bark, respectively, while parthenolide (2) represented 12.25, 10.00 and 0.16% of dry crude extracts in the same order. Epoxidation of parthenoilde (2) with *m*-chloroperbenzoic acid gave 1,10-epoxyparthenolide (3). The structure (Fig. 1) of costunolide (1), parthenolide (2) and parthenolide derivative, 1,10-epoxyparthenolide (3), was elucidated by using physico-chemical properties and spectroscopic methods, including high resolution mass spectroscopy (HR-MS), ^{1}H-NMR and ^{13}C-NMR.

Fig. 1. Chemical structure of sesquiterpene lactones (1-3)



costunolide (**1**) parthenolide (**2**) 1,10-epoxyparthenolide (**3**)

Table I. Fungicidal activity of dichloromethane and methanol extracts of *Magnolia grandiflora* against six plant pathogenic fungi

Fungus	EC_{50} (µg ml^{-1})	95% Confidence limits		Slope
		Upper	Lower	
	Dichloromethane stem bark extract			
Alternaria alternata	47.19	91.32	25.98	0.54
Helminthosporium spp.	21.72	62.31	8.47	0.29
Nigrospora spp.	41.17	61.30	28.20	0.84
Fusarium oxysporum	366.06	541.28	49.72	0.28
Fusarium culmorium	>1000	-	-	-
Rhizocotonia solani	24.37	63.34	10.40	0.32
	Methanol stem bark extract			
Alternaria alternata	569.68	807.46	404.00	1.04
Helminthosporium spp.	960.07	1139.70	808.97	2.99
Nigrospora spp.	>1000	-	-	-
Fusarium oxysporum	>1000	-	-	-
Fusarium culmorium	>1000	-	-	-
Rhizocotonia solani	>1000	-	-	-
	Dichloromethane leaves extract			
Alternaria alternata	21.50	32.72	14.29	0.67
Helminthosporium spp.	23.97	51.78	11.86	0.39
Nigrospora spp.	46.62	58.16	37.48	1.59
Fusarium oxysporum	>1000	-	-	-
Fusarium culmorium	198.15	404.15	102.29	0.82
Rhizocotonia solani	43.32	89.33	22.66	0.48

Fungicidal activity of sesquiterpene lactones (1-3). Fungicidal effects of the two isolated sesquiterpene lactones (1 & 2) and 1,10-epoxyparthenolide (3) against six plant pathogenic fungi are shown in Table II. Costunolide (1) revealed the strongest antifungal activity among the tested sesquiterpene compounds against three fungi, *Nigrospora* spp., *R. solani* and *Helminthosporium* spp., with EC_{50} values of 0.48, 2.92 and 2.96 µg mL^{-1}, respectively and the lowest activity against *F. oxysporum* and *F. culmorium* (EC_{50} = > 1000 µg mL^{-1} of each). Parthenolide (2) exhibited the highest antifungal activity against*A. alternata* and *F. culmorium* (EC_{50} = 4.07 & 50.27 µg mL^{-1}, respectively). The sesquiterpene lactones (1-3) showed a very weak antifungal activity against *F. oxysporum* ((EC_{50} > 1000 µg mL^{-1}). 1,10-Epoxyparthenolide (3) displayed the weakest antifungal activity among the tested compounds against all of tested fungi with two exceptions; the first case with *F. oxysporum* in which it showed similar activity of 1 and 2 and the second case with *F. culmorium* in which it exhibited higher activity than 1. The three sesquiterpene lactones (1-3) showed stronger antifungal activity (EC_{50} = 2.96, 3.85 &

28.55 μg mL^{-1}, respectively) than a reference fungicide, thiophanate-methyl (EC_{50} = 55.96 μg mL^{-1}), against *Helminthosporium* spp. Compound 1 (EC_{50} = 2.92 μg mL^{-1}) was also more potent than thiophanate-methyl (EC_{50} = 3.25 μg mL^{-1}) against *R. solani*.

DISCUSSION

Our results showed that dichloromethane extracts of leaves and the stem bark have a remarkable antifungal activity against four of the six tested fungi. Although the EC_{50} values are high compared with those of the thiophanate-methyl fungicide, these results are of interest since they have been obtained with the crude extracts and it is widely accepted that plant extracts that are active at EC_{50} values less than 100 μg mL^{-1} could be considered to have a good potency level (Rios *et al*., 1988). Methanol extract showed a weak or no antifungal activity against all of the tested fungi. The strong antifungal activity of dichloromethane extracts compared with methanol extract could be attributed to the presence of the two sesquiterpene lactones, costunolide (1) and parthenolide (2) at higher concentrations. These two compounds represented 14% of each dichloromethane extracts, while they represented only 0.27% of methanol extract.

The chemical structure (Fig. 1) of the isolated sesquiterpene lactones, costunolide (1) and parthenolide (2) and the parthenolide derivative, 1,10-epoxyparthenolide (3) was established by extensive analysis of ^{1}H-NMR, ^{13}C-NMR and mass spectrometry data and confirmed by comparison of the spectroscopic data with those reported in the literature (El-Feraly & Chan, 1978).

The antifungal activity of sesquiterpene lactones 1, 2 and 3 against six pathogenic fungi revealed that these compounds possessed a potent antifungal activity against four of the tested fungi. It should be pointed that costunolide (1) showed higher antifungal activity than the reference fungicide, thiophanate-methyl, against *Helminthosporium* spp. and *R. solani*. Moreover, parthenolide (2) and 1,10-epoxyparthenolide (3) were more active than thiophanate-methyl against *Helminthosporium* spp. It has been reported that costunolide (1), isolated from the roots of *Saussurea lappa*, showed strong antifungal activity (EC_{50} = 6.0 μg mL^{-1}) against the filamentous fungus *Cunninghamella echinulata* (Barrero *et al*., 2000). With regard to the structure-antifungal relationship, costunolide (1) with 4,5 double bond and parthenolide (2) with 4,5-epoxy group displayed stronger antifungal activity than 1,10-epoxyparthenolide (3) with 1,10 and 4,5-diepoxy groups against the four most sensitive fungi, *A. alternata*, *Nigrospora* spp, *R. solani* and *Helminthosporium* spp, suggesting that the presence of function groups and the polarity could play an important role on the antifungal potency of these compounds. This finding is in a good agreement with those reported on the antifungal activity of sesquiterpene melampolides against spores of the fungus *Pyricularia oryzae* in which the least polar one showed the greatest activity, suggesting a correlation between polarity and the antifungal activity of melampolides (Inoue *et al*., 1995). Similarly, Barrero *et al*. (2000) stated that the low polar sesquiterpene lactones the more potent antifungal activity. It is well known that the presence of α-methylene-γ-lactone is essential for potent antifungal activity of sesquitrpene lactones. Other function groups and their position on the skeleton may also enhance or reduce the activity of sesquitrpene lactones (Picman, 1986). It could be concluded that, besides the, α-methylene-γ-lactone, a relatively low polarity of costunolide (1) and parthenolide (2) is actually responsible for their strong antifungal activity. The low polarity of these compounds is matched with optimum lipophility degree required for passing through the fungal cell wall.

Table II. Fungicidal activity of sequiterpene lactones of *Magnolia grandiflora* against six plant pathogenic fungi

Fungus	EC_{50} (μg ml^{-1})	95% Confidence limits		Slope
		Upper	Lower	
		Costunolide (1)		
Alternaria alternata	7.50	9.90	5.64	1.02
Helminthosporium spp.	2.96	5.15	1.59	0.58
Nigrospora spp.	0.48	0.97	0.20	0.83
Fusarium oxysporum	>1000	-	-	-
Fusarium culmorium	>1000	-	-	-
Rhizocotonia solani	2.92	5.14	1.55	0.58
		Parthenolide (2)		
Alternaria alternata	4.07	4.84	3.41	2.13
Helminthosporium spp.	3.85	5.12	2.85	1.12
Nigrospora spp.	2.39	3.04	1.85	1.56
Fusarium oxysporum	>1000	-	-	-
Fusarium culmorium	50.27	86.89	30.33	0.65
Rhizocotonia solani	7.62	12.90	4.38	0.51
		1,10-epoxyparthenolide (3)		
Alternaria alternata	11.81	14.73	9.43	2.06
Helminthosporium spp.	28.55	37.34	21.75	1.08
Nigrospora spp.	123.97	152.58	100.90	1.72
Fusarium oxysporum	>1000	-	-	-
Fusarium culmorium	229.30	300.09	175.63	2.03
Rhizocotonia solani	178.72	230.63	138.88	1.73
		Thiophanate-methyl		
Alternaria alternata	0.34	0.43	0.27	1.54
Helminthosporium spp.	55.96	184.53	20.43	1.28
Nigrospora spp.	0.22	0.28	0.17	1.66
Fusarium oxysporum	6.42	7.74	5.33	1.64
Fusarium culmorium	7.58	9.99	5.79	0.92
Rhizocotonia solani	3.25	4.01	2.64	1.34

In conclusion, the present results clearly indicate that the dichloromethane extracts of *M. grandiflora* possessed significant antifungal activity. In addition, sesquiterpene lactones, costunolide (1) and parthenolide (2), proved to be promising antifungal agents against the foliar fungi, *A. alternata*, *Helminthosporium* spp, *Nigrospora* spp and the soil borne fungus, *R. solani*. To our knowledge, this is the first report on the antifungal activity of extracts and sesquiterpene constituents of *M. grandiflora* against plant pathogenic fungi. Therefore, intensive studies on the structure-antifungal activity relationships of this class of compounds are highly recommended.

REFERENCES

Barrero, A.F., J.E. Oltra, M. Alvarez, D.S. Raslan, D.A. Saude and M. Akssira, 2000. New sources and antifungal activity of sesquiterpene lactones. *Fitoterapia*, 71: 60–4

Baruah, N.C., J.C. Sarma, N.C. Barua, S. Sarma and R.P. Sharma, 1994. Germination and growth inhibitory sesquiterpene lactones and a flavone from *Tithonia diverifolia*. *Phytochem.*, 36: 29–36

El–Feraly, F.S., 1984. Melampolides from *Magnolia grandiflora*. *Phytochem.*, 23: 2372–4

El–Feraly, F.S. and Y.M. Chan, 1978. Isolation and characterization of the sesquiterpene lactones costunolide, parthenolide, costunolide diepoxide, santamarine and reynosin from *Magnolia grandiflora* (L.) *J. Pharmace Sci.*, 67: 347–50

El–Feraly, F.S. and W.S. Li, 1978. Phenolic constituents of *Magnolia grandiflora* (L.) seeds. *Lloydia*, 41: 442–9

El–Feraly, F.S., Y.M. Chan and D.A. Benigni, 1979. Magnolialide: a novel eudesmanolide from the root bark of *Magnolia grandiflora*. *Phytochem.*, 18: 881–2

Finney, D.J., 1971. *Probit analysis*, p. 318. 3rd edn., Cambridge University Press, London

Goren, N., H.J. Woerdenbag and C. Bozok–Johansson, 1996. Cytotoxic and antibacterial activities of sesquiterpene lactones isolated from *Tanacetum praeteritum* subsp *praeteritum*. *Planta Med.*, 62: 419–22

Hashim, M.S. and K.S. Devi, 2003. Insecticidal action of the polyphenolic rich fractions from the stem bark of *Streblus asper* on *Dysdercus cingulatus*. *Fitoterapia*, 74: 670–6

Inoue, A., S. Tamogami, H. Kato, Y. Nakazato, M. Akiyama, O. Kodama, T. Akatsuka and Y. Hashidoko, 1995. Antifungal melapolides from leaf extracts of *Smallanthus sonchifolius*. *Phytochem.*, 39: 845

Luo, X.D, S.H. Wu, Y.B. Ma, D.G. Wu and J. Zhou, 2001. Sesquiterpenoids from *Magnolia grandiflora*. *Planta Med.*, 67: 354–7

Mansilla, H. and J.A. Palenzuela, 1999. Minor eudesmanolides from *Artemisia canariensis*. *Phytochem.*, 51: 995–7

Nakano, T., 1954. The alkaloids of Magnoliaceous plants. XIII Alkaloids of *Magnolia grandiflora* L. *Pharm. Bull.*, 2: 326–8

Neerman, M.F., 2003. Sesquiterpene lactones: a diverse class of compounds found in essential oils possessing antibacterial and antifungal properties. *I. J. Aromath.*,13: 114–20

Pandey, D.K., N.N. Tripathi, R.D. Tripathi and S.N.Z. Dixit, 1982. Fungitoxic and phytotoxic properties of essential oil of *Hyptis suaveolens*. *PflKrankh. PflSchutz*, 89: 344–9

Parka, S.B., S.E. Leeb, W.S. Choia, C.Y. Jeongc, C. Songd and K.Y. Chod, 2002. Insecticidal and acaricidal activity of pipernonaline and piperoctadecalidine derived from dried fruits of *Piper longum* (L.) *Crop Protec.*, 21: 249–51

Picman, A.K., 1986. Biological activities of sesquiterpenes. *Biochem. System. Biol.,* 14: 255–81

Rao, K.V. and T.L. Davis, 1982. Constituents of *Magnolia grandiflora*. III. Toxic principle of the wood. *J. Nat. Prod.*, 45: 283–7

Rao, K.V. and W.N. Wu, 1978. Glycosides of *Magnolia grandiflora* III Structural elucidation of magnolenin C. *Lloydia*, 41: 56–62

Rios, J.L., M.C. Recio and A. Villar, 1988. Screening methods for natural products with antimicrobial activity: a review of the literature. *J. Ethnopharmacol.*, 23: 127–49

Wu, Z.Y., T.Y. Zhou and P.G. Xiao, 1988. Xinhua Compendium of Materia Medica. *Shanghai Sci. Technol.,* p. 58. Publishing House.

Zambonelli, A., A. Zechini, D. Aulerio, A. Bianchi and A. Albasini, 1996. Effects of essential oils on phytopathogenic fungi *in vitro*. *J. Phytopath*, 144: 491–4

(Received 20 March 2005; Accepted 10 June 2005)