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Control of phytopathogenic fungi by the essential oil and methanolic extracts of *Erigeron ramosus* (Walt.) B.S.P.

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Abstract The efficacy of the essential oil and methanolic extracts of *Erigeron ramosus* (Walt.) B. S.P. was evaluated for controlling the growth of some important phytopathogenic fungi. The hydro-distilled essential oil was analysed by GC-MS. Thirty one compounds representing 95.3% of the total oil were identified, of which β-caryophyllene (24.0%), α-humulene (14.5%), 1,8-cineole (9.0%), eugenol (7.2%), globulol (7.1%), caryophyllene oxide (5.2%), δ-cadinene (5.0%), α-copaene (4.9%) and widdrol (2.0%) were the major compounds. Thus, the monoterpenes and sesquiterpenes were the predominant portions of the oil. Essential oil and methanol extract of *E. ramosus* and the derived fractions of hexane, chloroform and ethyl acetate were tested for anti-fungal activity, which was determined by disc diffusion and minimum inhibitory concentration (MIC) determination methods. The oil (1,000 ppm) and methanolic extracts (1,500 ppm) displayed great potential of anti-fungal activity as a mycelial growth inhibition against the tested phytopathogenic fungi such as *Fusarium oxysporum* (KACC 41083), *Phytophthora capsici* (KACC 40157), *Colletotricum capsici* (KACC 410978), *Fusarium solani* (KACC 41092), *Rhizoctonia solani* (KACC 40111), *Sclerotinia sclerotiorum* (KACC 41065) and *Botrytis cinerea* (KACC 40573), in the range of 49.3–70.3% and minimum inhibitory concentration ranging from 125–500 μg ml^{-1}. The results obtained from this study may contribute to the development of new anti-fungal agents to protect the crops from fungal diseases.

Keywords Anti-fungal activity · β-Caryophyllene · *Erigeron ramosus* · Essential oil · Phytopathogenic fungi

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Introduction

Fungi have long been recognized as causal agents of plant diseases. Rice sheath blight (*Rhizoctonia solani*), grey mold rot (*Botrytis cinerea*), fruit rot (*Fusarium solani*), vascular wilt (*Fusarium oxysporum*), water soaked spot (*Sclerotinia sclerotiorum*) and fruit rot (*Phytophthora capsici*) are important plant diseases (Saini and Sharma 1978; Lee and Rush

1983; Agrios 1988; Rojo et al. 2007). Chemical fungicides are known to be highly effective to control the post-harvest diseases in various vegetables and fruits. However, they are not considered as long-term solutions due to the concerns associated with exposure risks, health and environmental hazards, residue persistence, and development of tolerance (Lingk 1991). The increasing recognition and importance of fungal infections and the difficulties encountered in their treatment have stimulated the search for alternatives to synthetic chemical fungicides. In recent years, researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that they have developed to antibiotics (Hunter and Reeves 2002).

Essential oils are made up of many different volatile compounds and have been shown to possess antimicrobial and fungicidal properties (Karmen et al. 2003; Cakir et al. 2004). Essential oils and plant extracts are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional uses (Sawamura 2000). So, essential oils and plant extracts are one of the most promising groups of natural compounds for the development of safer anti-fungal agents.

The genus *Erigeron* is a member of the Compositae (Asteraceae) family and contains more than 400 species. *Erigeron ramosus* (Walt.) B.S.P. is an indigenous weed from northern America and Canada, widely found in fields (Nesom 1989) and it has been introduced to many parts of the world (Holm et al. 1979). This species is also commonly found all over Korea. Secondary metabolites isolated from *E. annuus* showed inhibitory effects on seed germination with potential value as agriculturally useful products (Oh et al. 2002). The chemical constituents of the genus *Erigeron* plants such as *E. annuus*, *E. philadelphicus* and *E. sumatrensis* have been previously investigated and shown to contain monoterpenoids, diterpenoid, sesquiterpenoids, triterpenoids, sterols and phenolic compounds (Waddell et al. 1983; Oh et al. 2002; Iijima et al. 2003), but no study has been reported on the analyses of essential oil and anti-fungal activity of *E. ramosus* occurring in Korea.

Therefore, the aims of the present study were (a) to examine the chemical composition of the essential oil of *E. ramosus* by GC-MS; and (b) to determine the efficacy of essential oil and methanolic extracts of *E. ramosus* against some phytopathogenic fungi with emphasis for the possible future use of the essential oil and plant extracts as alternative anti-fungal compounds.

Materials and methods

Plant material

The leaves, stems and flowers of *E. ramosus* were collected from the Kyungsan city area of the Republic of Korea in June 2006. The plant was identified on the basis of morphological features and a voucher specimen has been deposited in the herbarium of the Department of Biotechnology, Daegu University, Republic of Korea.

Isolation of the essential oil

The air-dried flowers part (250 g) of *E. ramosus*. was subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous sodium sulphate and preserved in a sealed vial at 4°C until further analysis.

Preparation of methanolic extracts

The air-dried leaves and stems of *E. ramosus* were pulverized into powdered form. The dried powder (50 g) was extracted three times with 80% methanol (200 ml) at room temperature and the solvents from the combined extracts were evaporated by a vacuum rotary evaporator (EYELA N-1000, Japan). The methanol extract (5.7 g) suspended in water and extracted successively with hexane, chloroform and ethyl acetate to give hexane (1.98 g), chloroform (1.26 g) and ethyl acetate (0.88 g) and residual methanol fractions (0.78 g), respectively. Solvents (analytical grade) for extraction were obtained from commercial sources (Sigma–Aldrich, St. Louis, MO, USA).

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis of the essential oil was performed using a SHIMADZU GC-MS (GC-17A) equipped with

a ZB-1 MS fused silica capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was used as the carrier gas at a constant flow rate of 1 ml min^{-1}. Injector and MS transfer line temperature were set at 220°C and 290°C, respectively. The oven temperature was programmed from 50°C to 150°C at 3°C min^{-1}, then held isothermal for 10 min and finally raised to 250°C at 10°C min^{-1}. Diluted samples (1/100, v/v, in methanol) of 1.0 μl was injected manually in the splitless mode. The relative percentage of the oil constituents was expressed as percentages by peak area normalization.

Identification of compounds of the essential oil was based on GC retention time on ZB-1 capillary column, computer matching of mass spectra with those of standards (Wiley 6.0 data of GC-MS system) and whenever possible, by co-injection with authentic compounds (Adam 2001).

Fungal pathogens

The plant pathogenic fungi were obtained from the Korean Agricultural Culture Collection (KACC), Suwon, Republic of Korea. Cultures of each fungal species were maintained on potato-dextrose agar (PDA) slants and stored at 4°C. The fungal species used in the experiment were *Fusarium oxysporum* (KACC 41083), *Phytophthora capsici* (KACC 40157), *Colletotricum capsici* (KACC 410978), *Fusarium solani* (KACC 41092), *Rhizoctonia solani* (KACC 40111), *Sclerotinia sclerotiorum* (KACC 41065) and *Botrytis cinerea* (KACC 40573).

Preparation of spore suspension and test samples

The spore suspension of *F. oxysporum*, *P. capsici*, *C. capsici*, *F. solani*, *S. sclerotiorum* and *B. cinerea* were obtained from their respective 10 days old cultures, mixed with sterile distilled water to obtain a homogenous spore suspension of 1 x 10^5 spore ml^{-1}. Essential oil and plant extracts were dissolved in dimethyl sulfoxide (DMSO) separately to prepare the stock solutions with their respective known weights, which were further diluted to prepare test samples.

Determination of anti-fungal activity of essential oil and methanolic extracts

Anti-fungal assay

The essential oil and extracts were bio-assayed by the poisoned food technique (Nene and Thapliyal 1979). Essential oil was mixed with dimethyl sulfoxide (DMSO) so as to ease its incorporation into the agar medium in the proportion 1 volume oil to 9 volumes DMSO. The essential oil was tested at 1,000 ppm (that is μl l^{-1}). Methanolic extracts (diluted in DMSO) were tested at 1,500 ppm. The oil and plant extracts were incorporated to the autoclaved and cooled (50°C) PDA medium through 0.45 μm Millipore filters. The medium amended with oil or extracts was then poured into sterilized Petri dishes. A mycelial disc of 5 mm diameter of the test pathogens taken from 10 day old culture, with the help of a sterilized cork borer was placed at the centre of the medium. Some plates prepared as controls received no oil or extracts but only DMSO. The plates were then sealed with parafilm and incubated at 27±2°C for 5–7 days, time by which the growth of control would have reached the edges of the plates. Growth inhibition of each of the fungal strains was calculated as the percentage of inhibition of radial growth relative to the control along with the anti-fungal effect on fungal mycelium. The plates were used in triplicates for each treatment.

The growth inhibition of treatment compared to control was calculated by percentage, using the following formula:

$$\text{Inhibition}(\%) = \{1 - \text{radial growth of treatment (mm)}/\text{radial growth of control (mm)}\} \times 100$$

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the essential oil and methanol extracts against fungal pathogens was determined by agar dilution method as described before (Mitscher et al. 1972). Briefly, ten-milliliter aliquots of potato dextrose broth (PDB) were prepared in 25 ml Erlenmeyer flask. The oil and plant

extracts were dissolved with DMSO, sterile filtered (0.45 μm) and then added to the different flasks in order to obtain concentrations of 62.5 to 2,000 μg ml^{-1} of culture medium. The final concentrations of DMSO in the assay did not exceed 2%. In addition, one flask with uninoculated oil or extract-free medium, was included as a sterile control. Using a micropipette, an inoculum of 5 μl (10^5 spore ml^{-1}) of the spore suspension was inserted into each flask of medium containing a known concentration of samples, as well as samples-free medium. To prepare the mycelial suspension of *R. solani*, the liquid PDB culture broth was homogenized by a surface sterilized morter and 50 μl ml^{-1} mycelial suspension was inoculated for MIC determination. Sterile mineral oil (~0.5 ml) was layered on the inoculated medium to inhibit sporulation, and then the flasks were capped with cotton plugs. All cultures were incubated in a shaking incubator at 150 rpm for 3–8 days at 27±2°C or until good growth was apparent in the oil or extract-free control. The growth in all flasks was visually compared with that of the control in order to determine % inhibition. The growth was scored in the following manner: 4+, growth comparable to that of the sample-free control; 3+, growth approximately 75% that of the control; 2+, growth approximately 50% that of the control; 1+, growth 25% or less that of the control; and 0, no visible growth (data not shown). The minimum concentration at which no visible growth was observed was defined as the MIC, which was expressed in μg ml^{-1}.

Statistical analysis

The essential oil, methanol extract and derived fractions of methanol extract were assayed for evaluating the anti-fungal activity. Each experiment was run in triplicate, and mean values were calculated. A *t*-test was computed for the statistical significance of the results.

Results

Chemical composition of essential oil

The hydrodistillation of the air-dried flowers part of *E. ramosus* gave the dark yellowish oil with a yield of 0.4% (w/w). GC-MS analyses of the oil led to the identification of 31 different compounds, representing 95.3% of the total oil. The identified compounds are listed in Table 1 according to their elution order on a ZB-1 capillary column. The oil contained a complex mixture consisting of mainly oxygenated mono- and sesquiterpene hydrocarbons. The major compounds detected were β-caryophyllene (24.0%), α-humulene

Table 1 Percentage composition of the essential oil of *Erigeron ramosus* (Walt.) B.S.P.

No	Compound[a]	R_t[b]	Composition (%)
I	2-Phenethyl alcohol	2.912	0.6
2	Eugenol	5.690	7.2
3	β-Chamigrene	6.864	0.6
4	cc-Humulene	7.044	14.5
5	Nerolidol	7.214	0.8
6	ɣ-Cadinene	7.541	0.6
7	α-Copaene	7.632	4.9
8	Aromadendrene	7.696	1.3
9	β-Caryophyllene	7.811	24.0
10	δ-Cadinene	8.095	5.0
11	*trans*-β-Famesene	8.422	0.6
12	Aromadendrene epoxide	8.728	0.5
13	Caryophyllene oxide	8.813	5.2
14	3-p-Menthen-9-ol	8.911	0.4
15	(Z)-5-Pentadecen-7-yne	8.982	0.5
16	β-Selinene	9.023	0.5
17	Spathulenol	9.120	1.4
18	(Z)-6-Hexadecen-4-yne	9.173	0.4
19	Widdrol	9.503	2.0
20	Globulol	9.650	7.1
21	Viridiflorol	10.004	1.3
22	Ledol	10.071	0.6
23	n-Nonanal	10.320	0.5
24	Ledane	10.792	0.5
25	Geraniol	10.891	1.9
26	Myristic acid	10.927	1.0
27	Patchulane	11.581	0.2
28	1,8-Cineole (Eucalyptol)	11.871	9.0
29	Dibutylphthalate	12.468	0.3
30	Stearic acid	12.887	0.7
31	Palmitic acid	13.126	1.2
	Total		95.3

[a] Compound listed in order of elusion from a ZB-1 capillary column

[b] Retention time (as minutes)

(14.5%), 1,8-cineole (9.0%), eugenol (7.2%), globulol (7.1%), caryophyllene oxide (5.2%), δ-cadinene (5.0%), α-copaene (4.9%) and widdrol (2.0%). Geraniol (1.9%), spathulenol (1.4%), viridiflorol (1.3%), nerolidol (0.8%), *trans*-β-farnesene (0.6%), ledol (0.6%), β-selinene (0.5%) and n-nonanal (0.5%) were also found to be the minor components of *E. ramosus* oil in the present study.

Anti-fungal activity of essential oil and methanolic extracts

The essential oil of *E. ramosus* exhibited a moderate to high anti-fungal activity against all the tested fungi except *B. cinerea*. At the concentration of 1,000 ppm, the essential oil showed potent inhibitory effect on the mycelium growth of six phytopathogens such as *P. capsici* (70.3%), *S. sclerotiorum* (68.0%), *F. solani* (65.4%), *R. solani* (60.3%), *C. capsici* (56.6%) and *F. oxysporum* (56.3%), as shown in Table 2. Also, the crude methanol extract and its derived hexane, chloroform and ethyl acetate fractions (1,500 ppm) showed mycelium growth inhibition against some of the phytopathogens but not for all. According to the results given in Table 3, methanol extract of *E. ramosus* showed a great potential anti-fungal activity against *S. sclerotiorum* (70.3%), *P. capsici* (63.4%), *F. oxysporum* (58.2%), *C. capsici* (54.6%) and *R. solani* (54.6%). Hexane fraction showed weak inhibition (58.3–49.3%) against *F. oxysporum*, *P. capsici* and *S. sclerotiorum*. Chloroform fraction had good anti-fungal activity against *P. capsici*, *S. sclerotiorum, F. oxysporum*, *C. capsici* and *R. solani* with mycelium growth inhibition ranged from 49.3–65.5%, while ethyl acetate fraction inhibited 49.4–63.5% mycelium growth of *F. oxysporum*, *P. capsici*, *C. capsici* and *S. sclerotiorum*. However, the crude methanol extract and its hexane, ethyl acetate and chloroform fractions did not show any inhibitory effect against *F. solani* and *B. cinerea*.

Minimum inhibitory concentration (MIC)

According to the results given in Table 2, MIC of essential oil was found more effective against *P. capsici*, *F. solani* and *S. sclerotiorum* (125, 250 and 250 μg ml^{-1}, respectively) as compared to those of *F. oxysporum* and *C. capsici* (500 μg ml^{-1} for each). On the other hand, the crude methanol extract of *E. ramosus* (Walt.) B.S.P. and its chloroform fraction were found more susceptible than hexane and ethyl acetate fraction against the tested fungi (Table 4). As control, DMSO did not affect the growth of sample strains at the concentration used in this study. The MIC values of methanol extract and its chloroform fraction against *P. capsici*, *S. sclerotiorum, F. oxysporum* and *C. capsici* were found within the range125–500 μg ml^{-1}, whereas no inhibition was observed against *F. solani* and *B. cinerea*. The ethyl acetate fraction displayed anti-fungal activity against mycelium growth of *F. oxysporum*, *P. capsici*, *C. capsici* and *S. sclerotiorum* with MIC values of 250–500 μg ml^{-1}. However, the hexane fraction did not show activity against all the phytopathogens tested, other than *P. capsici* and *S. sclerotiorum* (MIC: 500 μg ml^{-1} for each).

Discussion

The increasing social and economic implications caused by fungal pathogens means there is a constant

Table 2 Growth inhibition of phytopathogenic fungi by the essential oil (1,000 ppm) *of Erigeron ramosus* (Walt.) B.S.P.

Fungal strains	Mycelial growth inhibition[a]		MIC (μg ml^{-1})
	mm	%	
F. oxysporum (KACC 41083)	19.0±0.5	56.3±1.5	500
P. capsic (KACC 40157)	13.4±0.5	70.3±1.5	125
C. capsid (KACC 410978)	19.6±0.5	56.6±0.7	500
S. scierotiorum (KACC 41065)	14.5±0.3	68.0±1.1	250
F. solani (KACC 41092)	15.6±0.4	65.4±1.2	250
R. solani (KACC 40111)	17.9±0.7	60.3±1.4	na
B. cinerea (KACC 40573)	nd	nd	na

nd no detection of anti-fungal activity, *na* not applicable

[a] Values are represented as the mean±S.D. of three experiments

Table 3 Growth inhibition of phytopathogenic fungi by the methanol extract and its derived fractions (1,500 ppm) of *Erigeron rcsmosus* (Walt.) B.S.P.

Fungal strains	Mycelial growth inhibition[a]							
	CME		HAF		CBF		EAF	
	mm	(%)	mm	(%)	mm	(%)	mm	(%)
F. oxysponim (KACC 41083)	18.7±05	58.2±1.1	22.8±0.3	49.3±05	203±05	54.6±1.4	19.0±0.4	56.3±05
P. capsici (KACC 40157)	165±0.4	63.4±1.1	18.8±05	58.3±1.1	15.5±05	655±05	16.6±0.4	63.4±1.1
C. capsici (KACC 410978)	20.3±0.5	54.6±1.3	nd	nd	203±0.5	54.6±0.7	22.7±0.5	49.4±0.6
S. sclerotiorum (KACC 4106)	13.5±0.6	70.3±1.2	18.8±05	58.3±1.3	15.5±0.4	655±0.5	161±0.4	635±1.2
F. solani (KACC 41092)	nd	nd	nd	nd	nd	nd	nd	nd
R. soiani (KACC 40111)	203±0.6	54.6±15	nd	nd	22.8±03	49.3±05	nd	nd
B. cinerea (KACC 40573)	nd	nd	nd	nd	nd	nd	nd	nd

CME Crude methanol extract, *HAP* Hexane fraction, *CEF* Chloroform fraction, *EAF* Ethyl acetate fraction, *nd* no detection of anti-fungal activity

[a] Values are represented as the mean±S.D. of three experiments

striving to produce safer food crops and to develop new anti-fungal agents. In general, plant derived essential oils are considered to be non-phytotoxic compounds and potentially effective against plant pathogenic fungi (Pandey et al. 1982). In recent years, interests have been generated in the development of safer anti-fungal agents such as plant-based essential oils and extracts to control phytopathogens in agriculture (Costa et al. 2000). Thus essential oils and plant extracts are promising natural anti-fungal agents with potential applications in agro-industries to control phytopathogenic fungi causing severe destruction of crops.

Table 4 Minimum inhibitory concentrations of methanol extract and its derived fractions of *Erigeron ramosus* (Walt.) B.S.P. against phytopathogenic fungi

Fungal strains	MIC (μg ml^{-1})			
	CME	HAF	CHF	EAF
F. oxysporum (KACC 41083)	500	nd	500	500
P. capthci (KACC 40157)	250	500	125	500
C. capsici (KACC 410978)	500	nd	500	500
S. scierotioruin (KACC 41065)	125	500	250	250
F. solarn (KACC 41092)	nd	nd	nd	nd
P. solani (KACC 40111)	na	na	na	na
B. cznerea (KACC 40573)	nd	nd	nd	nd

CME Crude methanol extract, *HAF* Hexane fraction, *CHF* Chloroform fraction, *EAF* Ethyl acetate fraction, *nd* no detection of anti-fungal activity, *na* not applicable

The hydrodistillation of the dried flowers part of *E. ramosus* gave dark yellowish oil with the major components of the oil having oxygenated mono- and sesquiterpenes, and their respective hydrocarbons. In recent years, several researchers have reported that mono- and sesquiterpene hydrocarbons and their oxygenated derivatives are the major components of essential oils from plant origin, which have enormous potential to inhibit microbial pathogens (Cakir et al. 2004; Nuñez et al. 2007). In general, the active antimicrobial compounds of essential oils are terpenes, which are plant secondary metabolites, and it would seem reasonable that their antimicrobial or anti-fungal mode of action might be also related to that of other compounds such as hydrocarbons, acids, esters and aromatics.

The essential oil of *E. ramosus* showed a remarkable anti-fungal effect against all the fungi tested except *B. cinerea*. This activity could be attributed to presence of β-caryophyllene (24.0%), α-humulene(14.5%), 1,8-cineole (9.0%), eugenol (7.2%), globulol (7.1%), caryophyllene oxide (5.2%), δ-cadinene (5.0%), α-copaene (4.9%) and widdrol (2.0%), which significantly (70.3%) inhibited the growth of all the phytopathogens tested, and /or other major and minor oxygenated mono- and sesquiterpenes present in the oil.

Some earlier papers on the anti-fungal properties of the essential oils of some species of various genera have shown that they have a varying degree of growth inhibition effects against some *Fusarium, Botrytis* and *Rhizoctonia* species (Alvarez-Castellanos

et al. 2001; Bouchra et al. 2003). Although the different compounds exhibited varying degrees of antifungal activity, β-caryophyllene and caryophyllene oxide were very fungitoxic against the studied *Fusarium* species (Cakir et al. 2004). Deba et al. (2008) reported that the essential oils of *B. pilosa* containing β-caryophyllene, caryophyllene oxide and cadinene had anti-fungal activities at 150 ppm doses against *F. solani*, *F. oxysporum* and *Corticum rolfsii*. Eugenol, β-caryophyllene and eugeyl acetate are the major components of clove oil, which exhibited potential anti-fungal activity against *Aspergillus flavus* (Omidbeygi et al. 2007). Salamci et al. (2007) reported that essential oils of *Tanacetum aucheranum* and *Tanacetum chiliophyllum*, which contain 1,8-cineole as a major component, showed potent anti-fungal activity against *S. sclerotiorum, R. solani* and *F. oxysporum*. δ-Cadinene, β-farnesene and spathulenol rich essential oil exhibited anti-fungal activity against *R. solani* (Cakir et al. 2005). Also, the oils of *Pistacia versa*, *P. terebinthus* and *P. lentiscus* had moderate activities at 750 ppm doses against *R. solani* (Alvarez-Castellanos et al. 2001). In spite of this, most of these oils are available for purchase as whole or as a part of pharmaceutical or cosmetic products, indicating that weak toxic properties do not prohibit their use. However, the ongoing investigation of toxic or irritant properties is imperative, especially when considering any new products for human use, whether medicinal or otherwise.

Certain plant extracts with their derived fractions and phytochemicals act in many ways on various types of disease complex, and may be applied to the crops in the same way as other agricultural chemicals. *E. ramosus* can also be used as a leading factor in a wide range of activities against many phytopathogens, where the pathogens have developed resistance against the specific fungicides (Elad 1991). In this study, the essential oil and the different extracts showed varying anti-fungal activity against some phytopathogenic fungi, which could be attributed to the presence of phenolic compounds and oxygenated monoterpenes and sesquiterpene hydrocarbons, and these finding are in agreement with the previous reports (Karmen et al. 2003; Omidbeygi et al. 2007). Volatile compounds, such as β-caryophyllene, α-humulene, 1,8-cineole, eugenol, globulol, caryophyllene oxide, δ-cadinene, α-copaene and widdrol have been claimed to contain the strong anti-fungal or antimicrobial properties (Azaz et al. 2002; Oumzil et al. 2002; Filipowicz et al. 2003; Cakir et al. 2005; Erdemgil et al. 2007; Nuñez et al. 2007; Salamci et al. 2007). Those claims are further supported by our findings; indicating high contents of β-caryophyllene, α-humulene, 1,8-cineole, eugenol, globulol, caryophyllene oxide, δ-cadinene, α-copaene and widdrol; comprising 78.9% of the oil (Table 1). Also, the anti-fungal activity of individual components of essential oils such as β-caryophyllene, caryophyllene oxide and 1,8-cineole have been reported previously (Cakir et al. 2004; Salgueiro et al. 2006). On the other hand, the components present in lower amounts, such as geraniol (1.9%), spathulenol (1.4%), viridiflorol (1.3%), nerolidol (0.8%), *trans*-β- farnesene (0.6%), ledol (0.6%), β- selinene (0.5%) and n-nonanal (0.54%), also contributed to anti-fungal activity of the oil (Gijsen et al. 1992; Bisignano et al. 2001; Bougatsos et al. 2004; Lee et al. 2007). It is also possible that the minor components might be involved in some type of synergism with the other active compounds (Marino et al. 2001). Therefore, it would also be interesting to study the effects of essential oils and crude extracts of *E. ramosus* against other important fungi for developing new anti-fungal agents to control serious fungal diseases in crops. In this regard, we have started a programme aimed at the evaluation of anti-fungal activity of essential oil and methanol extracts of *E. ramosus* and its derived fractions of hexane, chloroform and ethyl acetate, in the hope of finding new natural products to be used in the biocontrol of phytopathogens.

Thus, *E. ramosus* could become an alternative to synthetic fungicides for use in agro-industries and also to screen and develop such novel types of selective and natural fungicides in the treatment of many microbial phytopathogens causing severe destruction to crop and vegetables. A further study will evaluate the bio-active compounds present in methanolic extracts of *E. ramosus*.

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