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Antioxidant and antifungal properties of the essential oil of *Anisomeles indica* from India

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Essential oil of *Anisomeles indica* (Labiatae) growing in India was hydrodistilled and analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). A total of 26 compounds, representing 90.70% of the essential oil, were identified, and the major components were eugenol (17.63%), α-terpeneol (α–terpineol, 14.17%), β-pinene (8.11%), bornyl acetate (5.61%), etc. Essential oil was assayed for its antioxidant and antifungal activities. Antifungal activity of the essential oil was evaluated by poisoned food technique, that resulted in maximum activity against *Pithium aphanidermatum* (ED_{50} 51.58 µg/ml) followed by *Rhizoctonia bataticola* (ED_{50} 72.80 µg/ml). Antioxidant activity of the essential oil was evaluated by using 1,1-diphenyl-2-picrilhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) methods together with three antioxidant standards, ascorbic acid, tert-butyl-4-hydroxy toluene (BHT) and gallic acid.

Key words: *Anisomeles indica*, essential oil, sesquiterpenes, gas chromatography-mass spectrometry (GC-MS), antioxidant activity, antifungal activity.

INTRODUCTION

Plant-derived natural chemicals, known as secondary metabolites, are effective in their roles of protection, adaptation and pollination. Secondary metabolites are mainly used in food, pharmaceutical, chemical, cosmetic industries and agriculture (Philipson, 1990; Sokmen and Gurel, 2001). Plant-derived natural products are abundant in nature. Many of them exhibit numerous biological activities (Baratta et al., 1998 and 1998). Antioxidants of plant origin are an increasingly important ingredient in food processing (Madsen and Bertelsen, 1995). The most widely used synthetic antioxidants in food (butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA)) are very effective in their role as antioxidants. However, their use in food products has been falling off due to their instability, as well as due to a suspected action as promoters of carcinogenesis (Namiki, 1990; Ito et al., 1983). So, there is a growing interest in studies of natural healthy (non-toxic) additives as potential antioxidants (Tomaino et al., 2005).

Plants have an almost limitless ability to synthesize aromatic substances, most of which are terpenoids and polyphenolic derivatives. In the past decade, the essential oils and various extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the preservation of foods from the toxic effects (Scalbert et al., 2005). Naturally occurring terpenoids has been known to possess various biological activity, notably antibacterial, antifungal and antioxidant properties (Sagdic et al., 2003; Vardar-Unlu et al., 2003; Burt, 2004; Sokmen et al., 2004; Tepe et al., 2004). *Anisomeles indica* Linn. is found wide spread throughout the tropical and subtropical region of India, ascending to 6000 feets

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in the Himalayas. It is useful as carminative and astringent. The plant is well known for its medicinal, antipyretic, analgesic, anti-inflammatory, antibacterial and herbicidal activities (Dharmasiri et al., 2000, 2003). Essential oil was extracted from leaves of the plant and GC/MS analysis revealed eighteen mono and sesquiterpenoid constituents (Ushir et al., 2010). Methanol extract of the plant was reported to have antioxidative characteristics (Huang et al., 2012). The plant was reported to consist of diterpenoids, ovatodiolide, stigmasterol, β-sitosterol, stigmasterol, tetra cosine, tetra coranel, anisomlic acid, paraffins and fatty acids derivatives (Alam et al., 2000; Yadava and Barsainya, 1998). Due to lack of knowledge about antifungal and antioxidant activity of chemical constituents of *A. indica*, this work was carried out in order to evaluate the essential oil as potential antioxidant and antifungal agent. Antioxidant activities were evaluated using two complementary assays methods, 1,1-diphenyl-2-picrilhydrazyl (DPPH) radical scavenging potential and ferric ion reducing antioxidant power (FRAP) assays. Antifungal potentials were estimated against a panel of eight fungi. As far as our literature survey could ascertain, this study can be assumed as the first report on the antioxidant and antifungal activities of chemical constituents of *A. indica*.

MATERIALS AND METHODS

Reagents and chemicals

1,1-diphenyl-2-picrilhydrazyl (DPPH), 2,4,6-tris-2,4,6-tripiridyl-2-triazine (TPTZ), ferric chloride, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), L-ascorbic acid, tert-butyl-4-hydroxy toluene (BHT), and gallic acid were purchased from Sigma-Aldrich and used without further purification.

Plant

Leaves of *A. indica* Linn were collected from the forest area of Himachal Pradesh (India), in 2010. A voucher specimen (A-AI-06-10) of the plant was authenticated and deposited in the Herbarium of Department of Botany, Himachal Pradesh Agricultural University, and Himachal Pradesh, India.

Extraction and analysis

Essential oil from the fresh leaves of *A. indica* was isolated by hydro-distillation for 4 h, using a Clevenger apparatus according to the method described in the current European Pharmacopoeia (6th edition, 2008). The oil was dried over anhydrous sodium sulphate and stored under at 4°C. The yield of yellowish coloured essential oil was 0.65% (v/w).

Analytical gas chromatography was carried out on a Perkin Elmer 115 gas chromatograph fitted with a 30 m × 0.25 mm ID, 0.25 µm film thickness. Column temperature was initially kept at 70°C for 2 min, then gradually increased to 120°C at 2°C min^{-1} rate, held for 2 min and finally raised to 250°C at 5°C min^{-1}. Diluted samples (1/100 v/v, in hexane) of 1 µl were injected at 250°C. Flame ionization detection (FID) was performed at 280°C. GC-MS analysis was performed on an Agilent 6850 Series II apparatus, fitted with a fused silica HP-5MS non-polar capillary column (30 m × 0.25 mm ID, film thickness 0.25 µm). Carrier gas was helium at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 230 and 270°C, respectively. Ion source temperature was 200°C. The injection volume was 0.1 µl with a split ratio of 1:50. Mass range was from m/z 40 to 550 amu. Identification of components of the essential oils was based on GC retention indices and computer matching with the Wiley and TRLIB Library as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Adams, 1989; Swigar and Silverstein, 1981).

Determination of antioxidant capacity

Essential oil and analytical standards were dissolved in methanol in order to obtain 1 mg/100 ml solution, which was chosen as appropriate concentration for assessing antioxidant activity. For each antioxidant assay, a Trolox aliquot was used to develop a 0.5 to 10 mmol/L standard curve. All data were then expressed as Trolox Equivalents (mmol/L) and antioxidant activity referred to as Trolox Equvalents Antioxidant Capacity (TEAC).

DPPH assay

The antioxidant capacity of the essential oil was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH (Brand-Williams et al., 1995). Aliquots (200 µl) were added to 3 ml of DPPH solution (6×10^{-5} mol/L) and the absorbance was determined at 515 nm after 90 min. Methanol was used to zero the spectrophotometer. The absorbance of the DPPH radical without the antioxidant, that is, the control, was measured. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution (Blois, 1958). All determinations were performed in triplicate.

FRAP assay

The total antioxidant potential of the sample was determined using a ferric reducing ability (FRAP) assay (Benzie and Strain, 1996; Iris et al., 1999; Nilsson et al., 2005) as a measure of ‘‘antioxidant power’’. This assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe^{2+} tripyridyltriazine compound from colorless oxidized Fe^{3+} form by the action of electron donating antioxidants.

The working FRAP reagent was prepared by mixing solution of 10 mmol/L TPTZ in 40 mmol/L HCl and 12 mmol/L ferric chloride, diluted in 300 mmol/L sodium acetate buffer (pH 3.6) at a ratio of 1:1:10. Aliquot (200 µl) of oil solution was added to 3 ml of the FRAP solution, and allowed to react for 90 min at 37°C, before reading the absorbance at 593 nm. The change in absorbance between the final reading and the blank reading was selected for the calculation of FRAP values. Standard curve was prepared using different concentrations (0.1 to 5 mmol/L) of $FeCl_2.4H_2O$. All determinations were performed in triplicate.

Antifungal activity

Essential oil was tested for their antifungal activity against pathogenic fungi, namely, *Rhizoctonia solani* ITCC 5251, *Rhizoctonia bataticola* ITCC 4721, *Sclerotium rolfsii* ITCC 2330, *Fusarium udum* ITCC 2169, *Fusarium oxysporum* ITCC 5656, *Alternaria solani* ITCC 4342, *Pithium aphanidermatum* ITCC 3339 and *Aspergillus flavus* ITCC 1742 by the poisoned food technique (Nene and Thapliyal, 1979). A stock solution of 1000 µg/ml of the test compound was prepared, which was further diluted with

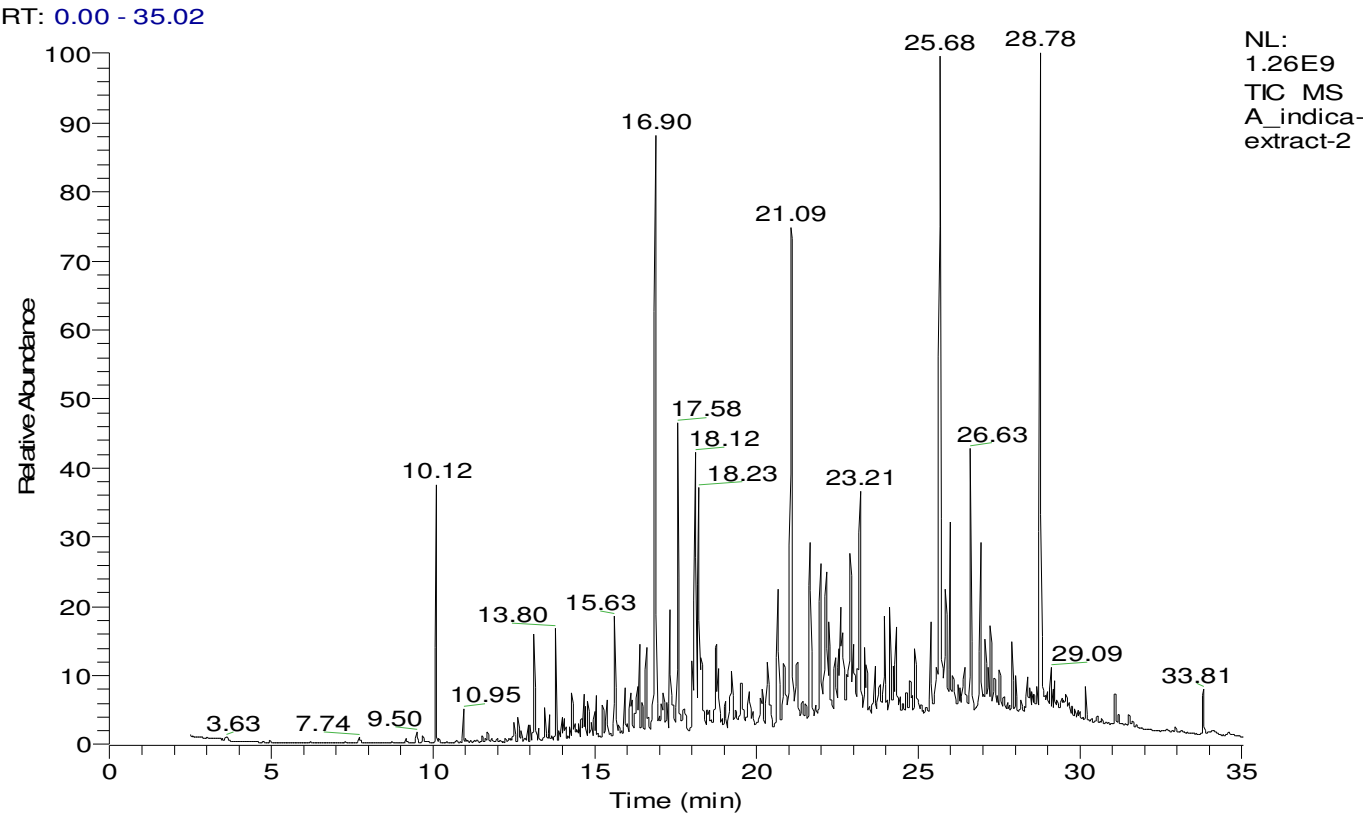

Figure 1. Total ion chromatogram (TIC) of leaf essential oil of *Anisomeles indica*.

acetone to give the required concentrations of 500 to 62.5 µg/ml. These solutions were added to the media (65 ml) contained in conical flasks to obtain the desired concentrations of the test compound in the media. The medium was poured into a set of two Petri dishes under aseptic conditions in a laminar flow. After solidification, a 5 mm mycelial disk cut from the actively growing front of a 2 weeks old colony of the desired pathogenic fungus was then placed with the inoculum side down in the centre of each treated Petri dish, aseptically. Treated Petri dishes were then incubated at 28°C until the fungal growth was almost complete in the control plates. All experiments were in quadruplicate for each treatment against each fungus.

The mean and standard deviation were calculated from the four replicates of each treatment and the percentage inhibition of growth (% I) was calculated using I (%) = (C-T)/C × 100.

For calculation of ED_{50} values, the percent inhibition was converted to corrected percent inhibition as corrected inhibition (%) = [(% I-C.F.) / (100-C.F.)] × 100, where CF is the correction factor obtained as correction factor (CF) = [(90-C)/C] × 100, where 90 is the diameter of the Petri dish in mm and C is the diameter of growth of the fungus in control plates. From the concentration (µg/ml) and corresponding corrected percent inhibition data of each compound, the ED_{50} (µg/ml) value was calculated statistically by Probit analysis with the help of Probit Package of MSTATC software.

Statistical analysis

All the experimental data of both antioxidant and antifungal activity were recorded as mean ± standard deviation. The analysis of variance was computed using Statistical Package for Social Sciences (SPSS version 10.0), and treatment means were compared by using Duncan's multiple range test (DMRT) at 5% levels.

RESULTS AND DISCUSSION

Chemical composition of essential oil

The hydro-distillation of leaves of *A. indica* yielded essential oil (0.65%; v/w). Upon GC/MS analysis, the essential oil was found to contain 26 constituents (Figure 1) accounting for 90.70% of the essential oil (Table 1). The volatile oil contained 75.83% monoterpenes and 14.87% sesquiterpenes constituents. The oxygenated compounds accounted for 52.73% of the essential oil, whereas hydrocarbon compounds were 37.97%. Monoterpenes constituted the major group in which eugenol (17.63%) was most abundant and characterised by the *m/z* 164. Other major monoterpene constituents were α-terpeneol (14.17%), β-pinene (8.11%), bornyl acetate (5.61%), etc. Among sesquiterpenes, caryophyllene (3.71%) and β-farnesene (2.77%) were identified as major constituents. The presence of eugenol, β-pinene, α-thujone and cymene as major constituents of the essential oil is supported by the earlier reports (Yogesh and Krishnakant, 2010).

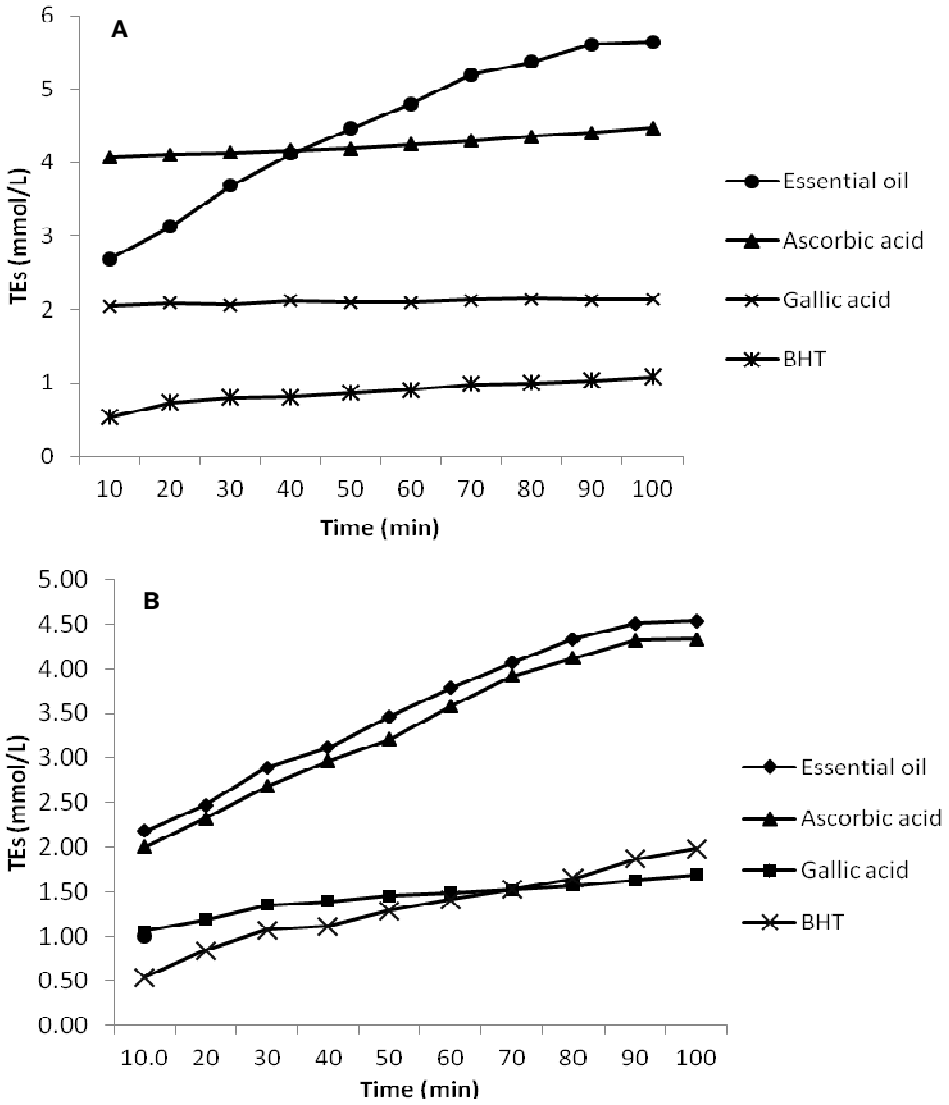

Figure 2. Antioxidant capacity of *A. indica* essential oil vs. three antioxidant standards measured by using (A) the FRAP method, and (B) the DPPH method.

Antioxidant activity

Owing to the complex reactive facets of phytochemicals, the antioxidant activities cannot be evaluated by only single method, but at least two test systems have been recommended for the determination of antioxidant activity to establish authenticity (Schlesier et al., 2002). Antioxidant activity of essential oil extracted from *A. indica* was performed by DPPH and FRAP tests and expressed as trolox equivalents (TEs). The reduction of DPPH absorption is indicative of the capacity of the oils to scavenge free radicals, independently of any enzymatic activity, while the FRAP method is used to determine the capacity of reductants in a sample. DPPH test showed antiradical activity of essential oil higher than that of all of standards. This capacity could be attributed to oil phenolic components (eugenol) and to their hydrogen donating ability by which they are considered potent free radical scavengers. As per DPPH test, essential oil exhibited highest radical scavenging activity (5.58 ± 0.4 mmol Trolox/L). A nearly identical trend was observed in FRAP assay (5.46 ± 0.1 mmol Trolox/L). The steady state was reached after 100 min in both assays and results are shown in Figure 2.

Antifungal evaluation

Essential oil of *A. indica* was evaluated for *in vitro* antifungal activity against eight pathogenic fungi. According to antifungal activity results reported in Table 2, the essential oil showed an interesting activity against

Table 1. Chemical composition of essential oil of *Anisomeles indica*.

Peak	Name	RT	RI	Content (%)
1	α-Thujane	9.50	1016	0.92
2	α-Pinene	10.95	1056	0.56
3	Camphene	10.12	1081	4.12
4	Cymene	13.80	1098	1.51
5	Citral	15.63	1107	0.79
6	Sabinene	16.28	1119	0.66
7	β-Pinene	16.90	1134	8.11
8	Linalyl acetate	17.58	1149	2.19
9	1,8-Cineol	18.12	1165	3.66
10	Limonene	18.23	1172	4.24
11	Myrcene	19.50	1190	0.50
12	Bornyl acetate	21.09	1208	5.61
13	Nerol	23.21	1218	3.34
14	Carveol	24.29	1233	1.22
15	Borneol	24.80	1239	1.65
16	α-Terpeneol	25.68	1257	14.17
17	Azulene	26.63	1288	4.57
18	Eugenol	28.78	1302	17.63
19	α-Terpenyl acetate	29.09	1317	0.83
20	β-Farnesene	29.57	1346	2.77
21	Germacrene-D	30.15	1419	0.92
22	Caryophyllene	30.72	1441	3.71
23	Bicyclogermacrene	32.98	1467	1.49
24	β-Bisabolene	33.81	1476	2.26
25	δ-Cadinene	35.29	1512	1.63
26	α-Cadinol	38.17	1526	2.09
Total essential oil content	90.70%			

RT=Retention time (min); RI = Relative index to *n*-alkanes (C_9-C_{21}) on HP-5MS column.

Table 2. Antifungal activity of essential oils of *Anisomeles indica* Linn against pathogenic fungi.

Fungi	ED_{50} (µg/ml)		
	Essential oil	α-Pinene	Hexaconazole
R. solani	57.90	32.68	37.80
R. bataticola	51.21	47.96	25.38
F. oxysporum	99.38	58.58	15.93
F. udum	98.05	57.70	19.22
A. solani	70.56	68.61	17.52
S. rolfsii	72.85	80.73	13.50
A. flavus	108.19	85.09	28.79
P. aphanidermatum	43.27	47.51	39.22

P. aphanidermatum which is comparable with the standard. The most sensible fungus was *P. aphanidermatum* (ED_{50} 43.27 µg/ml). Essential oil was also effective against *Rhizoctonia bataticola* (ED_{50} 51.21 µg/ml) and *R. Solani* (ED_{50} 57.90 µg/ml). It showed moderate effectiveness against *A. solani* (ED_{50} 70.56 µg/ml) and *S. rolfsii* (ED_{50} 72.85 µg/ml). Least activity was obtained against *A. flavus* (ED_{50} 108.19 µg/ml), *F. oxysporum* (ED_{50} 99.38 µg/ml) and *F. udum* (ED_{50} 98.05 µg/ml).

When these data are considered together with the composition of the essential oil, it is likely that antifungal activity could be mainly due to the presence of terpenoid constituents. Besides, other phenolic constituents like eugenol, terpeneol, nerol, 1,8-cineol also imparts higher antifungal potential. Although, this is the first report on the antifungal activity of *A. indica* essential oil, other members of the *Anisomeles* genus have been subjected to antimicrobial activity evaluation. Generally, higher activity of such plant is attributed to the presence of 1,8-cineole. Although, antifungal activity of essential oil is often attributed mainly to its major constituents, but synergistic and antagonistic effects of minor constituents in a mixture has to be considered.

Antioxidant activity along with antifungal action of *Anisomeles* essential oil is one of the most important examined features. Interestingly, it is the first information of antioxidant and antifungal activity of *A. indica* essential oil. Our data indicate that the essential oil possess good antioxidant activity and also shows potential antifungal activity against a number of pathogenic fungi. This study reveals the promising potent of essential oil of *A. indica* as a source of natural antioxidant.

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REFERENCES

Adams RP (1995). Identification of essential oil components by gas chromatography and mass spectroscopy. Carol Stream, IL: Allured Publ.

Alam M, Quader M, Rashid M (2000). HIV-inhibitory diterpenoid from *Anisomeles indica*. Fitoterapia 71:574-576.

Baratta MT, Dorman HJD, Deans SG, Figueiredo AC, Barroso JG, Ruberto G (1998). Antimicrobial and antioxidant properties of some commercial essential oils. Flavour Frag. J. 13:235-244.

Baratta MT, Dorman HJD, Deans SG (1998). Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oil. J. Essen. Oil Res. 10:618-627.

Benzie IF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as measurement of ‘‘antioxidant power’’: the Frap assay. Anal. Biochem. 239: 70-76.

Blois MS (1958). Antioxidant determinations by the use of a stable free radical. Nature 181:1199-1200.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol. 28:25-30.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods. Int. J. Food Microbiol. 94: 223-253.

Dharmasiri M, Thabrew M, Ratnasooriya W (2000). Anti-inflammatory effects of *Anisomeles indica*. Phytomedicine 7:97.

Dharmasiri M, Thabrew M, Ratnasooriya W (2003). Water extract of leaves and stems of *Anisomeles indica* possesses analgesic and anti-hyperalgesic activities in rats. Pharm. Biol. 41:37-44.

Huang H, Lien H, Ke H, Chang L, Chen C, Chang T (2012). Anti-oxidative characteristics of *Anisomeles indica* extract and inhibitory effect of ovatodiolide on melanogenesis. Mol. Sci. 13:6220-6235.

Iris FF, Benzie IF, Strain JJ (1999). Ferric reducing antioxidant power assay. Methods Enzymol. 299:15-27.

Ito N, Fukushima S, Hassegawa A, Shibata M, Ogiso T (1983). Carcinogenicity of butylated hydroxy anisole in F344 rats. J. Natl. Cancer Inst. 70:343-347.

Madsen HL, Bertelsen G (1995). Spices as antioxidants. Trends Food Sci. Tech. 6:271-277.

Namiki M (1990). Antioxidants/antimutagens in food. Crit. Rev Food Sci. Nutr. 29: 273-300.

Nene YL, Thapliyal PN (1979). Fungicides in plant disease control. Oxford and IBH Publishing Co., New Delhi, pp. 314-413.

Nilsson J, Pillai D, Önning G, Persson C, Nilsson A, Akesson B (2005). Comparison of the 2,2’-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and ferric reducing antioxidant power (FRAP) methods to assess the total antioxidant capacity in extracts of fruit and vegetables. Mol. Nutr. Food Res. 49:239–246.

Philipson JD (1990). Plants as sources of valuable products. In: Charlwood, B.V., Rhodes, M.J. (Eds.), Secondary Products from Plant Tissue Culture. Clarendon Press, Oxford pp. 1-22.

Sagdic O, Karahan AG, Ozcan M, Ozkan G (2003). Effect of some spice extracts on bacterial inhibition. Food Sci. Technol. Int. 9:353-356.

Scalbert A, Manach C, Morand C, Remesy C (2005). Dietary polyphenols and the prevention of diseases. Crit. Rev. Food Sci. 45:287-306.

Schlesier K, Harwat M, Bohm V, Bitsch R (2002). Assessment of antioxidant activity by using different in vitro methods. Free Rad. Res. 36:177-187.

Sokmen A, Gurel E (2001*).* Bitki Biyoteknolojisi “Plant biotechnology”. In: Babaoglu, M., Gurel, E., Ozcan, S. (Eds.), Sekonder Metabolit Uretimi (Secondary metabolite production). Selcuk University Press, Konya pp. 211-261.

Sokmen M, Serkedjieva J, Daferera D, Gulluce M, Polissiou M, Tepe B, Akpulat HA, Sahin F, Sokmen A (2004). *In vitro* antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and allus cultures of *Origanum acutidens*. J. Agric. Food Chem. 52:3309-3312.

Swigar A, Silverstein RM (1981). Monoterpenes: Infrared, Mass, 1H NMR and 13C NMR Spectra, and Kovats Indices. Aldrich Chemical Company, Inc., Milwaukee, Wisconsin.

Tepe B, Daferera D, Sokmen M, Polissiou M, Sokmen A (2004). *In vitro* antimicrobial and antioxidant activities of the essential oils and various extracts of *Thymus eigii* Zohary. J. Agric. Food Chem. 52:1132-1137.

Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pasquale A (2005). Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chem. 89:549-554.

Ushir YV, Tatiya AU, Surana SJ, Patil UK (2010). Gas chromatography-Mass spectrometry analysis and antibacterial activity of essential oil from aerial parts and roots of *Anisomeles indica* Linn. Int. J. Green Pharm. 4:98-101.

Vardar-Unlu G, Candan F, Sokmen A, Daferera D, Polissiou M, Sokmen M, Donmez E, Tepe B (2003). Antimicrobial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. etMey. var. pectinatus (Lamiaceae). J. Agric. Food Chem. 51:63-67.

Yadava RN, Barsainya D (1998). Chemistry and antimicrobial activity of the essential oil from *Anisomeles indica* (L). Ancient Sci. Life 18:228-230.

Yogesh U, Krishnakant P (2010). Chemical composition and antibacterial activity of essential oil from *Anisomeles* Species. Pharmacognosy J. 2:55-60.