Journal of Ecology and the Natural Environment Vol. 3(6), pp. 210-214, June 2011
Available online at http://www.academicjournals.org/jene
ISSN 2006-9847 ©2011 Academic Journals

Full Length Research Paper

Evaluation of the antifungal effects of rosemary oil and comparison with synthetic borneol and fungicide on the growth of *Aspergillus flavus*

M. Moghtader[1]*, H. Salari[2] and A. Farahmand[2]

[1]Department of Biodiversity, Research Institute for Environmental Sciences, International Center for Science, High Technology and Environmental Sciences, Kerman, Iran.
[2]Department of Ecology, Research Institute for Environmental Sciences, International Center for Science, High Technology and Environmental Sciences, Kerman, Iran.

Accepted 3 May, 2011

Rosemary (*Rosmarinus officinalis* L.) with more than 240 active pharmacological and nutritional compounds is important from the botanical point of view. Pharmacological studies show the antifungal properties of Rosemary plant. The essential oil analyses of the aerial parts of rosemary collected from Kerman province were obtained using gas chromatography and gas chromatography mass spectrometry. The study of antifungal effects of the oil sample tested against strain of *Aspergillus flavus* (PTCC = 5004) fungi by disc diffusion method via average inhibition zone. The results showed that yield of Rosemary oil from Kerman province was 3.2%. Forty-one compounds were identified in the essential oil concluded as 99.74% of the total oil. The major components were α-pinene (15.52%), camphor (11.66%), verbenone (11.10%) and 1, 8-cineole (10.63%). The results showed that essential oil from rosemary plant at 1, 1/2 and 1/4 oil dilutions exhibited strong antifungal activity than gentamycin antibiotic on *A. flavus* and exhibited moderate of borneol was at 10% dilution. Benomil fungicide at 10% dilution had no inhibitory effect on *A. flavus*. Large percentage antifungal activities of Rosemary oil are related with α-pinene of monoterpenes as the main compound.

Key words: *Rosmarinus officinalis* L., essential oil, *Aspergillus flavus*, aflatoxin, borneol.

INTRODUCTION

Aspergillus sp. are the most common fungal species which are able to produce mycotoxins in food and feedstuffs. Mycotoxins are known to be potent hepatocarcinogens in animals and humans. The presence and growth of fungi may cause spoilage and result in a reduction in quality and quantity of foods. It is a common mold in the environment, and can cause storage problems in stored grains. It can also be a human pathogen, associated with aspergillosis of the lungs and sometimes causing corneal, otomycotic, and nasoorbital infections. Many strains produce significant quantities of aflatoxin (Klich, 2007), a carcinogenic and acutely toxic compound. *A. flavus* spores are allergenic. *A. flavus* sometimes causes losses in silkworm hatcheries. *A. flavus* is the second most common agent of aspergillosis, the first being *Aspergillus fumigatus*. *A. flavus* may invade arteries of the lung or brain and cause infarction. *A. flavus* also produces a toxin (aflatoxin) which is one of the aetiological agents for hepatocellular carcinoma. *A. flavus* grows as a yellow-green mold in culture. Like other *Aspergillus* species it produces a distinctive conidiophore composed of a long stalk supporting an inflated vesicle. Conidiogenous cells on the vesicle produce the conidia.

Many strains of *A. flavus* exhibit a greenish fluorescence under UV light that is correlated with levels of aflatoxin production. *A. flavus* is particularly common

*Corresponding author. E-mail:moghtader18@yahoo.com Tel: +983426226611. Fax: +983426226617.

on corn and peanuts, as well as water damaged carpets, and is one of several species of mold known to produce aflatoxin which can cause acute hepatitis, immunosuppression, and hepatocellular carcinoma. The absence of any regulation of screening for the fungus in countries which also have a high prevalence of viral hepatitis highly increases the risk of hepatocellular carcinoma (Crawford, 2005).

Borneol is a bicyclic organic compound and a terpene. The hydroxyl group in this compound is placed in an endo position. Borneol is easily oxidized to the ketone yielding camphor. One historical name for borneol is Borneo camphor which explains the name. Borneol can be synthesized by reduction of camphor by the Meerwein-Ponndorf-Verley Reduction. The same reduction but then fast and irreversible with sodium borohydride gives isoborneol as the kinetically controlled reaction product. Borneol is a component of many essential oils.

Rosemary plant with the scientific name of *Rosmarinus officinalis* L. is of Lamiaceae (Labiatae) family. This type has an evergreen bush which is a local plant of Mediterranean region with pharmacological and decorative value. It is a sustainable plant, so aromatic and has wooden stalks with 50 cm up to 2 m height, growing in Mediterranean region and in particular in the littorals region through the minor Asia areas widely. Its leaves facing each other are turned down, narrow, long, thick, sharp-pointed and with a tough appearance. The flowers appearing besides the leaves from May to June are in light blue and rarely white. Its fruit is achene and brown in color (Ghahreman, 1993).

In traditional medicine, Rosemary is used to treat different diseases including: depression, insomniac, gout and arthritic pains (Zargari, 1995).Its dried leaves are also used to prepare soups and sauces. With due attention to their antioxidant, antibacterial and antifungal effects and that they give flavor to meat, fish and chicken they are used to keep the quality of fats and meats. Today, this plant or its essential oil is used in different industries including cosmetic and sanitary industries to reinforce the hairs, to fix the hair color and also is utilized in non-alcoholic drinks (Aeenechi, 1991)

The essential oil of Rosemary plant has been studied in Iran and in the world. The chemical compounds, genetic differences, antimicrobial and antifungal impacts of Rosemary plant have been studied (Angioni et al., 2004). The use of plants is as old as the mankind. Natural products are cheap and claimed to be safe. They are also suitable raw material for production of new synthetic agents.

Rosemary (*R. officinalis* Linn.) is a common household plant grown in many parts of the world. It is used for flavouring food, a beverage drink, as well as in cosmetics; in folk medicine it is used as an antispasmodic in renal colic and dysmenorrhoea, in relieving respiratory disorders and to stimulate growth of hair. Extract of Rosemary relaxes smooth muscles of trachea and intestine, and has choleretic, hepatoprotective and antitumerogenic activity.

The most important constituents of rosemary are caffeic acid and its derivatives such as rosmarinic acid. These compounds have antioxidant effect. The phenolic compound, rosmarinic acid, obtains one of its phenolic rings from phenylalanine via caffeic acid and the other from tyrosine via dihydroxyphenyl-lactic acid. Relatively large-scale production of rosmarinic acid can be obtained from the cell culture of Coleus blumei Benth when supplied exogenously with phenylalanine and tyrosine. Rosmarinic acid is well absorbed from gastrointestinal tract and from the skin. It increases the production of prostaglandin E2 and reduces the production of leukotriene B4 in human polymorphonuclear leucocytes, and inhibits the complement system. It is concluded that Rosemary and its constituents especially caffeic acid derivatives such as rosmarinic acid have a therapeutic potential in treatment or prevention of bronchial asthma, spasmogenic disorders, peptic ulcer, inflammatory diseases, hepatotoxicity, atherosclerosis, ischaemic heart disease, cataract, cancer and poor sperm motility (Al-Sereiti et al., 1999).

This study evaluated and identified the chemical compounds of rosemary mainly. Also antifungal activity of Rosemary has been compared with synthetic borneol, benomil fungicide and standard gentamicin antibiotic on culture of *A. flavus.*

MATERIALS AND METHODS

Plant material and isolation procedure

The leaves with young branches of *R. officinalis* plant were obtained from shrubs grown in Kerman province, Iran in May 2008 before blooming. The samples were cleaned in shade condition to prevent hydrolysis of the existing materials and to keep the natural color of the sample fixed. The leaves were dried in the lab temperature and were powdered and kept at appropriate conditions from the viewpoint of temperature and light until the essential oil taking stage. Afterwards, essential oil was taken from 150 g of the powdered sample in hydro distillation method with the help of Clevenger set for three hours. Following the sample oils were dried with anhydrous sodium sulfate and kept in sterile sample tubes in refrigerator. The oil yields were calculated on a dry weight basis as 3.2%.

Gas chromatography

GC analysis was performed using a model Agilent-6890 gas chromatograph equipped with column DB-5 in 40 m length; internal diameter of 0.18 mm and film thickness 0.25 µm. Oven temperatures was from 60 to 210°C at a rate of 5°C slope per minute. Injector temperature was 280°C and detector (FID) temperature was 270°C and carrier gas was helium.

Gas chromatography/ mass spectrometry

In order to analyze and identify the combinations forming the

essential oil, the chromatograph gas set attached to a mass spectrometry, model Shimadzu-QP5050A was used. The conditions of analysis and specifications of the GC/MC set were as follows: Capillary column DB5-MS in 40 m length, internal diameter of 0.18 mm and layer thickness of 0.18 µm, thermal program of oven (5 min) in 60°C, 60 to 275°C with a 5°C slope per minute, then 10 min in 275°C, the temperature of injection at 280°C, gas conveying helium, the speed of gas move at a rate of 0.9 ml per minute, the ratio of fission (1 to 43), the rate of injection (0.1 µl), temperature of the reservoir of ionization (230°C), ionization mode EI and ionization energy 70 eV. The series of normal Alkans C8-C28 were also injected to the set under the same condition with that of essential oil injection to calculate restrictive index (RI) of components of essential oil. The Restrictive Index of components of the sample was calculated by using a computerized program. Finally, the components of essential oil was identified by comparing the mass spectrums obtained with the existing standard mass spectrums at electronic library of Wiley 2000 existing in Absolution software of GC/Ms set and calculation of standard Restrictive Index in accordance with C_8-C_{28} Alkans and comparing them with the existing standard figurers in references (Adams, 2001).

Antifungal assay

The solvent showing no antifungal activity from DMSO was selected as a diluting medium for the oil. Undiluted oil was taken as dilution 1, 1/2, 1/4, 1/8 and 1/16 dilutions of the oil were made DMSO. For antifungal activity 50 µl of each dilution was used. The antifungal activity of the essential oil was evaluated by disc diffusion method using Mueller Hinton Agar (Baron and Finegold, 1995) and determination of inhibition zones at different oil dilutions against *A. flavus* (PTCC=5004). The fungal strains under experiment were obtained from the Center for Fungi and Bacteria of Iranian Scientific and Industrial Researches Organization. The antifungal property of the oil was tested by agar well diffusion method using Sabouraud Dextrose Agar (SDA). Standard reference antibiotic was used in order to control the sensitivity of the tested fungi (gentamicin 8 mg/ml). The incubation conditions used was 48 to 72 h at 24°C for fungi. All the experiments were carried out in triplicate and averages were calculated for the inhibition zone diameters.

RESULTS AND DISCUSSION

The study of the analysis of Rosemary plant essential oil under investigation showed that the output of essential oil is 3.2%. The essential oil complex identified as restrictive index (RI), and quantitative percentage of the compounds. 41 compounds being identified in the essential oil of this plant with 99.74%, the combinations of α-pinene, Camphor, Verbenone and 1, 8-cineol with 48.91% constitute the highest percentage of essential oil. The results of studying the antifungal impacts of the Rosemary essential oil shows that the oil of this plant has an inhibitory effect in 1, 1/2, 1/4, 1/8 and 1/16 dilution with average diameter growth of respectively 32, 26, 21, 16 and 10 mm. The results with standard antibiotics gentamicin (8 mg/ml) with a diameter of 19 mm had inhibitory effect. Borneol synthetic in 1% dilution had no inhibitory effect on *A. flavus* growth but at 10% dilution had a moderate inhibitory of fungi growth. Using 10% dilution of benomil fungicide had no inhibitory effect on *A. flavus*. Large percentages of antifungal activities of rosemary oil are related with α-pinene of monoterpens as the main compound. The essential oil of Rosemary plant has been studied in Iran and in the world as the essential oil of the aerial parts of *R. officinalis* collected from Konya, Turkey was analyzed by gas chromatography and gas chromatography-mass spectrometry. The oil yield of dried plant (volume/dry weight) obtained by hydro distillation was 1.9%. Twenty compounds representing 99.93% of the oils were identified. The main constituents of the oils were p-cymene (44.02%), linalool (20.5%), gamma-terpinene (16.62%), thymol (1.81%), beta-pinene (3.61%), alpha-pinene (2.83%) and eucalyptol (2.64%). The oil consisted of monoterpenic hydrocarbons, oxygenated monoterpenes and sesquiterpene hydrocarbons. Also, the inhibition effect of rosemary oil was investigated against *Alternaria alternata*, Botrytis cinerea and Fusarium oxysporum. The experiment was carried out *in vitro* using disc diffusion to investigate the antifungal action of the oil. Oil tested on potato dextrose agar plates exhibited an inhibitory effect. The extent of inhibition of fungal growth varied depending on the levels of essential oil used in experiment (Ozcan and Chalchat, 2008).Rosemary leaf extracts were obtained by supercritical fluid extraction (SFE) and Soxhlet extraction. Their chemical compositions were evaluated by GC-MS. The extracts were analyzed for compounds reported in the literature as showing antimicrobial and antioxidant activities. The Rosemary extracts were tested with regard to antioxidant (DPPH radical scavenging and total phenolic content – Folin-Denis reagent), antibacterial (Gram-positive bacteria – *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 11778 – and Gram-negative bacteria – *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and antifungal (*Candida albicans*) activities. Antioxidant, antibacterial and antifungal activities of the SFE extracts were confirmed (Genena et al., 2008). A detailed analysis of *R. officinalis* L. essential oil from Sardinia and Corsica (α-pinene/verbenone/bornyl acetate chemotype) was carried out using GC-RI, GC-MS and ^{13}C-NMR, on the bulk sample or after repeated chromatography. Fifty-eight compounds were identified. The antimicrobial activity of two Sardinian samples was investigated and both exhibited a moderate antibacterial activity. Gram-positive bacteria were more sensitive (MIC 2.5 to 4 mg/ml) than Gram-negative bacteria. Killing time experiments demonstrated that prolonged times (60 min) are needed to completely inactivate the bacterial inoculum (Pintore et al., 2002).

The purpose of this study is to identify the antimicrobial activity of the essential oil of Rosemary plant with tetracycline antibiotic and resistant microorganisms. The aerial parts of rosemary were collected from Research Center for Medicinal Plants at Shahid Bahonar University of Kerman prior to the blooming stage. After drying the plant materials in shade, essential oil was obtained by hydro-distillation method. The study of antimicrobial effect

of the oil sample was conducted on nine strains of pathogenic bacteria resistant by disc diffusion assay and measuring the diameters of zones of inhibition from growth. The 41 components were identified in the essential oil of Rosemary, the main constituents are α-pinene (15.52%), camphor (11.66%), and verbenone (11.10%) and 1, 8- cineole (10.63%). The results showed that the essential oil of Rosemary has an effective controlling and antimicrobial power against all positive and negative bacteria. The antimicrobial impacts of the essential oil of Rosemary plant under investigation can be related with the high percentage of α-pinene, camphor, verbenone and 1, 8-cineole (Moghtader and Afzali, 2009).

Plant-derived antimycotics are attracting the attention of mycologist because the increased resistance of fungi to azoles. The aim of this study was to investigate the anticandidal activity of *Zataria multiflora* (thyme), *Pelargonium graveolens* (geranium), *Artemisia sieberi* besser (Artemisia), *R. officinalis* (rosemary) and *Lavandula stoechas* (lavender) oils against some clinical isolates of *C.albicans* Disc diffusion method and macro broth dilution assay were employed to evaluate the antifungal activity of these oils. Essential oils were analyzed by GC which led to the identification of these main components. Carvacrol (39.8%), Citronellol (45.2%), α- pinene (23.7%), 1, 8-cineol (30.2%) and, α-thujone (38.8%) are the main components of thyme, geranium, Rosemary, lavender and artemisia oils respectively. Thyme oil showed strong antifungal activity (34 to 50mm, MIC562.57g/ml), while geranium oil had good antifungal activity (12-29.5 mm, MIC>62.5 7 g/ml) but lavender, Rosemary and artemisia oils showed only a moderate effect (zone inhibition<12 mm). The inhibition zone of thyme oil is larger than Amphotricin B. Results showed that thyme and geranium oils may be useful in the clinical management of candidal infection. Further clinical trials are required to validate their use as therapeutic, alternatives for candidal infection (Mahboubi et al., 2008).

Aflatoxin B1 (AFB1) is a highly toxic and carcinogenic metabolite produced by *Aspergillus* species on food and agricultural commodities. Natural products may regulate the cellular effects of aflatoxins and evidence suggests that aromatic organic compounds of spices can control the production of aflatoxins. With a view to controlling aflatoxin production, the essential oils from *R. officinalis* and *Trachyspermum copticum* L. were obtained by hydrodistillation. Antifungal activities of the oils were studied with special reference to the inhibition of *Aspergillus parasiticus* growth and aflatoxin production. Minimal inhibitory (MIC) and minimal fungicidal (MFC) concentrations of the oils were determined. *T. copticum* L. oil showed a stronger inhibitory effect than *R. officinalis* on the growth of A. parasiticus. Aflatoxin production was inhibited at 450 ppm of both oils with that of *R. officinalis* being stronger inhibitor. The oils were analyzed by GC and GC/MS. The major components of *R. officinalis* and *T. copticum* L. oils were Piperitone (23.65%), alpha-pinene (14.94%), Limonene (14.89%), 1, 8-Cineole (7.43%) and thymol (37.2%), P-Cymene (32.3%), gamma-terpinene (27.3%) respectively. It is concluded that the essential oils could be safely used as preservative materials on some kinds of foods to protect them from toxigenic fungal infections (Rasooli et al., 2008).

R. officinalis is widely found in the lands of Aegean and Mediterranean regions of Turkey. The goal of this work was to test the antimicrobial activity of the essential oils and methanolic extracts of *R. officinalis* collected from three different regions at four different time intervals of the year against *S. aureus*, *Proteus vulgaris*, *P.aeruginosa*, *Klebsiella pneumonia*, *Enterococcus feacalis*, *E. coli*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *C. albicans*. Essential oils were obtained from the aerial parts of the plant by using a Clevenger apparatus for 4 h. After distillation, the distillates were filtered, air-dried and then extracted by using a Soxhlet apparatus for 9 h to obtain the methanolic extracts. The antimicrobial activities of the methanolic extracts were tested by the disc diffusion technique. The antimicrobial activities of the essential oils obtained from *R. officinalis* were determined by minimum inhibitory concentration (MIC). The results indicated that the tested bacteria were sensitive to the essential oils and partially to the methanolic extracts. The antimicrobial activities of the essential oils against the tested bacteria differed, depending on location and seasonal variations (Soliman and Badeaa, 2002).

Essential oils of 12 medicinal plants were tested for inhibitory activity against *A. flavus*, *A. parasiticus*, *Aspergillus ochraceus* and *Fusarium moniliforme*. The oils of thyme and cinnamon (< or = 500 ppm), marigold (< or = 2000 ppm), spearmint, basil, quyssum (3000 ppm) completely inhibit all the test fungi. Caraway was inhibitory at 2000 ppm against *A. flavus*, *A. parasiticus* and 3000 ppm against *A. ochraceaus* and *F. moniliforme*. *A. flavus*, *A. ochraceus*, *A. parasiticus* and *F. moniliforme* were completely inhibited by anise at< or = 500 ppm. However, chamomile and hazanbul at all concentrations were partially effective against the test toxigenic fungi. The results indicate that the test toxigenic fungi are sensitive to the 12 essential oils, and particularly sensitive to thyme and cinnamon. The results also showed that the essential oils of thyme, cinnamon, anise and spearmint have more effect on fungal development and subsequent mycotoxin production in wheat grains. The extent of inhibition of fungal growth and mycotoxin production was dependent on the concentration of essential oils used (Soliman and Badeaa, 2002).

The effects of 16 essential oils from aromatic plants were tested for their inhibitory effect on *A.flavus* IMI 242684 on PDA. The results showed that the essential oil of white wood (*Melaleuca cajeputi*) gave the highest

inhibition followed by the essential oils of cinnamon (*Cinnamomum cassia*) and lavender (*Lavandula officinalis*), respectively. Furthermore, the inhibitory effects of these three essential oils at different concentrations were examined. It was found that the essential oil of white wood at 1.5625% (v/v) and of cinnamon and lavender at 50% (v/v) were the optimum concentrations for fungal growth inhibition. The essential oil of white wood at 25% (v/v) completely inhibited the growth of *A. flavus* IMI 242684 on PDA for 28 days (Thanaboripatet al., 2007).

The effect of 20 essential oil constituents on *A. flavus* growth and aflatoxin production was tested at the level of 1000 ppm. Some of the tested oils exhibited inhibitory effects on fungal growth and toxin formation. Five oils, namely geraniol, nerol and citronellol (aliphatic oils), cinnamaldehyde (aromatic aldehyde) and thymol (phenolic ketone), completely suppressed growth and aflatoxin synthesis. Trials for determining the minimum inhibitory concentration (MIC) of these oils revealed that geraniol, nerol and citronellol were effective at 500 ppm, while thymol and cinnamaldehyde were highly effective at doses as low as 250 and 200 ppm, respectively. It was observed that citral, citronellol and eugenol prevented fungal growth and toxin formation for up to 8 d. However, after 15 d of incubation, toxin production was greater than the controls (Mahmoud, 1994).

The results of the study of the antifungal impacts of the essential oil of Rosemary plant against *A. flavus* (PTCC=5004) (With diameter of zone of inhibition from growth of 34 mm) have a considerable antifungal impacts. The results show the high controlling and antifungal power of Rosemary essential oil under investigation. The antifungal effects of Rosemary essential oil can be attributed to the Monoterpens combination and in particular α-Pinene whose antifungal effects of this combination has been proved (Okamura et al., 1994). So with regard to the antimicrobial effects of Rosemary essential oil under investigation as compared with gentamycin antibiotic, this essential oil can be used as a combination with antifungal effects and natural origin.

ACKNOWLEDGEMENT

This study has been conducted under the financial support of the International Center for Science, High Technology and Environmental Sciences, Kerman. Hereby, the executive and colleagues of the research project expresses their thanks for the support extended to them.

REFERENCES

Adams RP (2001). Identification of essential oil components by gas chromatography mass spectroscopy. Illinois Allured Publication Corporation, pp. 9-456.

Aeenechi Y (1991). Medical Singulars and Medical Plants of Iran, Tehran University Press.

Al-Sereiti MR, Abu-Amer KM, Sen P (1999). Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. Indian J. Exp Biol., 37(2): 124-130.

Angioni A, Barra A, Cereti E, Barile D, Coisson JD, Arlorio M (2004). Chemical composition, plant genetic, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L. J Agric Food Chem., 52(11): 3530-3535.

Baron EJ, Finegold SM (1995). Bailey and Scott's Diagnostic Microbiology, 8th ed. Mosby, St. Louis, MO, USA, pp. 171-193.

Celiktas OY, Hames KEE, Bedir E, Vardar SF, Ozek T, Baser KHC (2007). Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. Food Chem., 100(2): 553-559.

Crawford JM (2005). Liver and Biliary Tract. Pathologic Basis of Disease, ed. Kumar V, et al., Philadelphia: Elsevier Saunders, p. 924.

Genena AK, Hense H, Juniour AS, Souza SM (2008). Rosemary (*Rosmarinus officinalis*) a study of the composition, antioxidant and antimicrobial activities of extracts obtained with supercritical carbon dioxide. Ciênc. Tecnol. Aliment. Campinas, 28(2): 463-469.

Ghahreman A (1993). Chromophites of Iran (Botanical Systematic), University Publication Center, Tehran.

Klich MA (2007). *Aspergillus flavus*: the major producer of aflatoxin. Molecular Plant Pathol., 8(6): 713-22.

Mahboubi M, Feizabadi MM, Safara M (2008). Antifungal activity of essential oils from *Zataria multiflora*, *Rosmarinus officinalis, Lavandula stoechas*, *Artemisia sieberi* Besser and *Pelargonium graveolens* against clinical isolates of *Candida albicans*. Pharmacognosy Mag., 4(15): 15-18.

Mahmoud ALE (1994). Antifungal action and antiaflatoxigenic properties of some essential oil constituents. Lett. Appl. Microbiol., 19(2): 110-113.

Moghtader M, Afzali D (2009). Study of the Antimicrobial Properties of the Essential Oil of Rosemary. American-Eurasian J. Agric. Environ. Sci., 5(3): 393-397.

Okamura N, Haraguchi H, Hashimoto K, Yagi A (1994). Flavonoids in *Rosmarinus officinalis* leaves. Phytochemistry, 37(5): 1463-1466.

Ozcan MM, Chalchat JC (2008). Chemical composition and antifungal activity of rosemary (*Rosmarinus officinalis* L.) oil from Turkey. International J. Food Sci. Nutr., 59(7-8): 691-698.

Pintore G, Usai M, Bradesi P, Juliano C, Boatto G, Tomi F, Mario C, Riccardo C, Joseph C (2002). Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. Flavour and Fragrance J., 17: 15-19.

Rasooli I, Fakoor MH, Yadegarinia D, Gachkar L, Allameh A, Rezaei MB (2008). Antimycotoxigenic characteristics of *Rosmarinus officinalis* and *Trachyspermum copticum* L. essential oils. Int. J. Food Microbial., 122(1-2): 135-139.

Soliman KM, Badeaa R (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem. Toxicol., 40: 1669-1675.

Thanaboripat D, Suvathi Y, Srilohasin P, Sripakdee S, Patthanawanitchai O, Charoensettasilp S (2007). Inhibitory effect of essential oils on the growth of *Aspergillus flatus*. KMITL Sci. Tech. J., 7: 1.

Zargari A (1995). Medical Plants, 5th Edition, Tehran University Press.