INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 11, No. 4, p. 164-172, 2017

RESEARCH PAPER OPEN ACCESS

Effective antifungal and antioxidant properties of essential oil extracted from the leaves of *Laurus nobilis* L. wild-growing in Algeria

Hicham Boughendjioua*

Department of Natural Sciences, High School Professors Technological Education, Skikda, Algeria

Key words: *Laurus nobilis*, Essential oil, Chemical composition, Antifungal activity, Antioxidant activity

http://dx.doi.org/10.12692/ijb/11.4.164-172 Article published on October 21, 2017

Abstract

The food industry is constantly challenged by consumers seeking for increasingly healthy foods and less use of synthetic chemical additives such as pesticides or preservatives. In this context the aim of the present work was to study the antifungal activity of Laurel essential oilin different treatments against two phytopathogenic fungus: *Aspergillus niger* and *Fusarium oxypouim*. These strains are among the most contaminating genera of mycotoxin dry and mainstream producers. The extraction was carried out by hydrodistillation and molecular characterization by GC/MS. The essential oil of the *Laurus nobilis* L. leaves shows a great inhibition of mycelial growth (*in vitro*). The 1,8-Cineole (49.43 %), Terpinyl acetate (14.90 %)and Sabinene (09,88%) possess the most important inhibitory powers. The essential oil was also subjected to a biological screening for its possible antioxidant activities by means of DPPH radical scavenging test and b-carotene/linoleic acid test, the sample tested showed a good antioxidant activity in comparison with the positive control (Ascorbic acid and Butylhydroxytoluene (BHT)).

* **Corresponding Author:** Hicham Boughendjioua ✉ boughendjioua.hicham@yahoo.com

Introduction

The fight against fungi by the application of natural fungicides has taken a very important place in the alternative strategies to synthetic fungicides (Laplace, 2006). Since ancient civilizations, the use of various spices to enhance the flavor of foods and beverages was a common practice. In addition, an increase in the shelf life of these foods was also observed, suggesting that some of these spices would be able to inhibit spoilage and pathogenic microorganisms (Racowski *et al.*, 2016).

The purpose of the present work was to evaluate the antifungal and antioxidant activities of *Laurus nobilis* L. essential oil cultivated in Algeria. And relate them with their chemical composition, for further application in food as natural valuable products.

Materials and methods

Vegetal material

The leaves of *Laurus nobilis* L. growing wild in Azzaba located at Skikda city (North-east of Algeria) were collected on December 2016.The taxonomic identity of the plant was confirmed by the well-known Algerian flora of Quezel and Santa (1962).

Extraction of the essential oil

The aerial parts (100 g) were dried at 25°C in the shade and subjected to hydrodistillation using a Clevenger-type apparatus for 4 h(Clevenger, 1928). The oil was dried with anhydrous sodium sulfate, weighed and stored at 4°C in dark until use.

Chromatographic analysis (GC/MS)

Component identification was performed based on their Kováts indices (KI) and gas chromatography coupled to mass spectrometry (GC-MS). The latter is carried out on a Hewlett Packard type gas chromatograph (HP 6890 series) coupled with a mass spectrometer (HP series 5973). The fragmentation is carried out by electronic impact at 70 eV. The column used is an HP-5MS capillary column (30 mx 0.25 mm), the film thickness is 0.25 µm. The temperature of the column is programmed from 50 to 250°C. at a rate of 4 ° C.min^{-1}. The carrier gas is helium whose flow rate is set at 1.5 ml.min^{-1}.

The injection mode is in the split mode (leakage ratio: 1/70). The apparatus is connected to a computer system managing a NIST 98 mass spectrum library.

Evaluation of antifungal activity

The direct contact method has been applied to test the sensitivity of molds to essential oil (Hamrouni *et al.*, 2014; Messgo-Moumene *et al.*, 2014; Sameza *et al.*, 2014). The technique consists in adding the oil at different concentrations to the still liquid culture medium at a temperature of 56°C. After solidification of the culture medium, for each mold, a mycelial disc of 6 mm diameter is deposited aseptically on the surface of the agar medium in the center of the 9 cm diameter petri dish made of glass. The volume of the medium used is 20 ml / dish. In parallel with controls composed of PDA "potatoes dextrose agar" oil-free control. The incubation was carried out in an oven at a temperature of 25 ± 2°.

DPPH assay

The DPPH test was carried out as described (Cuendet *et al.*, 1997; Burits and Bucar, 2000). 50 ml of various dilutions of the extract was mixed with 5 ml of a 0.004% methanol solution of DPPH. After an incubation period of 30 min, the absorbance of the samples was read at 517 nm in Biospec-mini Shimadzu spectrophotometer. Butylhydroxytoluene (BHT) and ascorbic acid were used as positive controls. Inhibition of free radical DPPH in percent (I%) was calculated in following way:

$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$

A_{blank} is the absorbance of control reaction (without test compound) and A_{sample} is the absorbance of test compound. The concentration of extract which provide the 50% was calculated from the graph plotting inhibition percentage against extract concentration.

Carotene/linoleic acid assay

This assay was used to visual instrumentally bleaching of the carotene/linoleic acid solution. The model test mixture was prepared by dissolving 0.5 mg b-carotene in 1 ml chloroform, 25 µl of linoleic acid and 200 mg Tween 40 were added to the b-carotene solution.

Chloroform was removed using a rotary evaporator at 50°C. 100 ml of distilled water saturated with oxygen during 30 min, flow rate 100 ml min-1, was added and the mixture was vigorously shaken. 250 µl of this model test mixture was distributed in each of the test tube. 35 µl of an ethanolic solution of the extract was added to each test tube. The test tubes were then placed in an incubator at 55°C for 48 hr. After incubation period the absorbance was measured in Biospec-mini Shimadzu spectrophotometer at 490 nm.

An equal amount of ethanol was used for the blank samples and BHT used as synthetic antioxidant as explained before (Dapkevicius *et al.*, 1998).

Results and discussion

The average yield of essential oil extracted from the plant is studied in the range of 0.8%.This performance is comparable to that obtained for the same species studied in Iran whose yield was: by hydrodistillation, were 0.7%, 0.8%, 1.1% and 0.6% in the vegetative, bud, flowering and seed-bearing stages, respectively, calculated on a dry weight basis (Verdian-rizi and Hadjiakhoondi, 2008).

Table 1. Chemical composition of leaf essential oil of *Laurus nobilis* L.

Compounds	KI	Aera %
α-Thujene	933	0.10
α-Pinene	941	04.05
Camphene	955	0.11
Sabinene	977	09,88
β-Pinene	981	03.09
Myrcene	992	0.09
3-Carene	1013	0.22
α-Terpinene	1020	0.12
p-Cymene	1028	0.43
Limonene	1032	0.19
1,8-Cineole	1035	49.43
γ-Terpinene	1064	0.91
cis-Sabinene hydrate	1070	0.30
Terpinolene	1089	0.45
trans-Sabinene hydrate	1100	0.70
δ-Terpineol	1166	0.71
Borneol	1167	0.39
4-Terpineol	1180	0.79
α-Terpineol	1190	01.50
Nerol	1227	0.90
Linalool	1258	04,13
Isobornyl acetate	1286	0.20
Terpinen-4-yl acetate	1291	0.91
Terpinylacetate	1351	14.90
Eugenol	1356	02.99
	Total	97.49

In contrast, this performance was revealed lower than that given by the same species from different regions in Morocco whose performance can reach 1.86% (Derwich *et al.*, 2009).

There are many studies on the chemical composition of the essential oil obtained from leaves of Mediterranean and European *Laurus nobilis* L. (Riaz *et al.*,1989).

The essential oil composition showed a similar pattern to those published for other geographical regions: 1.8-cineole was reported as the major component in the essential oil from Turkey (Ozcan and Chalchat, 2005), Tunisia (Bouzouita *et al.*, 2001), Croatia–Serbia (Simic *et al.*, 2004), Italy (Flamini *et al.*, 2007), the Netherlands (Hokwerda *et al.*, 1982) and Argentina (Huergo and Retamar, 1978).

Table 2. Antifungal activity of the essential oil of *Laurus nobilis* L.

	Dose (mg/ml)							
Strains	1/100 v/v	1/250 v/v	1/500 v/v	1/1000 v/v	1/2000 v/v	1/3000 v/v	1/5000 v/v	C
Aspergillus niger	-	-	-	-	+	+	+	+
Fusariumoxypouim	-	-	-	-	+	+	+	+

C: control; (-): inhibition; (+): Growth.

The percentage composition of the essential oilis listed in Table 1.The chromatographic analyses resulted in the identification of 25 compounds, representing 97.49% of the oil, 1,8-Cineole (49.43%), Terpinyl acetate (14.90%)and Sabinene (09,88%) were the major components.

Verdian-riziand Hadjiakhoondi (2008) identified

Thirty-seven components accounting for 95.8% of the total composition were identified in the vegetative stage. The major constituents of this oil were 1,8-cineole (35.7%), trans-sabinene hydrate (9.7%), α-terpinyl acetate (9.3%), methyl eugenol (6.8%), sabinene (6.5%) and eugenol (4.8%). In the volatile of the bud stage, thirty-six compounds amounting 98.8% of the total components were identified which included 1,8-cineole (34.9%), α-terpinyl acetate (12.1%), trans-sabinene hydrate (11.9%), methyleugenol (8.1%), sabinene (6.0%) and eugenol (3.8%) as main components. In the oil obtained from the flowering stage, thirty-six components were identified, which represented about 95.5% of the total composition. 1,8-Cineole (31.4%), α-terpinyl acetate (11.4%), trans-sabinene hydrate (9.8%), methyleugenol (9.4%), sabinene (5.8%) and eugenol (5.5%) were the principal components of this oil. In the seed-bearing stage, thirty-seven constituents accounting for 97.3% of the total oil were identified that included 1,8-cineole (35.7%), trans-sabinene hydrate (11.4%), α-terpinyl acetate (10.4%), methyl eugenol (7.9%), sabinene (5.9%) and eugenol (4.3%) as main components.

Table 3. Antioxidative capacities of the essential oil of *Laurus nobilis* L. measured in DPPH and b-carotene/linoleic acid assays.

Extract and controls	DPPH [a] (mg ml^{-1})	b-Carotene /linoleic acid[b]
Essential oil	94,93	70,82
BHT	1,55	90,64
Ascorbic acid	0,42	NT [c]

[a] IC_{50} values of DPPH assay, [b] % inhibition rate of linoleic acid oxidation, [c] Not tested.

Twenty-six compounds were identified by Derwich *et al.*, (2009), which accounted which made up (98.59%) of the total essential oil. The most abundant constituents were 1,8-cineole (52,43%), terpinyl acetate (8,96%), sabinene (6,13%), limonene (5,25%), Apinene (3.72%) and β-pinene (3.14%).

Goudjil*etal.*,(2015)reports that the investigations and research on the essential oil; extracted from the dried leaves of *Laurus nobilis* Lauraceae, which was harvested in the region of Skikda (East of Algeria); and separated by gas chromatography coupled by a mass spectrometry (GC/MS); resulted in obtaining Twenty-two constituents, representing 99.7% of the essential oil of *Laurus nobilis*. The main compounds identified are 1,8-Cineole (45.36%), followed by bornylene (17.25%), linalool (8.13%), and sabinene (7.48%).

Antifungal activity of essential oil

Growth of fungi in food leads to food spoilage, causing great economic damages. On the other hand, toxin-producing species of genera *Aspergillus* (*A. carbonarius, A. flavus, A. ochraceus, A. oryzae, A. parasiticus, A. versicolor*), *Penicillium* (*P. nordicum, P. expansum, P. viridicatum, P. verrucosum*),

Fusarium (*F. culmorum, F. graminearum, F.oxysporum, F. verticillioides, F. proliferatum*), *Alternaria* (*A. alternata, A. solani, A. brassicae, A. tenuissima, A. tomato*) as well as teleomorphs of the class *Ascomycetes* (*Petromyces alliaceus, Emericella nidulans*, etc.) can biosynthesize toxic secondary metabolites - mycotoxins (aflatoxins, ochratoxin A, sterigmatocystin, patulin, fumonisins, zearalenone, deoxynivalenol, alternariol, alternariol monomethyl ether, tenuazonic acid)(Kocić-Tanackov and Dimić, 2013).

The chemical structure of the main essential oil components and their antifungal properties are related. The presence and position of the hydroxyl group in the molecule, the presence of the aromatic nucleus, solubility in fats and spatial orientation affect the antifungal activity (Singh*etal.*,2005; Sudhakar *et al.*, 2009).

The fungal colonies were identified based on morphological and microscopic characters according to Pitt and Hocking, (2009).

The results of the antifungal activity of the essential oil of the leaves of *Laurus nobilis* L. are summarized in Table 2. The essential oils exerted an important inhibitory activity against fungi, the two fungal strains; *Aspergillus niger* and *Fusarium oxypouim* were inhibited from the minimum concentration of 1/2000 v / v.

Mady A. Ismail *et al.*, (2014) reported that treatment of cheese with of the essential oil of *Laurus nobilis* L. caused either increase or decrease in fungal counts.
We also noted that, Zuzarte *et al.* (2009), Bhuiyan *et al.* (2011), Arnusch *et al.*, (2012) and Ben Mansoura *et al.* (2013) reported an important anti-fungal activity against *Aspergillus niger*.

However, Biondi *et al.*, (1993) observed that when compared to the oil of other aromatic plants of the same family, commercial laurel oil is much more inefficient when tested against various microorganisms, including fungi *Aspergillus niger*, *A. terreus*, *Candida albicans* and *Fusarium* sp.

Burt, (2004), Ceylan and Fung, (2004) and Tajkarimi *et al.*, (2010) indicates that the antifungal activity of the essential oil of *Laurus nobilis* L. is related to Cineole.

Research show that essential oil in antifungal and antimycotoxigenic protection of food can be applied as surface protection (by smearing or applying in the form of biofilm) (Amiri *et al.*, 2008; Xing *et al.*, 2010), as an addition to the modified atmosphere packaging (Nielsen and Rios, 2000;Suhr and Nielsen, 2003), or as an addition to food (Omidbeygi *et al.*, 2007).

It is undeniable that essential oil has the potential to control the development of fungi which contaminate food, as well as the production of mycotoxin by the toxin-producing species. However, the general ascertainment of researches who deal with antifungal testing of essential oilis that the methods of determining the antifiungal activity should be standardized. It is necessary to complete the antifungal and antimycotoxigenic research of combined effects of essential oil*in vivo* and *in vitro,* since stronger antifungal effects can be achieved through the application of lower concentrations of combined essential oil in food or in active packaging. Science and practice should provide the answers to which of the essential oil and which combinations are to have strong antifungal effect in food, while not being harmful to human health, having a suitable effect on the sensory quality of the product, and being economically profitable (Kocić-Tanackov and Dimić, 2013).

Antioxidant activity

Total antioxidant activity of the plant extracts is recommended to carry out by employing two or more methods (Politeo *et al.*, 2007). The potential antioxidant activity of the essential oil from *Laurus nobilis* L. were determined by employing two complementary tests, namely DPPH free radical scavenging and b-carotene/linoleic acid test systems. The IC_{50} value of essential oil is 94,93 mg ml-1 which is higher than the synthetic antioxidant agents, BHT (1.55 mg ml^{-1}) and ascorbic acid (0.42 mg ml^{-1}), the

essential oil seemed to inhibit the oxidation of linoleic acid with the value of 70.82%inhibitions which is close enough to the synthetic antioxidant, BHT (90.64%)(Table 3). The results of this study confirm the previous results which have been showing the antioxidant properties of essential oil from *Laurus nobilis* L. (Ruberto and Barata, 2000).

Several studies (Dall'Acqua*etal*., 2009; Saab *etal*., 2012; Ramos *et al*., 2012)have been conducted to clarify the possible substances involved in antioxidant properties of the essential oil. Among the identified compounds in the essential oil from laurel, methyl eugenol may be considered the main contributors to the antioxidant activity. The antioxidant activity of eugenol has been reported several times. And there are reports that, monoterpene hydrocarbons (sabinene, a-terpinene) and oxygenated monoterpenes (1.8-cineole, a-terpinenol, terpinen-4-ol) have shown antioxidant activity and it's likely that the activity of essential oil of *Laurus nobilis* L. is due to this compounds (Simic *et al*., 2003).

Conclusion

The essential oil extracted from the leaves of *Laurus nobilis* L. grown in the region of Azzaba, Skikda city (Algeria), showed, *in vitro*, an antifungal activity against both fungi; *Aspergillus niger* and *Fusarium oxypouim* tested. This bioactive power observed in oil is attributed mainly to their contents of 1,8-Cineole, Terpinyl acetate and Sabinene known for their inhibitory properties of mycelial growth.

The results obtained confirm that the good antioxidant potential of the essential oil of this plant according to others. These results keep an open perspective for research of formulations on the basis of essences of the *Laurus nobilis* L. in place of other synthesis preservatives or antioxidant on the basis of plant used in the field of food industry, pharmaceutical and cosmetics industry.

References

Amiri A, Dugas R, Pichot AL, Bompeix G. 2008. *In vitro* and *in vivo* activity of eugenol oil (*Eugenia caryophylata*) against four important postharvest apple pathogens. International Journal of Food Microbiology **126**, 13-19. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.04.022

Arnusch CJ, Ulm H, Josten M, Shadkchan Y, Osherov N, Sahl HG, Shai Y. 2012. Ultrashort Peptide Bioconjugates Are Exclusively Antifungal Agents and Synergize with Cyclodextrin and Amphotericin B. Antimicrobial Agents and Chemotherapy **56(1),** 1-9. http://dx.doi.org/10.1128/AAC.00468-11.

Ben Mansoura M, Balti R, Rabaoui L, Bougatef A, Guerfel M. 2013. Chemical composition, angiotensin I-converting enzyme (ACE) inhibitory, antioxidant and antimicrobial activities of the essential oil from south Tunisian *Ajuga pseudoiva* Rob. Lamiaceae. Process Biochemistry **48(4),** 723-729.

Bhuiyan MNI, Varshney VK, Shiam C, Varshney Tomar A, Akter F. 2011. Composition of essential oil of the leaf and inflorescence of *Pogostemon benghalensis* (Burm.f.) Kuntze. International Research Journal of Plant Science **2 (9),** 271-275.

Biondi D, Cianci P, Geraci C. 1993. Antimicrobial activity and chemical composition of essential oils from sicilian aromatic plants. Flavour and Fragrance Journal **8,** 331-337.

Bouzouita N, Nafti A, Chaabouni M, Lognay G, Marlier M, Zghoulli S, Thonart P. 2001. Chemical compositions of *Laurus nobilis* oil from Tunisia. Journal Essential Oil Research **13,** 116-117.

Burits M, Bucar F. 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy Research **14,** 323-328.

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods, a review. International Journal of Food Microbiology **94 (3)**, 223-253.

Ceylan E, Fung YCD. 2004. Antimicrobial activity of spices. Journal of Rapid Methods and Automation in Microbiology **12,** 1-55.

Clevenger JF. 1928. Apparatus for the determination of volatile oil. Journal of the American Pharmacists Association**17**, 346-1.

Cuendet M, Hostettmann K, Potterat O, Dyatmiko W. 1997. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helvetica Chimica Acta **4**, 1144-1152.

Dall'Acqua S, Cervellati R, Speroni E, Costa S, Guerra MC, Stella L, Greco E, Innocenti G. 2009.Phytochemical composition and antioxidant activity of *Laurus nobilis* L. leaf infusion. Journal of medicinal food **12(4),** 869-76. https://doi.org/10.1089/jmf.2008.0119

Dapkevicius A, Venskutonis R, Van Beek TA, Linssen JPH. 1998. Antioxidant activity of extracts obtained different isolation procedures from some aromatic herbs grown in Lithuania. Journal of the Science of Food and Agriculture **77**, 140-146.

Derwich E, Benziane Z, Boukir A. 2009. Chemical Composition and Antibacterial Activity of Leaves Essential Oil of *Laurus nobilis* from Morocco. Australian Journal of Basic and Applied Sciences **3(4),** 3818-3824.

Flamini G, Tebano M, Cioni P, Ceccarini L, Simone S, Longo I. 2007. Comparison between the conventional method of extraction of essential oil of *Laurus nobilis* L. and a novel method which uses microwaves applied *in situ*, without resorting to an oven. Journal of Chromatography A **1143,** 36-40.

Goudjil MB, Ladjel S, Bencheikh SE, Zighmi S, Hamada DJ. 2015. Study of the chemical composition, antibacterial and antioxidant activities of the essential oil extracted from the leaves of Algerian *Laurus nobilis* Lauraceae. Journal of Chemical and Pharmaceutical Research **7(1),** 379-385.

Hamrouni L, Hanana M, Amri I, Romane A, Gargouri S, Jamoussi B. 2014. Allelopathic effects of essential oils of *Pinus halepensis* Miller: chemical composition and study of their antifungal and herbicidal activities. Archives of Phytopathology and Plant Protection. **48(2),** 1-14.

Hokwerda H, Ros R, Tattje D, Malingre T. 1982. Composition of essential oils of *Laurusnobilis* L. *nobilis* var. *angustifolia* and *Laurusazorica*. Planta Medica **44,** 116-119. http://dx.doi.org/10.1055/s-2007-971415.

Huergo H, Retamar J. 1978. El aceiteesencial de bay (*Laurus nobilis* L). Rivistaitaliana EPPOS **60**, 635-637.

Kocić-Tanackov SD, Dimić GR. 2013. Antifungal activity of essential oils in the control of food-borne fungi growth and mycotoxin biosynthesis in food. Microbial pathogens and strategies for combating them: science, technology and education (A. Méndez-Vilas, Ed.) 838-849.

Laplace JP. 2006. Agriculture *et al*imentation Réflexions croisées. Cahiers Agricultures **15(4),** 375-78.

Mady A, Ismail Al-Zahraa M, Darwish, Nemmat A. 2014. Hussein & Soumia M. Darwish. In vivo Effect of Essential Oils from *Laurus nobilis*, *Anethum Graveolens* and *Mentha piperita* on Mycobiota Associated with Domiati Cheese During Storage. Food and Public Health **4(3),** 110-122.

Messgo-Moumene S, Li Y, Bachir K, Houmani Z, Bouznad Z, Chemat F. 2014. Antifungal power of citrus essential oils against potato late blight causative agent. Journal of Essential Oil Research **27 (2),** 169-176.

Nielsen PV, Rios R. 2000. Inhibition of fungal growth on bread, by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. International Journal of Food Microbiology **60**, 219-229.

Omidbeygi M, Barzegar M, Hamidi Z, Naghdibadi H. 2007. Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus*in liquid medium and tomato paste. Food Control **18,** 1518-23.

Ozcan M, Chalchat J. 2005. Effect of different locations on the chemical composition of essential oils of bay (*Laurus nobilis* L.) leaves growing wild in Turkey. Journal of Medicinal Food **8,** 408-411. http://dx.doi.org/10.1089/jmf.2005.8.408.

Pitt JI, Hocking AAD. 2009. Fungi and Food Spoil*age*, 3rd edition, Springer Science plus Business Media, LLC, 233 Spring Street, New York, NY 10013, USA 524.

Politeo O, Jukic M, Milos M. 2007. Chemical composition and antioxidant activity of free volatile aglycones from bay (*Laurus nobilis* L.) compared to its essential oil. Croatia Chem. Acta **80,** 121-126.

Quezel P, Santa S. 1962. Nouvelles Flores d'Algérie et des Régions Désertiques Méridionales. CNRS, Paris. Éditions du centre national de la recherche scientifique **2,** 793.

Racowski I, Foramiglio VL, Teodoro JA, Freire VT. 2016. Antifungal Activity of Infusions from Fresh Oregano, Laurel and Rosemary Leaves and Their Commercial Essential Oils against *Acremonium* sp. Journal of Microbiology Research. **6 (2),** 35-39. www.dx.doi.org/10.5923/j.microbiology.20160602.02.

Ramos C, Teixeira B, Batista I, Matos O, Serrano C, Neng NR, Nogueira JMF, Nunes ML, Marques A. 2012. Antioxidant and antibacterial activity of essential oil and extracts of bay laurel *Laurus nobilis* Linnaeus (Lauraceae) from Portugal. Natural Product Research **26(06),** 518- 529. http://dx.doi.org/10.1080/14786419.2010.531478.

Riaz M, Ashraf CM, Chaudhary FM. 1989. Studies of the essential oil of the Pakistani *Laurus nobilis* Linn in different seasons. Pakistan Journal of Scientific and Industrial Research **32,** 33 D35.

Ruberto G, Barata MT. 2000. Antioxidant activity of selected essential oil components in two lipid model system. Food Chemistry **69,** 167-174.

Saab AM, Tundis R, Loizzo MR, Lampronti I, Borgatti M, Gambari R, Menichini F, Esseily F, Menichini F. 2012. Antioxidant and antiproliferative activity of *Laurus nobilis* L. (Lauraceae) leaves and seeds essential oils against K562 human chronic myelogenousleukaemia cells. Natural Product Research **26(18),** 1741-1745. http://dx.doi.org/10.1080/14786419.2011.608674

Sameza ML, Boat MAB, Nguemezi ST, Mabou LCN, JazetDongmo PM, Boyom FF, Menut C. 2014. Potential use of *Eucalyptus globules* essential oil against *Phytophthora colocasiae* the causal agent of taro leaf blight. European Journal of Plant Pathology **(1402),** 243-250.

Simic A, Sokovic M, Ristic M, Grujic-Jovanovic S, Vukojevic J, Marin P. 2004. The Chemical Composition of some Lauraceae essential oils and their antifungal activities. Phytotherapy Research **1,** 713-717.

Simic M, Kundakovic T, Kovacevic N. 2003. Preliminary assay on the antioxidative activity of *Laurus nobilis* extracts. Fitoterapia**74,** 613-616.

Singh G, Marimuthu R, De Heluani CS, Catalan C. 2005. Antimicrobial and antioxidant Potentials of essential oil and acetone extract of *Myristica fragrans* Houtt. (Aril part). Journal of Food Science **70,** M141-M148.

Sudhakar P, Latha P, Sreenivasulu Y, Reddy B VB, Hemalatha TM, Balakrishna M, Reddy KR. 2009. Inhibition of *Aspergillus flavus* colonization and aflatoxin (AfB1) in peanut by methyleugenol. Indian Journal of Experimental Biology **47(1),** 63-67.

Suhr IK, Nielsen VP. 2003. Antifungal activity of essential oils evaluated by two different application techniques against rye bread spolage fungi. Journal of Applied Micrbiology **94,** 665-674.

Tajkarimi MM, Ibrahim SA, Cliver DO. 2010. Antimicrobial herb and spice compounds in food. Food Control **21(9)**, 1199-1218.

Verdian-rizi M, Hadjiakhoondi A. 2008. Essential Oil Composition of *Laurus nobilis* L. of Different Growth Stages Growing in Iran. Verlag der Zeitschriftfür Naturforschung, Tübingen 785-788.

Xing Y, Li X, Xu Q, Yun J, Lu Y. 2010. Antifungal activities of cinnamon oil against *Rhizopusnigricans*, *Aspergillus flavus*and *Penicillium expansum in vitro* and *in vivo* fruit test. International Journal of Food Science and Technology **45,** 1837-1842.

Zuzarte M, Gonã§ Alves M J, Cavaleiro C, Dinis AM, Canhoto JM, Salgueiro LGR. 2009. Chemical composition and antifungal activity of the essential oils of *Lavandula pedunculata* (Miller) Cav. Chemistry & biodiversity **6,** 1283-1292.