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Full Length Research Paper

Evaluation of the antibacterial effects of essential oil from the leaves of *Laurus nobilis* L. in Kerman Province

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The antibacterial activity of the essential oil of *Laurus nobilis* L. on human pathogenic bacteria by disc diffusion method via average inhibition zone was studied. The chemical composition of the essential oil of the leaves of *L. nobilis* L. (Lauraceae) were obtained by hydrodistillation method and analyzed by gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS). Thirty three compounds, accounting for 95.75% of the total oil with 1.8% (v/w) oil yield were identified in the essential oil of the leaves. The major components were 1,8-cineole (25.7%), sabinene (8.7%) and α-pinene (5.25%). To study the antibacterial activity, the essential oil tested against 9 bacteria strains such as three Gram positive bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus faecalis* and six Gram negative bacteria: *Pseudomonas aeroginosa, Shigella flexneri, Klebsiella pneuomoniae, Salmonella typhi, Serratia marcescens* and *Escherichia coli* were studied. Effect of the essential oil of *L. nobilis* on bacteria tested was more than that of tetracycline antibiotic. The results showed that the essential oil of *L. nobilis* had strong anti-bacterial effects.

Key words: *Laurus nobilis* L., human pathogenic bacteria, antibacterial activity, 1,8-cineole, gas chromatography mass spectrometry (GC/MS).

INTRODUCTION

Pathogenic bacteria are important causes of disease in humans and farm products. Pathogenic bacteria contribute to other global important diseases, such as pneumonia, which can be caused by bacteria such as *Streptococcus* and *Pseudomonas*, and foodborne illnesses, which can be caused by bacteria such as *Shigella* and *Salmonella*. Side effects of drugs, use of chemical preservatives and the use of a special blend of natural oils to prevent the growth of bacteria need to be researched on. Sweet bay or bay laurel with scientific name, *Laurus nobilis* L. (Lauraceae) is an evergreen shrub growing to 12 m (39ft) by 10 m (32ft) at a slow rate and indigenous to the south parts of Europe and Mediterranean area. It is hardy to zone 8. Its leaf appears in January and flower from April to May. The flowers are dioecious (individual flowers are either male or female, but only one sex is found on any plant; so both male and female plants must be grown if seed is required) and are pollinated by bees. The plant is not self-fertile. Leaves are fresh or dried. As a spice, with aromatic flavouring, bay leaves are commonly used as flavouring for soups, stews, etc and form an essential ingredient of the herb mix 'Bouquet Garni' (Huxley, 1992). This plant is cultivated in the northern part of Iran. In folk medicine, the leaves of this plant are used to treat epilepsy, neuralgia, Parkinsonism, hemorrhoid and rheumatic pains (Weiss and Fintelmann, 2000). The leaves can be used either fresh or dried after summer harvesting and drying. The flavour of freshly dried, crushed or shredded leaves of the plant is stronger than that of fresh leaves, but the leaves should not be stored longer than a year since they will lose their flavour (Bown, 2001). The dried fruit is used as flavouring. The dried leaves are brewed into herbal tea. An essential oil obtained from the leaves is used as food

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flavouring. Yields can vary from 1 to 3% oil (Chiej, 1984). The bay tree has a long history of folk use in the treatment of many ailments, particularly as an aid to digestion and in the treatment of bronchitis and influenza (Phillips and Foy, 1990). It has also been used to treat various types of cancer (Duke *et al.,* 2002). The fruits and leaves are not usually administered internally, other than as a stimulant in veterinary practice, but were formerly employed in the treatment of hysteria, amenorrhoea, flatulent colic, etc (Bisset and Wichtl, 2001). It is also reported that the leaves are used mainly to treat upper respiratory tract disorders and to ease arthritic aches and pains (Barnes et al., 2002). It is settling to the stomach and has a tonic effect, stimulating the appetite and the secretion of digestive juices. The leaves are antiseptic, aromatic, astringent, carminative, diaphoretic, digestive, diuretic, emetic in large doses, emmenagogue, narcotic, parasiticide, stimulant and stomachic. The fruit is antiseptic, aromatic, digestive, narcotic and stimulant. An infusion has been used to improve appetite and as an emmenagogue (Grieve, 1984). The fruit has also been used in making carminative medicines and was used in the past to promote abortion. A fixed oil from the fruit is used externally to treat sprains, bruises etc, and is sometimes used as ear drops to relieve pain (Grieve, 1984). The essential oil from the leaves has narcotic, antibacterial and fungicidal properties (Lewis et al., 2003). An essential oil from the fruit is used in soap making. The plant is highly resistant to pests and diseases, it is said to protect neighbouring plants from insect and health problems (Holtom and Hylton, 1979). The leaves are highly aromatic and can be used as an insect repellent, the dried leaves protect stored grain, beans, etc from weevils. It is also used as a strewing herb because of its aromatic smell and antiseptic properties (Chiej, 1984). Very tolerant of clipping, it can be grown as a screen or hedge in areas suited to its outdoor cultivation. Wood-sweetly-scented, does not wear quickly. Used for marqueterie work, walking sticks and friction sticks for making fires. The antibacterial activity of essential oil of the leaves of *L. nobilis* L. plant has been studied in other countries and in Iran but the antibacterial of the essential oil of the leaves of *L. nobilis* L. that grow in Kerman Province has not yet been studied.

In the present work, the antibacterial properties of the oil of the leaves of *L. nobilis* L. that grows in Kerman Province in Iran were studied and then the results were compared with others from other countries.

MATERIALS AND METHODS

Plant material and isolation procedure

The leaves of *L. nobilis* L. plant were obtained from plants grown in a village in Kerman Province, Iran at full flowering stage in April 2011. The samples were cleaned in shade condition to prevent hydrolyzation of the existing materials and to keep the natural color the help of Clevenger set for three hours. The obtained essential oils were dried with anhydrous sodium sulfate and kept in sterile sample tubes in refrigerator. The oil yields from leaves was calculated as dry weight percentage, 1.8% (v/w).

Analysis of essential oil

Gas chromatography

GC analysis was performed using a model HP-439 gas chromatograph equipped with column CP Sil. 5CB with 25 m length, internal diameter of 0.25 mm and film thickness of 0.39 µm. Oven temperature was from 60 to 220°C at a rate of 7°C slope per minute. Injector temperature was 280°C and detector (FID) temperature was 270°C and carrier gas was helium.

Gas chromatography/mass mass spectrometry

In order to analyze and identify the combinations forming the essential oil, the chromatograph gas set attached to a mass spectrometry, Model Hewlett Packard-5973 was used. The conditions of analysis and specifications of the GC/MC set were as follows: Capillary column HP 5MS of 60 m length, internal diameter of 0.25 mm and layer thickness of 0.25 µm, thermal program of oven (3 min) at 60°C, then 60 to 220°C with a 6°C slope per minute, then 3 min in 220°C, the temperature of place of injection 280°C, gas conveying helium, the speed of gas moved by 1.0 ml per minute, the ratio of fission is 1 to 43, the rate of injection is 0.1 µl, temperature of the reservoir of ionization is 230°C, ionization mode EI, ionization energy is 70 eV. The series of normal Alkans C8-C17 were also injected into the set under the same condition with that of essential oil injection to calibrate retention index (RI) of components of essential oil. The retention index of components of the sample was calculated. 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 was in accordance with C8-C17 Alkans and when comparing them with the existing standard figurers in literature (Adams, 2001).

Antibacterial assay

The antibacterial activity of laurel oil on 9 resistant pathogenic bacterial strains such as three Gram positive bacteria *Staphylococcus aureus* (PTCC = 1431), *Staphylococcus epidermidis* (PTCC = 1436) and *Streptococcus faecalis* (PTCC = 1237) and six Gram negative bacteria *Pseudomonas aeroginosa* (PTCC = 1430), *Shigella flexneri* (PTCC = 1716), *Kellebsiella pneuomoniae* (PTCC = 1053), *Salmonella typhi* (PTCC = 1609), *Serratia marcescens* (PTCC = 1187) and Escherichia *coli* (PTCC = 1533) was determined. The bacteria under experiment were obtained from the Center for Fungi and Bacteria of Iranian Scientific and Industrial Researches Organization. The antibacterial activity of the essential oil was evaluated by disc diffusion method (Baron and Finegold, 1995). The bacteria were cultured for 24 h on the media of Muller Hinton Agar. A suspension with a dilution of 0.5 Mac Farland at the culture media of Muller Hinton Broth was prepared. Then 1 ml of suspension of each bacteria was cultured using spread plate method. Then, the blank sterile discs containing 30 µl of 1.5 dilution of essential oil diluted with DMSO were placed on culture media. The diameters of zones of inhibition of growth after 24 h of plate incubation at 37°C were measured. At that time, the antibacterial impacts of essential oil of sweet bay when compared with tetracycline antibiotic (8 mg/ml) as evidence was studied. All

Table 1. Combinations identified in the essential oil of the leaves of *L. nobilis* L.

Compound no.	Compound name	Restrictive index (RI)	Percentage (%)
1	(E)-2-Hexenal	845	1.32
2	Tricyclene	925	2.27
3	α-Thujene	928	0.38
4	α-Pinene	937	5.25
5	Camphene	958	3.86
6	Sabinene	983	8.7
7	β-Pinene	1005	3.99
8	Myrcene	1018	1.68
9	α-Phellandrene	1022	0.37
10	α-Terpinene	1025	2.12
11	*P*-Cymene	1029	0.31
12	Limonene	1032	3.47
13	1,8-Cineole	1037	25.7
14	γ-Terpinene	1068	3.48
15	Terpinolene	1092	0.22
16	Linalool	1123	1.56
17	Sabinol	1138	2.45
18	Borneol	1154	2.37
19	δ-Terpineol	1175	0.19
20	Terpinene-4-ol	1192	1.21
21	α-Terpineol	1201	3.79
22	Bornyl acetate	1257	1.79
23	γ-Terpinyl acetate	1321	0.24
24	Eugenol	1368	1.69
25	B-elemene	1395	2.30
26	β-Caryophyllene	1425	0.87
27	α-Humulene	1476	2.19
28	Germacrene A	1498	1.53
29	γ-Cadinene	1514	2.68
30	Germacrene D-4-ol	1549	1.59
31	Spathulenol	1573	3.38
32	Caryophyllene oxide	1589	0.58
33	Humulene epoxid-2	1597	2.22
Total			95.75

The restrictive index was calculated by injecting the mixture of normal hydrocarbons (C_8-C_{17}) into HP-5MS column.

the experiments were carried out in triplicate and averages were calculated for the inhibition zone diameters.

RESULTS

The study of the analysis of *L. nobilis* L. essential oil under the present investigation showed that the output ofessential oil was 1.8% (v/w). The identified compounds in essential oil, restrictive index (RI) and quantitative percentage of the compounds from leaves are presented in Table 1. Thirty three compounds identified in *L. nobilis* essential oil, comprises 95.75%, they are 1,8-cineole (25.7%), sabinene (8.7%) and α-pinene (5.25%) with 39.65% constituting the highest percentage of essential oil.

Antibacterial activity of essential oil from the leaves of *L. nobilis* L. on human pathogenic bacteria by disc diffusion method with measurement of average inhibition zone was studied in the Kerman Province. The antibacterial effects of this essential oil was determined against three Gram positive bacteria: *S. aureus* (PTCC = 1431), *S. epidermidis* (PTCC = 1436) and *S. faecalis* (PTCC = 1237) and six Gram negative bacteria: *P. aeroginosa* (PTCC = 1430), *S. flexneri* (PTCC = 1716), *K. pneuomoniae* (PTCC = 1053), *S. typhi* (PTCC = 1609), *S. marcescens* (PTCC = 1187) and *E. coli* (PTCC = 1533).

Table 2. The results of the antibacterial effects of the essential oil of the leaves of *L. nobilis* L.

Bacteria	The diameter of zone of inhibition of growth (mm)	Tetracycline (8 mg/ml)
Staphylococcus aureus (1431)	29	15
Staphylococcus epidermidis (1436)	26	21
Streptococcus faecalis (1237)	18	16
Pseudomonas aeroginosa (1430)	24	22
Shigella Flexneri (1716)	27	19
Kellebsiella pneuomoniae (1053)	21	14
Salmonella typhi (1609)	18	17
Serratia marcescens (1187)	26	13
Escherichia coli (1533)	28	12

The results of studying the antibacterial impacts shows that the oil of this plant has a 29, 26 and 18 ml diameter of zone of inhibition of growth on positive bacteria of *S. aureus*, and *S. epidermidis* and *S. faecalis*, respectively and has a diameter of zone of inhibition of growth of 24, 27, 21, 18, 26 and 28 ml on negative bacteria of *P. aeroginosa*, *S. flexneri*, *K. pneuomoniae*, *S. typhi*, *S. marcescens* and *E. coli*, respectively (Table 2). The effect of the essential oil of *L. nobilis* on bacteria tested was more than that of tetracycline antibiotic. The results showed that the essential oil of *L. nobilis* had strong anti-bacterial effects.

DISCUSSION

The antibacterial activity of the essential oil of *L. nobilis* L. in this study was compared with that of other researches in the world. In a research, the essential oil of *L. nobilis* L. exhibited a very strong antibacterial activity against the tested bacteria ($P < 0.05$) (Dadalioglu and Evrendilek, 2004). The results of antimicrobial activity against both Gram positive and negative bacteria species showed that *L. nobilis* induced the highest toxicity at 0.3 mg/g and affected energy metabolism and oxidative stress (Malti and Amarouch, 2009). In this study, the antibacterial and antifungal activities of *L. nobilis* L. (Lauraceae) was investigated. The microbial effect of this plant was tested by a disk diffusion method using *Bacillus megaterium* DSM 32, *Bacillus brevis* FMC 3, *Bacillus subtilis* IMG 22, *Bacillus cereus* FMC 19, *E. coli* DM, *Enterobacter aerogenes* CCM 2531, *P. aeruginosa* DSM 50071, *S. aureus* Cowan 1, *Listeria monocytogenes* Scott A and *Micrococcus luteus* LA 2971, *Candida tropicalis* and *Candida albicans* CCM 314. The results showed that the growth of *S. aureus* was inhibited by this plant extract (Digrak et al., 2001). In a research, eleven ethanolic extract from species of *L. nobilis* was assayed for the *in vitro* antibacterial activity against 3 Gram positive (*B. subtilis, S. aureus* and *S. epidermidis*) and 2 Gram negative bacteria (*E. coli* and *P. aeruginosa*), using agar dilution methods. The minimum inhibition concentration (MIC) of the *L. nobilis* ethanolic extracts was 5 mg/mL for all the microorganisms tested (Erturk, 2006). The essential oils from *L. nobilis* (L.) has been characterized and tested against two bacteria (*Lactobacillus plantarum* and *E. coli*) using a submerged broth culture method. The results obtained showed that *E. coli* was more inhibited than *L. plantarum* by essential oil of *L. nobilis* (L.) (Bouzouita et al., 2003). The antibacterial activity of *L. nobilis* was studied *in vitro* in tree bacterial strains: *S. aureus*, *S. intermedius* and *K. pneumonia.* The major component was 1,8-cineole (52.43%). The bacterial strains tested were found to be sensitive to essential oils studied and showed a very effective bactericidal activity with minimum inhibitory concentrations (MIC) ranging from 0.01 to 1 mg/ml (Derwich et al., 2009). In a study, the bacteriostatic and bactericidal activities of laurel oil against seven bacteria (*Aerobacter aerogenes, B. subtilis, E. coli, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus albus* and *S. aureus*) were evaluated. The results showed that the essential oil tested varied in their antibacterial activity. Laurel oil was active against all tested bacteria (Kivanc and Akgul, 1986). This study was carried out to determine the *in vitro* antimicrobial activity of the essential oil, seed oil and methanolic extract of seed oil obtained from *L. nobilis* L. (Lauraceae). The methanolic extract of seed oil exhibited more effective antibacterial activity as compared to the essential oil and seed oil. GC-MS analyses of the essential oil resulted in the identification of 25 compounds. 1.8-Cineol (44.72%), a-terpinyl acetate (12.95%) and sabinene (12.82%) were the main components. Methanolic extract of seed oil, showed 64.28% inhibition (Ozcan et al., 2010). Leaf extracts of *L. nobilis* L. showed antimicrobial activity against Gram-positive bacteria (*S. aureus* and *S. pyogenes*) (Fukuyama et al., 2011). In a research, the essential oil exhibited strong antibacterial activity against all tested foodborne spoilage and pathogenic bacteria (Ramos et al., 2012). In a study, kaempferol glycosides isolated from *L. nobilis* L., kaempferol-3-*O*-α-L-(2″,4″-di-*E*-*p*-coumaroyl)-rhamnoside (C2) and kaempferol-3-*O*-α-L-(2″-*E*-*p*-coumaroyl-4″-*Z*-*p*-coumaroyl)-rhamnoside (C3), showed strong antibacterial

activities against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (Liu et al., 2009). An extract from *L. nobilis* L. (Lauraceae) leaves showed antibacterial activity against methicillin-resistant *S. aureus* (Otsaka et al., 2008). 1,8-Cineole laurel oil has antimicrobial activity on several strains of bacteria and yeasts as well as some molds (Akgul et al., 1989).

Conclusion

The antibacterial properties of the essential oil of *L. nobilis* L. has been studied in other countries but the antibacterial effects of the essential oil of *L. nobilis* L. grown in Kerman Province is yet to be studied. The present study results showed that the major oil components of the leaves of *L. nobilis* L. from Kerman Province, Iran were 1,8-cineole, sabinene and α-pinene which were reported as the main constituents in other areas. The results of this study could be of interest for further phytochemical and biological investigation of *L. nobilis* L. taking into account that 1,8-cineole oil showed marked antimicrobial activity (van Vuuren and Viljoen, 2007). 1,8-Cineol is a natural organic compound which is a colorless liquid. It is a cyclic ether and a monoterpenoid with antibacterial effects (Cowan, 1999). 1,8-Cineole was reported as important constituents of *L. nobilis* L. *L. nobilis* has a higher antibacterial effect than tetracycline antibiotic. The strong antibacterial activity of *L. nobilis* oil and their components (1,8-cineole, sabinene and α-pinene) can be explained by the high percentage of these components in the oil.

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