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Physicochemical characteristics and antioxidant activity of *Lavandula bipinnata* seed oil

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Abstract

The aim of this work was to analyze physicochemical characteristics and fatty acid profile of seed oil of *Lavandula bipinnata*. The total lipid content of *L. bipinnata* seed was 27.2%. The oil was liquid at room temperature, with a specific gravity and refractive index of 0.971 and 1.480 respectively. The acid value (5.764 mg NaOH/g), saponification number (188.75 mg KOH/g), ester value (182.98 mg KOH/g), iodine value (169.2 mg/100 g), free fatty acid (2.88) and peroxide value (7.62 mq/kg) were comparable with that of sunflower oil. The seed oil possess high concentration of linolenic acid (66.25%) followed by linoleic (18.05%), oleic (7.20%) and palmitic (5.46%). *L. bipinnata* seeds accumulate a high amount polyunsaturated fatty acid (84.31%) and a monounsaturated fatty acid concentration of 7.29%. The seed oil failed to possess any antagonistic activity against the tested bacteria but a radical scavenging activity (IC_{50}) of 8.15 µg/µl was observed against DPPH.

Introduction

Lavandula genus is an important member of family *Lamiaceae* comprised of 39 species and several hybrids of woody perennial plants some of which have long been grown for their essential oil (Urwin and Mailer, 2008). *Lavandula bipinnata* (Roth) Kuntze is used in folk traditions for a variety of medicinal purposes and research till date was focused on essential oil composition and its antimicrobial activity (Hanamathagouda *et al.*, 2010). The lavender seed is known to possess a high content of oil on a dry weight basis (Urwin and Mailer, 2008), little work was focused on fatty acid profile and no work were concentrated on the physicochemical properties of the seed oils of this genus. Till date no attempts have been made in analyzing the physicochemical characteristics and fatty acid profile of the seed oil in *L. bipinnata.* The aim of present work is to analyze fatty acid composition of seed oil of *L. bipinnata* with its antioxidant activity.

Material and Methods

Plant material

Lavandula bipinnata (Roth) Kuntze seeds were obtained from Asangihal village in Sindagi taluk of Bijapur district, India and grown in the Botanical garden, Department of Botany, Karnatak University, Dharwad, India for three consecutive years. Prior to extraction of oil approximately 100 g of seed was dried overnight at 80°C and ground in a coffee grinder. The seed powder thus obtained was used for extraction of oil.

Oil extraction

The oil was extracted from the ground lavender seed powder using petroleum ether (b.p. 40–60°C) as the solvent for 8 h. The extracted oil was filtered and excess solvent was removed using a rotary evaporator at 40°C. Finally, the seed oil thus obtained was subsequently used for further analysis.

Physical analyses of seed oil

Oil from the seed was subjected to physical characterization. The color and state of the oil at room temperature were noted by visual inspection, while specific gravity was determined by the method of the Association of Official Analytical Chemists (AOAC, 2000a). The refractive index of the oil at room temperature was estimated using the Abbe refractometer as outlined in Association of Official Analytical Chemists (AOAC, 2000b).

Chemical composition

Acid value, free fatty acids, iodine value, saponification number, peroxide value and unsaponifiable matter were measured following a previously described method (AOAC, 2000c). The analysis was done for the freshly extracted seed oil.

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The samples were analyzed in triplicates; mean and standard error were calculated.

Fatty acid analysis

The fatty acid composition of the oil sample was analyzed by GC after transesterification. Fatty acid methyl esters was analyzed on a Chemito G.C. 8610 gas chromatograph equipped with flame ionization detector and capillary column B P × 70 (50 m × 0.32 mm × 0.25 µm films). The detector temperature was programmed for 260°C with flow rate of 0.3 ml/min. The injector temperature was set at 240°C. Nitrogen (purity 99.95%) was used as the carrier gas. Identification of the peaks was performed by comparing retention times with those of genuine standards analyzed under the same conditions.

Antimicrobial activity test

Antimicrobial activity of seed oil of *L. bipinnata* was tested by the paper disc diffusion method according to the National Committee for Clinical Laboratory Standards Guidelines (Anonymous, 2001) with some modifications, using 100 µl of suspension of the test microorganisms, containing 2.0 x 10^6 colony forming units (cfu/ml) for inoculating the plates. The antimicrobial activity of *L. bipinnata* seed oil was tested towards 15 different microorganisms. Six Gram positive bacteria namely *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, Vancomycin Resistant *Enterococcus* (VRE) ATCC 51299, *Bacillus subtilis* SDMC 025, *Micrococcus* spp. SDMC 016 and *Staphylococcus epidermidis* SDMC 097. Six Gram-negative bacteria, namely *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* SDMC 042, *Salmonella paratyphi* A SDMC 014, *Salmonella paratyphi* B SDMC 011 and *Providencia alcalifaciens* SDMC 056 were used. *Aspergillus niger* SDMC 052, *Penicillium notatum* SDMC 064 and *Candida albicans* SDMC 033 were the three fungi used for the study. The microorganisms used for the analysis were obtained from American Type Culture Collection and cultures maintained at S.D.M. Medical College (SDMC), Dharwad, Karnataka, India. Oil sample was prepared in DMSO and loaded (10 µl) onto sterile filter paper discs (6 mm diameter, Hi-Media Laboratories Pvt. Limited, India), with a final concentration of 50 µg/µl per disc.

Antioxidant activity using 1, 1-diphenyl-2-picrylhydazyl (DPPH)

The abilities of the oil to scavenge DPPH free radicals were measured by a previously reported method (Brand-Williams *et al.*, 1995). The DPPH solution (7.6 x 10^{-5} M) in dichloromethane was prepared fresh daily prior to UV measurements. Various concentration (33, 66, 166 and 333 µg ml^{-1}) of the oil solutions was dissolved in dichloromethane and placed into the vials. The DPPH solution (1 ml) was added to each of these vials. After sample solutions were allowed to stand for 30 min in the dark (25°C), the absorbance was measured at 517 nm using a UV spectrophotometer. Blank samples containing the same amount of methanol and DPPH solution were also prepared and measured. Ascorbic acid and butylated hydroxyanisole (BHA) was also monitored for radical-scavenging activity. The extracted oil in triplicates was analyzed separately. Inhibition of free radical by DPPH in percent was calculated using the following formula: Scavenging DPPH (%) = 100 x (A_{blank} - A_{sample}/A_{blank}), where A_{blank} is the absorbance of the control reaction (containing all reagents except the oil) and A_{sample} is the absorbance of the sample. The percentage of scavenged DPPH was plotted versus the concentration of antioxidants and the concentration of antioxidant required to obtain 50% inhibition (50% inhibition concentration or IC_{50}) was obtained from the graph.

Statistical analysis

Results are expressed as the means and standard errors of three separate estimations. The oil sample of each year was estimated for its physicochemical, fatty acid analysis, antimicrobial and antioxidant assay in triplicates.

Results and Discussion

The physicochemical properties of lavender seed oil extracted are given in Table 1. The oil content obtained from the seeds was 27.2% on a dry weight basis. The seed oil was colorless and consistently liquid at room temperature (25.0 ± 2.0°C) (Table 1). The specific gravity and refractive index of the oil were 0.97 and 1.48, respectively. The refractive index of the oil was in the range of edible oils. The total acidity, expressed as acid value, was 5.76 mg NaOH/g. The low level of percent FFA (2.88) in the seed oil suggests that the oil could be a good source of edible oil that can be stored for a long time without spoilage via oxidative rancidity. The high iodine value (169.20 mg/ l00 g) of lavender seed oil is due to the presence of high amounts of unsaturated fatty acids such as linolenic and linoleic acid (Table 2). The iodine value of the lavender seed oil was higher than that of watermelon, sunflower and soybean oil (Zahara and Ali, 2010). The obtained iodine value of lavender seed oil has been found satisfactory for

Table 1. Proximate and physicochemical properties of oil extract from *Lavandula bipinnata* seed

Constituents (g/100 g)	*L. bipinnata* seed oil ± Standard error
Total oil (%)	27.2 ± 1.36
Acid value (mg NaOH/g oil)	5.76 ± 0.36
Saponification number (mg KOH/g oil)	188.75 ± 3.36
Iodine value (mg/ l00g)	169.20 ± 1.21
FFA (%) as oleic acid [a]	2.88 ± 0.19
Ester value (mg/KOH)	182.98 ± 3.16
Peroxide value	7.62 ± 0.39
State at RT [b]	Liquid
Colour	Colourless
Specific gravity	0.97 ± 0.01
Refractive index at RT [b]	1.48

[a] FFA (%) = Free fatty acid (%)
[b] RT = Room temperature

Table 2. Fatty acids composition of *Lavandula bipinnata* seed oil

Fatty acid	*L. bipinnata* seed oil ± Standard error
C14:0 Myristic	0.02 ± 0.002
C16:0 Palmitic	5.46 ± 0.11
C18:0 Stearic	2.28 ± 0.09
C16: 1 Palmitoleic	0.08 ± 0.006
C18:1 Oleic	7.20 ± 0.42
C18:2 Linoleic	18.05 ± 0.29
C18:3 Linolenic	66.25 ± 1.04
C20:0 Arachidic	0.17 ± 0.01
C22:0 Behenic	0.13 ± 0.005
Total saturates	8.05
Total unsaturates	91.95

exploring its use as a source of essential fatty acids. The oil had high peroxide values of 7.62 meq/Kg, and this can be attributed to the presence of higher amounts of polyunsaturated fatty acids such as linolenic and linoleic acid (Table 2). Lavender seed oil had saponification values of 188.75. This clearly suggests that lavender seed oil consist mainly of medium chain fatty acids (i.e. C16 and C18). The ester value of the oil was found to be 182.98 ± 3.16 mg KOH/g oil.

The fatty acid compositions of the lavender seed oil are given in Table 2, which shows the principal fatty acid components in the lavender seed oil to be linolenic (66.25%), linoleic (18.05%), oleic (7.20%), palmitic (5.46%) and stearic (2.28%) acids, in decreasing order, while myristic (0.02%), arachidic (0.17%) and behenic acids (0.13%) were present in fairly low concentration. The presence of essential fatty acids, namely linoleic acid and linolenic acid confers on the oil considerable nutrition value (Eromosele and Eromosele, 2002). Especially, linoleic acid is important for its metabolic role in the synthesis of prostaglandins (Eromosele and Eromosele, 2002). In this study, the level of palmitic acid (5.46%) was lower than the sesame (8.7%) (Mohammed and Awatif, 1998), cotton (26%) and palm seed oil (40%) (Aparico and Aparico-Ruiz, 2000). *L. bipinnata* seeds accumulate a high concentration of polyunsaturated fatty acid (84.31%) and a monounsaturated fatty acid concentration of 7.29%. The total lipid composition obtained was similar to the results shown for other *Lavandula* species, but slight variations were

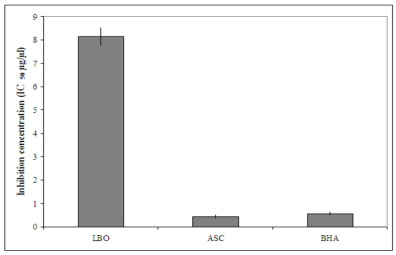

Figure 1. Antioxidant activity (IC_{50}) of *L. bipinnata* seed oil (LBO), ascorbic acid (ASC) and butylated hydroxyanisole (BHA)

observed in the concentrations of linoleic and oleic acids (Urwin and Mailer, 2008). The oil possesses a higher concentration of linolenic acid (66.25%) when compared to sunflower seed oil (5.5-10%) and the chemical properties was comparable with the chemical properties of sunflower seed oil (Lidefelt, 2007), thus it would therefore seem that commercial production of seeds or seed oils from *Lavandula* species may have some merit and give growers new products and at the same time help in minimizing waste disposal problems.

The seed oil failed to show any antagonistic activity against the tested microbes. In the DPPH assay, the radical scavenging ability of the oil and the positive controls (butylated hydroxyanisole (BHA) and ascorbic acid) was analyzed in triplicates. The oil was able to reduce the stable radical DPPH to the yellow colored DPPH-H with an IC_{50} value of 8.15 ± 0.32 µg/µl (Figure 1). BHA and ascorbic acid exhibited high antioxidant activity with IC_{50} values 0.425 ± 0.09 µg/µl and 0.566 ± 0.06 µg/µl, respectively.

The fatty acid composition of vegetable oil is highly variable in different plant species. In lavender, linolenic and linoleic acids (PUFAs) are the predominant fatty acids and form more than 80% of the total fatty acids. The high levels of polyunsaturated fatty acids (PUFAs) increase the quality of the oil for human consumption. Moreover, high levels of PUFAs, reduce blood cholesterol and play an important role in preventing atherosclerosis (Ghafoorunissa, 1994).

Conclusion

The physicochemical properties of *L. bipinnata* oil compare favorably with those of conventional edible oils; percent free fatty acids and peroxide value are below the maximum desirable limit and this suggests the suitability of the oil as edible oil. Lavender seed oil might be an acceptable substitute for highly unsaturated fatty acid and its radical scavenging activity. The seed oil of *L. bipinnata* can be used as an important source of PUFA, thus

nutritionally valuable. Therefore, results obtained in this study provide useful background information for using lavender seed as an important new source of oil for its high concentration of PUFA which would be beneficial for combating human health problems.

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