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RESEARCH ARTICLE

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VARIATION IN BIOACTIVE COMPOUNDS IN DIFFERENT PLANT PARTS OF LEMON BASIL (OCIMUM BASILICUM VAR CITRIODORUM)

ABSTRACT

Basil is a rich source of dietary antioxidants. Present study was conducted to study variation in bioactive compounds in different plant parts of lemon basil. Essential oil yield was higher (0.28%) in flowers followed by leaves and stem. TLC screening confirmed the presence of phenols, flavonoids and terpenes in stem, leaves and flowers. Leaves contained highest total phenols and flavonoids i.e., 215.43 mg GAE/100 g DW and 221 mg CE/100 g DW, respectively. Highly significant differences were observed for antioxidant activity among plant extract/essential oil, plant parts and different concentrations. Essential oil possessed higher antioxidant activity than plant extract and lemon basil leaves were found to be having highest antioxidant potential (65.62%) followed by flowers and stem.

Key Words: Lemon basil, essential oil, total phenols, total flavonoids, antioxidant activity, DPPH assay, TLC

INTRODUCTION

The genus Ocimum of Lamiaceae family is comprised of 200 species differentiated from each other on the basis of morphological features and chemical constituents [1]. Lemon basil and sweet basil is used mainly as spice in Thai cuisine. Basil is cultivated for its extraordinary essential oil which display many therapeutic usages such as in medicinal application, herbs, culinary, perfume for herbal toiletries, aromatherapy treatment and as flavouring agent. Use of the basil leaves in food is beneficial to health because of antifungal, anti-inflammatory, antimicrobial and antioxidant activities [2,3] which are attributed to the presence of different bioactive compounds including phenols and flavonoids [4,5]. The chemical constituents fluctuate among basil cultivars. The major constituents are linalool, methyl chavicol, eugenol, 1,8-cineole, geranial, neral, methyl cinnamate [6].

Basil leaves are reported to possess antioxidant activities [2, 7] but little is known about variation in bioactive compounds concentration in different plant parts i.e., leaves, stem and flowers. In this context, present study was aimed to investigate the concentration of essential oil, total phenols and flavonoids in stem, leaves and flowers of lemon basil. Antioxidant activity of plant extracts and essential oil extracted from stems, leaves and flowers was also explored. The information spawned will be helpful in exploring the medicinal uses of lemon basil.

Materials and Methods

Plant material

Lemon basil was harvested at flowering stage from clonal repository of medicinal plants at Plant Genetic Resources Institute, NARC, Islamabad. Leaves, stems and flowers were separated and dried in shade for three days.

Preparation of plant extract

Dried and crushed plant material (400g) was extracted with methanol in Soxhlet apparatus for 72h [8]. Plant extracts were concentrated by rotary evaporator after filtration with Whatman No. 1 filter paper and residue was stored at -20˚C for further analysis.

Extraction of essential oil

Essential oil was extracted from dried stems, leaves and flowers through hydro-distillation using Clevenger type apparatus for three hours Essential oil yield was calculated on dry weight basis by using following formula.

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$$EO\ (\%) = \frac{\text{Volume of essential oil (ml)}}{\text{Weight of dried plant material (g)}} \times 100$$

The essential oil was stored at 4ºC in amber glass vials for use in experiments.

Thin Layer Chromatography (TLC)

TLC was carried out for the screening of major components present in plant extracts and essential oil extracted from stem, leaves and flowers. TLC Silica gel 60 F254 (20x20 cm) glass plates were activated at 110ºC for 30 minutes. Mobile phase consisted of toluene and ethyl acetate (19:1) for resolution of components. Essential oils and plant extract samples were prepared by dissolving 10µl in toluene (50µl). Samples (3µl) were applied at 1.5cm distance from the base of the plate. After air drying the developed TLC plates were sprayed with methanolic solution of vanillin and heated at 100ºC for fifteen minutes. Retention factor (R_f) values were recorded for visible spots.

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front TLC plates}}$$

Total Phenolics

Total phenol contents were determined by following Sulaiman and Balachandran [9]. Plant extract (500µl) of extract and deionised water (was added to a 20 ml test tube, containing 4.5ml distilled deionised water. Folin-Cicalteau reagent (500µl) was added to the mixture and shaken. After five minutes 7% Na_2Co_3 solution (5 ml) was added to the reaction mixture. Final volume was made up to 12.5ml by adding ddH_2O. Blank sample was prepared by using ddH_2O. Different concentrations of gallic acid (20, 40, 60, 80, 100, 120, 140 mg/l) were used for standard curve. Samples were incubated at room temperature for 90 minutes and absorbance was taken 750nm using UV-Vis Spectrophotometer Lambda 5. The total phenolics in extracts of lemon basil stem, leaves and flowers were expressed as milligrams of gallic acid equivalents per 100g dry weight (mg GAE/100 g DW).

Total flavonoids

For total flavonoids determination, method described by Park et al., [10] was used. Plant extract and catechin standard solution (20, 40, 60, 80, and 100 mg/l) was added to a 10 ml volumetric flask containing 4ml distilled water in an aliquot of one ml. To the flask was added 0.3ml 5% $NaNO_2$. After 5 minutes 0.3ml of 10% $AlCl_3$ was added. Two milliliters of 1M NaOH were added and the total volume was made up to 10ml with ddH_2O. The solution was mixed well and the absorbance was measured against a prepared reagent blank at 510nm with a UV-VIS Spectrophotometer Lambda 5. Total flavonoid contents were expressed as milligrams of catechin equivalent per 100 grams dry weight (mg CE/100 g DW).

Antioxidant Activity (DPPH assay)

The antioxidant activity of methanolic extracts and essential oils extracted from stem, leaves and flowers of lemon basil grass was measured in terms of hydrogen donating or radical scavenging ability using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). DPPH stock solution was prepared in methanol by dissolving 3.96 mg (x 4) of DPPH into 20 ml of methanol. DPPH (1ml) was added to 0.5 ml of sample solution. These solution mixtures were incubated for 30 minutes in dark at room temperature and absorbance was

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measures at 517 nm. Lower absorbance of the reaction mixture indicated higher free radicals scavenging activity. Finally the radical scavenging activity was calculated as percentage of DPPH discoloration using the equation

$$\% \text{ scavenging DPPH free radical} = \frac{\text{Abs of Control} - \text{Abs of Sample}}{\text{Abs of Control}} \times 100$$

Statistical Analysis

The data was recorded from triplicate experiments and analyses of variance were calculated using the statistical software Statistix 8.1 with least significant differences at the 5% probability level. The statistical differences among means were calculated by using Duncan's multiple range test (DMRT).

Results and Discussion

Essential Oil Yield

Mean values for essential oil yield from different parts of lemon basil are presented in Fig.1. Flowers contained highest quantity of essential oil (0.28%) followed by leaves (0.16%). Stems were found to contain minimum quantity of essential oil. Lamiaceae plants are a source of aromatic oil responsible for specific flavour and aroma. Sweet basil essential oil contents are reported to be 0.57-0.71% [11].

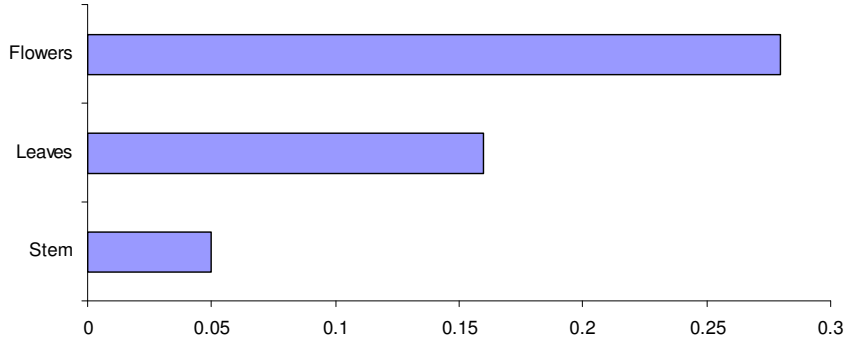

Fig. 1 Essential oil yield from different parts of lemon basil

TLC

Phytochemical screening of essential oils and plant extracts gives information regarding the presence of secondary metabolites and drug potential of a plant. Significant chemical constituent is separated through a chromatographic technique. Preliminary phyto-chemical screening through TLC of plant extract and essential oil of different parts of lemon basil revealed the presence of terpenes and phenols when plates were developed in toluene and ethyl acetate (19:1). R_f values shown in Table 1 indicated the polar nature of the constituents. Several researchers have used TLC as useful tool for separating the bioactive constituents of medicinal plant extracts [12-14].

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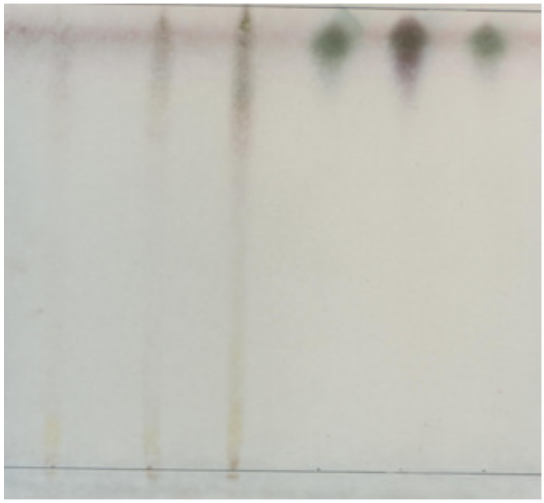

Fig. 2 TLC fingerprinting of plant extract and essential oil of lemon basil. (a: plant extract from stem, b: plant extract from leaves, c: plant extract from flowers, d: essential oil from stem, e: essential oil from leaves, f: essential oil from flowers)

Plant Extract/Essential Oil	Plant Part	R_f Value	Colour of the Spot
Plant Extract	Stem	0.87	Brown
	Leaves	0.90	Light green
	Flower	0.94	Deark green
Essential Oil	Stem	0.85	Dark green
	Leaves	0.79	Brown
		0.88	Dark green
	Flower	0.88	Dark green

Table 1. R_f values of plant extracts and essential oils from different plant parts of lemon basil

Total Phenols and Flavonoids

Considerably higher phenol and flavonoid contents were detected in different parts of lemon basil. Leaves contained highest total phenols and flavonoids i.e., 215.43 mg GAE/100 g DW and 221 mg CE/100 g DW, respectively (Fig. 3). Lowest total phenols and flavonoids were detected in stems (Fig.3). Leaves contained higher proportion of total flavonoids (141.2 mg CE/100 g DW) than phenols (99.76 mg GAE/100 g DW). Phenol sand flavonoids have been implicated as natural antioxidants in fruits and vegetables, possessing health maintaining and protecting abilities [15]. Javanmardi et al., [4] reported higher phenolic contents in basil than other lamiacae plants including thyme and rosemary. Taie et al., [11] reported a higher quantity of total flavonoids (7.03 mg/g quercetin) in basil.

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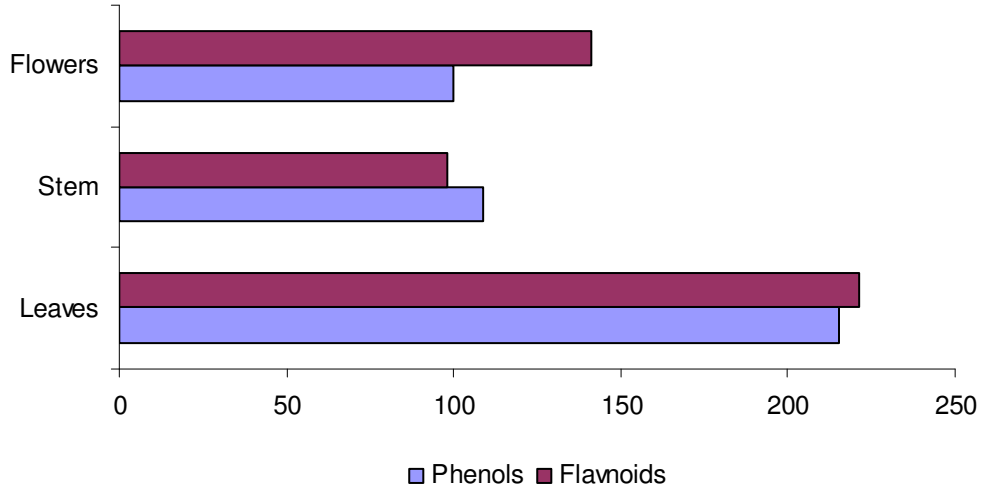

Fig.3 Total phenols and flavonoids in different parts of lemon basil

Antioxidant Activity of Plant Extracts and Essential Oils

Highly significant differences were observed for antioxidant activity among plant extract/essential oil, plant parts and different concentrations (Table 2). The results presented in Table 3 showed that essential oil possessed higher antioxidant activity than plant extract and lemon basil leaves were found to be having highest antioxidant potential followed by flowers and stem. Mean values of antioxidant activity possessed by different concentrations of plant extract and essential oil of lemon basil are presented in Table 4. Highest antioxidant activity (74.11%) was recorded for 200ppm concentration of essential oil followed by 100ppm of essential oil (70.27%). It is clear from the table that each tested concentration of essential oil performed better than respective concentration of plant extract. Thymus capitatus essential oil was stated to possess more antioxidant potential as compared to plant extract in a preceding study [16]. Many researchers have used DPPH assay to determine the antioxidant potential of medicinal plants [17,18]. 57% antioxidant activity has been reported by Taie et al., in sweet basil [11]. Higher antioxidant activity may be due to higher levels of phenols and flavonoids in leaves than flowers and stem. These findings are supported by previous studies in which correlation between total phenols and antioxidant activity has been reported [19].

Source	DF	F value
PE/EO	1	44.07^{**}
Plant Part	2	13.96^{**}
Conc.	3	24.39^{**}
PE/EO x Plant Part	2	48.09^{**}
PE/EO x Conc.	3	0.41^{ns}
Plant Part x Conc.	6	0.94^{ns}
Error	6	
Total	23	

PE: plant extract; EO: essential oil. **highly significant at 5%; $^{ns:}$ non-significant

Table 2 Analysis of variance for antioxidant activity of plant extract and essential oil of different plant parts of lemon basil.

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PE/EO		Plant Part		
Plant Extract	**Essential Oil**	**Stem**	**Leaves**	**Flowers**
51.89[b]	64.83[a]	54.21[b]	65.62[a]	55.24[b]

Table 3 Mean values for antioxidant activity of plant extract and essential oil of different plant parts of lemon basil.

*Means with same letters are not statistically different

Plant Extract/Essential Oil	Concentration (ppm)	Antioxidant Activity (%)
Plant Extract	25	41.12[f]
	50	46.81[ef]
	100	55.17[c-e]
	200	64.44[bc]
Essential Oil	25	53.50[de]
	50	61.44[b-d]
	100	70.27[ab]
	200	74.11[a]

*Means with same letters are not statistically different

Table 4 Mean values for antioxidant activity of different concentrations of plant extract and essential oil

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Riffat Tahira[1]*, Tauseef Rehan[1], Ata-ur-Rehman[2] and Muhammad Naeemullah[1]
[1]Plant Genetic Resources Institute, NARC, Park Road, Islamabad.
[2]Pakistan Science Foundation, 1-Constitution Avenue,Sector G-5/2, Islamabad.