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Antioxidant activity of methanol extract of three species of *Leucas* (Lamiaceae) having restricted distribution in South India

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Abstract
The present study aims to carry out the phytochemical screening and to check the antioxidant activity of three endemic species of *Leucas* from South India. Aqueous, methanol, ethanol and chloroform extracts of each plant were subjected to qualitative phytochemical screening. The total phenols, flavonoids and tannins were quantified in the methanolic extract by standard spectrophotometric methods. The antioxidant capacities of the extract were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay. Among three *Leucas* species, *Leucas lanceaefolia* shows higher antioxidant acivity (IC 50 value is 24.64 ± 1.29) followed by *Leucas prostrata* (58.90 ± 1.03) and *Leucas aspera* (108.55 ± 7.45). A positive correlation between the antioxidant activity potential and total phenolic levels are noticed in the species studied.

Keywords: Antioxidant, DPPH, Extract, *Leucas*, Phytochemical screening, Phytoconstituents,

Introduction
Plants have always been an attractive source of drugs. Currently 25% of drug molecules are obtained from plant sources. Traditional knowledge and use of plants are exploited as a source of base information to isolate chemotherapeutic agents and drugs from plants. Studies on natural product by exploration of existing scientific knowledge, traditional uses and discovery of potential chemotherapeutic agents are aimed to determine medicinal values of plants. Phytochemicals are used as the motifs for lead optimization programs, which are intended to make effective and safe drugs [1]. The genus *Leucas* R.Br. is one of the largest genera of the subfamily Lamioideae of the angiosperm mint family Lamiaceae with about 100 species found in dry or disturbed regions of Tropical to Southern Africa, India, South China, Iran South East Asia up to Australia [2]. *Leucas* is well known for its medicinal value, and widely used to cure many diseases in Ayurveda. The whole plant is used for curing stomach pain, gas trouble and piles. Flowers are used for curing hiccups.
The objective of the present study was to investigate the antioxidant activity *in vitro* of the crude extract of leaves of three *Leucas* species grows only in south India. Total phenolic, flavonoid and tannin content was also determined in order to evaluate a coefficient of correlation between the antioxidant activity and these compounds.

Materials and Methods
Plant material
Fresh leaves of *L. aspera*, *L. lanceaefola*, *L. prostrata* (Figure 1) were collected from different regions of Western Ghats and were identified. Voucher specimens have been deposited in Calicut University Herbarium (CALI). The leaves were dried at ambient temperature and powdered. The Plant powder was extracted exhaustively with methanol in a Soxhlet extractor.
The leaf extracts of three species of *Leucas* were analysed for the presence of alkaloids, flavonoids, phenols, tannins, terpenoids, saponins, carbohydrates, glycosides, proteins and amino acids according to standard methods [3].

Total phenolics
The total phenolic content was estimated by Folin-Ciocalteu method [4]. An aliquot (1 ml) of extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 µg/ml) was added to a 25 ml volumetric flask, containing 9 ml of distilled water. A reagent blank was prepared using distilled water. One milliliter of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na2CO3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 90 min at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV/Vis spectrophotometer. Total phenolics content was expressed as mg gallic acid equivalents (GAE).

Flavonoid content
Total flavonoid content was measured by the aluminium chloride colorimetric assay [5]. An aliquot (1 ml) of the extracts or standard solutions of quercetin (50, 100, 150, 200 and 250 µg/ml) was added to a 10 ml volumetric flask containing a 4 ml of distilled water. To the flask, 0.30 ml of 5% NaNO2 was added and after 5 min, 0.3 ml of 10 % AlCl3 was added. After 5 min, 2 ml of 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE).

Total tannin content
In this method, an aliquot (1 ml) of the extracts or standard solutions of tannic acid (1, 2.5, 5.0, 10 µg/ml) was added to a 10 ml volumetric flask containing a 4 ml of distilled water. A reagent blank was prepared using distilled water. 500 µl of Folin-Denis reagent was added to the mixture and shaken. After 5 min, 1 ml of 7 % Na2CO3 was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 775 nm. The concentration of tannins was expressed as tannic acid equivalents in milligram per gram (TAE mg/g) of crude extract.

Fig 1: A; *L. aspera*, B; *L. lanceifolia*, C; *L. prostrata*

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity

The effect of the extracts on DPPH radical was estimated using the method of Liyana-Pathiranan and Shahidi [6]. A solution of 0.1 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol containing 10 - 300 µg of the extract. The reaction mixture left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid and BHT were used as references. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$ where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract /standard.

The IC50 value (µg/mL) is the effective concentration at which DPPH radicals were scavenged by 50% and the value was obtained by interpolation from linear regression

Results and Discussion

Qualitative analysis was done to identify the presence of the following phytoconstituents - alkaloids, flavonoids, phenols, tannins, terpenoids, saponins, carbohydrates, glycosides, proteins and amino acids (Table 1).

Estimation of total phenol, flavonoid and tannin content

Methanolic extracts of leaves of *L. aspera*, *L. lanceaefola* and *L. prostrata* were prepared to examine the total phenolic content, flavonoids and tannins. The results are tabulated in Table 2[7].

The total phenolic content is given based on the absorbance values of extracted solutions reacted with the Folin–Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents. *Leucas lanceaefola* is having higher count of total phenolics compared other two species (37 ± 0.71), followed by *Leucas prostrata* (15.86 ± 0.18) and lastly by *Leucas aspera* (10.63 ± 1.78). Similarly, *Leucas lanceaefola* is having higher quantities of total flavonoids (66 ± 5.61) and tannins (6.9 ± 0.28). *Leucas prostrata* is having lesser quantity of flavonoids (32.13 ± 3.01) whereas *Leucas aspera* is having lesser quantity of tannins (1.78 ± 0.15). The concentration of total tannins is significantly lesser compared to total phenolics and flavonoids.

Various factors such as the genetic potential of individual species for polyphenol biosynthesis variation in total phenol content may be the reason for the variations in total phenol content [8]. Apart from this, maturation stage may also be critical in this respect [9] and also the influence of the extractability of solvents of phenolic compounds. Pure methanol is an effective solvent for antioxidant extraction, especially for phenolic compounds [10,11]. The phenolic compounds act as antioxidants because of their redox properties [12]. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants *in vivo* [13,14]**.** Tannins were reported to exhibit anti-tumour, antiviral and antibacterial activities and also it was reported that certain tannins were able to inhibit HIV replication selectively.

DPPH radical-scavenging activity

Antioxidant activity of the leaf methanolic extracts of three species of *Leucas* was tested by the DPPH radical scavenging assay where the consumption of a stable free radical was measured. Antioxidant activity of leaf extracts was compared with a known high antioxidant activity standard compound i.e. Ascorbic acid. Figure 2 shows the dose response curves of the methanolic extracts of three species of *Leucas*. Lower IC50 value indicates higher antioxidant activity. IC50 value for ascorbic acid was found to be 14.14 ± 0.50 µg/mL. *Leucas lanceaefola* is showing greater antioxidant activity since the IC50 value is very low

compared to other species (24.64 ± 1.29 µg/mL). *Leucas aspera* is having lower antioxidant activity (IC50 value is 108.55 ± 7.45 µg/mL) and *Leucas prostrata* is showing moderate antioxidant activity (IC50 value is 58.90 ± 1.03).

There is a strong correlation between antioxidant activity and Total Phenol content and several researches had confirmed the importance of polyphenols as a potential antioxidant biomolecule [15, 16, 17]. Recent reports show a highly positive relationship between total phenol and antioxidant activity appears to be the trend in many plant species [18]. In this study, it appeared that the higher Total Phenol content of the plant extracts resulted in higher antioxidant activity.

A significant and linear relationship existed between the antioxidant activity and phenolic content of the extracts, thus indicating that phenolic compounds are major contributors to antioxidant activity.

Our data indicated that the methanolic extracts of the *Leucas* species studied possess good antioxidant activity and are the potential sources of secondary metabolites too. Further extensive investigations are needed to be done evaluate the *in vivo* potential of these extracts in animal models and also isolation and characterization of the active antioxidant compounds. Determination of the antioxidant compounds in essential oils and plant extracts will help to develop new drug supplement for antioxidant therapy.

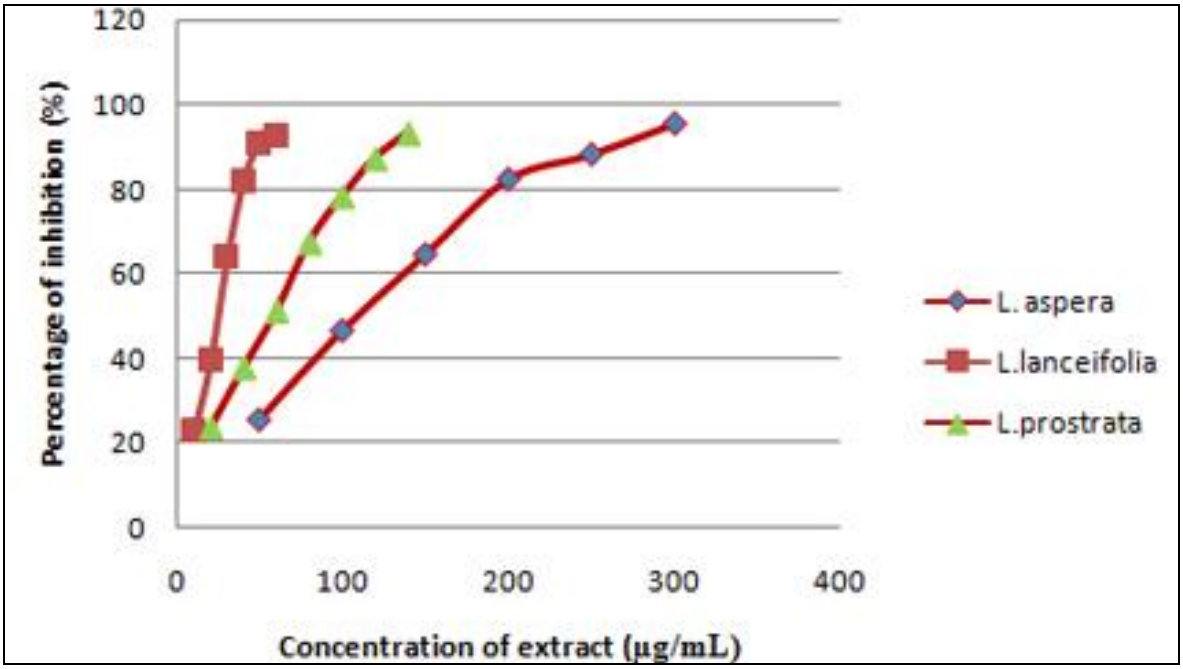

Fig 2: Dose response curves of the methanolic extracts of *L. aspera, L. lanceifolia* and *L. pros-trata*

Table 1: Phytochemical composition of the leaves of *L. aspera, L. lanceaefola* and *L. prostrata* in aqueous, methanol, ethanol and chloroform extracts.

Plant Name	Crude extract	Phyto-constituents								
		Alkaloids	Flavanoids	Phenols	Tannins	Terpenoids	Saponins	Carbohydrates	Proteins & Aminoacids	Glycosides
Leucas aspera	AE	++	+++	+++	-	++	++	+++	++	+++
	ME	++	+++	+++	+	+++	++	+++	-	++
	EE	-	+	-	-	-	++	+++	+++	++
	CE	-	-	-	-	+	++	+	-	+
Leucas lanceaefola	AE	+	+++	+++	-	++	+	+++	++	+++
	ME	-	+++	+++	+	+	+	+++	-	+++
	EE	-	-	++	-	+	+	+++	+++	++
	CE	-	-	-	-	-	+	++	++	++
Leucas prostrata	AE	+	+++	+++	-	+++	++	+++	+++	+++
	ME	+	+++	+++	+	++	++	+++	-	+++
	EE	-	-	+	-	+	++	+++	+++	+++
	CE	-	-	-	-	-	++	+++	++	++

AE: Aqueous extract, CE: Chloroform extract, EE: Ethanol extract, ME: Methanol extract,
+: Present, ++: Moderately present, +++: Appreciable amount, -: Absent

Table 2: Quantitative analysis for total phenolics, flavonoids and tannins

Plants	Phenolics [a]	Flavonoids [b]	Tannins [c]
Leucas aspera	10.63 ± 1.78	38.63 ± 0.18	1.78 ± 0.18
Leucas lancaefolia	37 ± 0.71	66 ± 5.61	6.9 ± 0.28
Leucas prostrata	15.86 ± 0.18	32.13 ± 3.01	2.38 ± 0.18

All values are the mean of three measurements.

a Total phenolic content expressed as mg of GAE/g of dry weight (DW) (mg GAE/g DW).

b Flavonoids content expressed as mg of QE/g of dry weight (DW) (mg QE/g DW).

c Condensed tannins content expressed as mg of TAE/g of dry weight (TAE) (mg CE/g DW).

Table 3: Fifty percent inhibitory concentration (IC50) of different plants extracts.

Methanolic extract	IC50 (µg/ml)
Leucas aspera	108.55 ± 7.45
Leucas lanceaefola	24.64 ± 1.29
Leucas prostrata	58.90 ± 1.03

The results are expressed as means SD (n=3).

Conclusion

The phytochemical screening and the antioxidant activity of three endemic species of *Leucas* were analyzed in the present study. Among the three *Leucas* species studied, *Leucas lanceaefolia* exhibited the highest antioxidant activity (IC_{50} = 24.64 ± 1.29), followed by *Leucas prostrata* (58.90 ± 1.03), while *Leucas aspera* showed the lowest activity (108.55 ± 7.45). The species studied exhibited a positive correlation between antioxidant activity potential and total phenolic content. The methanolic extracts of the three *Leucas* species demonstrate significant antioxidant potential, underscoring their phytochemical richness and highlighting their promise as natural sources of bioactive compounds for future pharmacological and conservation-oriented studies.

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Conflict of Interest

The authors declare no conflict of interest.

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