



Pharmacognostic Study, Chemical Analysis and Antioxidant Potential of *Leucas indica* L. (R. Br.)

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ABSTRACT

Background & Aim: *Leucas indica* L. (R. Br.) is a lesser known aromatic herb of family Lamiaceae. Traditionally it was used to cure cough and cold by tribals of Southern India. The present work was aimed to investigate its pharmacognostic characters, major phytochemicals and antioxidant potential as possible replacement of its allied members to reduce their exploitation.

Experimental: The fine details of stem and leaves of *L. indica* were noted including vasculature, cortical and epidermal depositions, trichome structure and stomata. The fluorescence analysis of leaf powder was done using various laboratory chemicals and reagents. The methanol leaf extract was analyzed using HPTLC to identify major active compounds and antioxidant potential was studied using standard method.

Results: Both simple and glandular trichomes were present on stem as well as leaves. The fluorescence analysis of powdered drug material after interaction with different laboratory chemicals showed distinct colorations. The preliminary analysis revealed the phytochemical richness of the plant. Most phytoconstituents were found to be extracted in methanol solvent. The HPTLC analysis of methanol extract showed presence of a range of phenolics and flavonoids. The chromatogram showed availability of rutin, ferulic acid, catechin and apigenin which makes this plant as a possible antioxidant drug candidate.

Recommended applications/industries: The rich chemical diversity of this plant and significant antioxidant potential could be used as good natural source for herbal pharmaceuticals.

1. Introduction

Leucas is a common genus of family Lamiaceae represented by its distinct aroma. *Leucas indica* is relatively uncommon plant in Maharashtra state (India). It is a small aromatic herb with known traditional medicinal utility (Udayan et al., 2007) against cough and cold. However, other species of *Leucas* especially

L. aspera and *L. cephalotes* are also used for their antioxidant property (Ali et al., 2013) and their antimicrobial and anti-inflammatory properties were also investigated (Ai et al., 2012; Tahareen et al., 2016).

Present study is focused on pharmacognostic evaluation with fluorescent analysis of leaf powder and its HPTLC profiling to identify the chemical

compounds which could be further exploited as drug material.

2. Materials and Methods

The plant was collected from Narnala forest area and identified using flora of Marathwada (Naik, 1998).

The pharmacognostic study was done by taking transvers sections of stem and leaves of fresh material. The shade dried leaves were powdered and used for fluorescent analysis (Chase and Pratt, 1949), preliminary phytochemical analysis (Horborne, 1973; Krishnaiah *et al.*, 2009) and HPTLC analysis was done using CAMAG TLC Scanner 4. The chromatograms were analyzed using standard literature considering Rf values as compound indicator. The antioxidant activity was determined by modified method of Bloiss (1958).

3. Results and discussion

L. indica is an annual herb growing in tropical and subtropical area. Its microscopic analysis, fluorescent study of leaf powder, HPTLC of methanol extract and antioxidant potential was investigated.

3.1. Microscopic features

In transverse section of *L. indica* stem, it appears quadrangular in outline. Epidermis is parenchymatous, covered with thick cuticle; both simple trichomes and sessile glandular trichomes with broad base and 3 to 4 celled head were observed. Cortex parenchyma forms 3 to 7 rows, with thick walled cells. The thickness of cortex was more below ridges. Endodermis distinct, pericycle is characterized by slight larger parenchymatous cells. Vascular bundles were conjoint collateral, vascular elements were well developed in secondary structure. The phloem elements were more in furrow region while at ridges phloem was more compressed. Central pith was parenchymatous; cells were larger and some with calcium oxalate crystals (Figure 1A).

Transverse section of *L. indica* leaf passing through midrib shows single layered upper epidermis interrupted by both simple and glandular trichomes. The epidermal cells were of slight larger size. It was followed by single layered palisade cells in lamina portion. In the center at midrib portion 2-4 collateral

vascular bundles were seen. The lower portion of the midrib is occupied by collenchymatous cells. Both simple and glandular trichomes are present, simple trichomes were uniseriate with pointed apex and glandular trichomes stalked with unicellular head. Stomata on lower side of leaf were anomocytic type (Figure 1B).

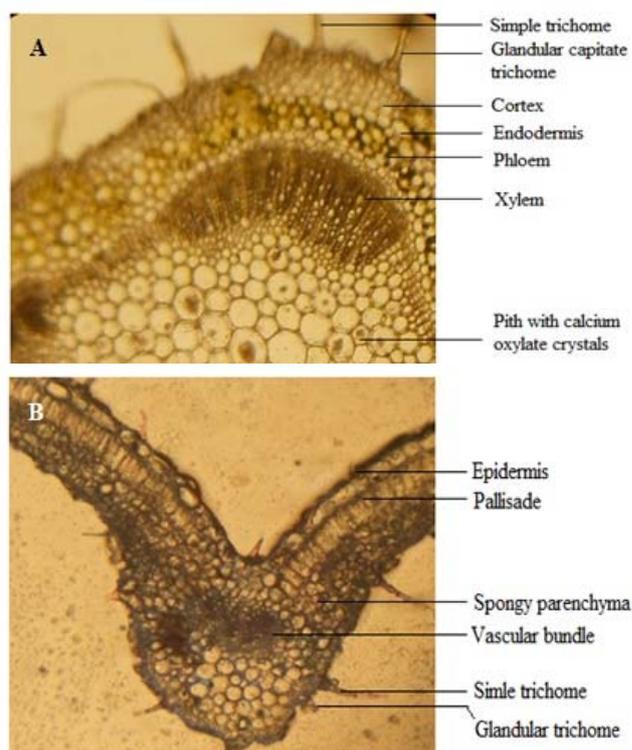


Figure 1. Anatomical study of stem and leaf of *L. indica*. A: Transverse section of *L. indica* stem; B: Transverse section of *L. indica* leaf.

3.2. Florescent analysis

The powdered material of shade dried leaves of *L. indica* was analyzed for its physical properties especially its coloration. It was also added with some routine chemicals available in the laboratory to observe the color change in direct sun light as well as in UV-light. The results showed that there is drastic color change when the reaction mixture was expose to both sun light and UV light. It is important criterion to decide the authenticity of the market available crude powdered drug of traditional medicinal plants (Table 1).

Table 1. Florescent analysis of crude powder of *L. indica* leaves.

Reaction with chemicals	<i>Leucas indica</i> leaf powder	
	Sun light	UV- light
Powder as such	Green	Dark green
Powder + Picric acid	Deep green	Bluish green
Powder + HCl	Blackish green	Dull green
Powder + HCl (50%)	Deep green	Dull green
Powder + H ₂ SO ₄	Dark brown	Greenish black
Powder + H ₂ SO ₄ (50%)	Dull brown	Grayish black
Powder + NaOH solution	Brownish yellow	Brownish
Powder + Ferric chloride (5%)	Green	Blackish green
Powder + Nitric acid (50%)	Reddish brown	Greenish black

3.3. Preliminary Phytochemistry

The preliminary phytochemical analysis of *L. indica* leaf extract showed that, it is rich in phytoconstituents. The tests were done in three solvents i. e. aqueous, methanol and ethanol. When compared methanol was observed to be more useful for extraction of phytochemicals than rest of the solvents. The methanol

extract showed presence of alkaloids, glycosides, phenols, flavonoids, tannins, terpenoids, steroids, saponins and reducing sugar (Table 2). In aqueous extract alkaloids, glycosides, tannins, terpenoids and steroids were absent and while ethanol extract showed positive tests for alkaloids, phenolics, saponins, reducing sugars, carbohydrate and proteins.

Table 2: Preliminary phytochemical analysis of *L. indica* leaf extracts.

Plants species	Extract	Test for phytoconstituents										
		Alkaloids	Glycosides	Phenolics	Flavonoids	Tannins	Terpenes	Steroids	Saponins	Red. Sug	Carb rates	Proteins
<i>L. indica</i>	AE	-	-	+	+	-	-	-	+	+	+	+
	ME	+	+	+	+	+	+	+	+	+	+	+
	EE	+	-	-	-	-	-	-	+	+	+	+

Note: AE= Aqueous extract; ME= Methanol extract; EE= Ethanol extract

3.4. HPTLC analysis

HPTLC chromatogram of methanolic leaf extract of *L. indica* at 366 nm showed total 09 peaks (Figure 2).

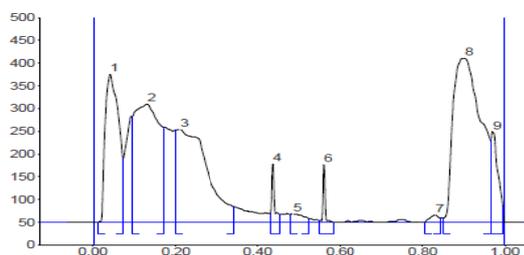


Figure 2. HPTLC chromatogram of methanol extract of *L. indica* leaf at 366 nm.

On the basis of correlation with the Rf values of compounds, they are predicted as Rutin (peak number 2 and Rf value 0.13) and apigenin (peak number 8 and Rf

value 0.90), respectively. At 540 nm HPTLC chromatogram of *L. indica* methanol leaf extract showed 20 peaks (Figure 3). Out of these, four peaks were identified on the basis of their Rf values. These are peak number 2 (Rutin, Rf value 0.13), peak number 6 (catechin, Rf value 0.37), peak number 14 (Ferulic acid, Rf value 0.74) and peak number 19 (Apigenin, Rf value 0.91).

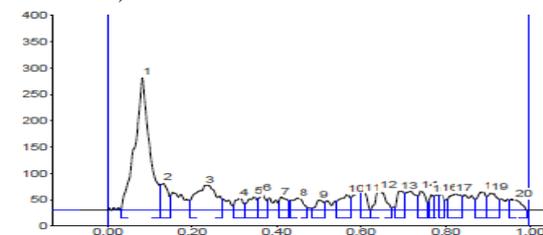


Figure 3. HPTLC chromatogram of methanol extract of *L. indica* leaf at 540 nm.

3.5. Antioxidant activity

Comparative DPPH scavenging and total antioxidant activity of *L. indica* was found to be significant. In both DPPH scavenging and total antioxidant potential, IC₅₀ values for methanol extract of *L. indica*, were comparable with positive controls i.e. *L. aspera* and *L. cephalotes* (Table 3) which are traditionally being used as potent antioxidants. The methanol extract showed higher antioxidant activity than the standard antioxidant.

Table 3: Antioxidant potential of *L. indica* leaf extract

Crude methanol leaf extracts	IC50 values for antioxidant activity	
	DPPH Scavenging	Total antioxidant
<i>L. aspera</i>	18.25	16.22
<i>L. cephalotes</i>	18.85	17.29
<i>L. indica</i>	21.17	19.48
Standard (Ascorbic acid)	26.08	17.25

Note: The presented values are the average of triplicate analysis.

Above stated results clearly indicates that *L. indica* has specific characters to identify the species in laboratory condition with both glandular and non-glandular trichomes and anomocytic stomata. The florescent analysis of powder was unique, which could be used as marker to note the adulteration in market available crude drug powder. Earlier, few workers have done pharmacognostic evaluation of *Leucas species* (Makhija *et al.*, 2011; Itoria *et al.*, 2011; Paul and Saha, 2012; Sudhakara Reddy *et al.*, 2017). However, each report differs in their observation as per species diversity. It's richness in phytochemicals probably responsible for its diverse biological activities. It has significant level of phenolic compounds responsible to its antioxidant potential (Kahkonen *et al.*, 1999; Menon *et al.*, 2012 and Tahareen *et al.*, 2016) and could be used as possible antioxidant drug.

4. Conclusion

L. aspera has unique microscopic characters with both simple and glandular trichomes. It is rich in phytochemical composition especially phenolics and flavonoids. Its methanolic extracts of leaves showed significant antioxidant activity as compare to standard antioxidant and thus could be used as possible replacement of *L. aspera* and *L. cephalotes* as antioxidant which will help to reduce the burden of overexploitation of these species from nature.

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