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Phytochemical analysis of *Leucas urticifolia* (Vahl) R. Br. ex Sm.: A traditional medicinal herb

Rajput Nutan and Satya Veena

Abstract
The present paper shows the phytochemical analysis of the traditional medicinal plant *Leucas urticifolia* (Vahl) R. Br. ex Sm. belonging to family Lamiaceae. The Preliminary Phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoids, tannins, terpenoids, steroids, saponins and phenolics. Alkaloids were present at low quantity but phenolics were present in high quantity as compare to flavonoids. During HPTLC analysis total 11 compounds were identified i.e., Ferulic acid, Kaempferol, p-Caumarin, Cinnamic acid, Chlorogenic acid, Catechin, Myricetin, Gallic acid, Apigenin, Quercetin and Vanillic acid.

Keywords: Medicinal herb, phytochemical

Introduction
Ancient tribal societies around the world have learnt to utilize the neighbourhood plants for curative as well as offensive purposes. Their folklore knowledge become basic leads or clues for Chemical, Pharmacological, Clinical and Biochemical trials, which ultimately gives birth to modern drugs. Therefore, it is essential to investigate such plants from different unexplored regions for their medicinal potential. In addition, the information regarding the presence of various chemical constituents in plants becomes important in discovering the authentic value of folklore medicines (Mojab *et al*., 2003) [10].

Medicinal plants are generally known as "Chemical Gold mines". Medicinal plants and various products, obtained from them, being act as a valuable source of both traditional as well as modern medicine, are authentically helpful for primary health care over years (WHO 1993) [19].

The medicinal properties of the plant are unique to particular plant species or groups and consistent with the concept that the combination of secondary product in a particular plant is taxonomically distinct. (Wink *et al.*, 1999) [20]. These are synthesized during various metabolic path ways in the life cycle of plants.

Natural products of plants are useful in pharmacy. More than 20% of drugs in developed countries are derived directly or indirectly from plants. The most important of these chemically active constituents of plants are alkaloids, flavonoids and phenolic compound. Research in natural product chemistry is the back bone of herbal industry responsible for promoting the use of herbs in modern medicine.

Lamiaceae or mint family is one of the largest family comprising 252 genera and 7000 species. (Plant- list 2013). In India the family is represented by 65 genera and 400 species. Nearly all the members of this family possess ethnomedicinal properties. Most of the ethnomedicinally important plants of family have been explored for their various active principles and phytochemical properties, they possess. However, some of the members remained unexplored in spite of their excellent medicinal values. In the present study, the author has selected the under explored plant, *Leucas urticifolia* (Vahl) R.Br. ex Sm.

About Study Area
Dhar dist. is situated in the south-western part of Madhya Pradesh. The dist. comprises Badnawar, Dhar, Dharampuri, Kukshi and Mandu tehsils. District is situated between latitude 22^0 1˝14" to 23^0 08˝49" North and longitude 74^0 28˝15" to 75^o42˝43" East. It is bounded by the dist. of Ratlam to the north, Ujjain to the northeast, Indore to the east, Khargone (West Nimar) to the southeast, Barwani to the south, and Jhabua to the west. It is part of the Indore Division of Madhya Pradesh. The total geographical area of the district is 8,153 km^2, of which forest area occupy 117000 hectares. The recorded forest area of the dist. is 137 km^2 moderately dense forest and 597 km^2 open forest.

Materials and Methods

Collection of Plant Material

Different areas of Dhar district was explored in order to collect the plant material. Meanwhile, the local people and medicine men called local vaidyas were interviewed to investigate the ethnomedicinal importance.

Preparation of extract

It was carried out by soxhlation techniques by using different solvents according to increasing order of polarity *viz*, Ethyl acetate, Methanol and distilled water. The different parts (stem, leaves & inflorescence) of the plants *Leucas urticifolia* were dried in shade, collected in powder form. The extraction procedure was adopted from Harborne's 1984 [5].

Preliminary Phytochemistry

In order to determine the presence of alkaloids, glycosides, flavonoids, tannins, terpenoids, steroids, saponins, phenolics, fats and sugars, a preliminary phytochemical study (color reactions) with various plant extracts were performed. (Harborne, 1973; Sofowora, 1993 and Krishnaiah *et al.*, 2009) [4, 15, 7].

Crude quantification of the major Phytochemicals

Only alkaloids, Phenolics, flavonoids and Saponins from the plants under study were quantified.

Thin Layer Chromatography (TLC) analysis

The TLC analysis was done as per standard protocols (Harborne, 1973) [4]. Synthetic TLC plates (Merk) were used for the analysis. The powdered material was extracted in aqueous solution, ethyl acetate and methanol and then loaded on the TLC place about 1 cm from below.

High Performance Thin Layer Chromatography (HPTLC)

The HPTLC analysis of methanolic leaf extracts of sample was done using CAMAG LINOMATE TLC scanner 4. The solvent systems used were- (1) Petroleum ether: Acetone: Formic acid (35:10:05) and (2) Chloroform: Methanol (7.5:2.5) and the concentration of each sample was 5.00 ml. The interpretation of Chromatograms obtained was done in the light of recent standard references and using online library for Rf values of phenolic compounds considering the Rf values of sample bands obtained.

Observations

Preliminary phytochemical analysis (Qualitative analysis)

Preliminary phytochemical analysis of *Leucas urticifolia* (Vahl) R. Br. ex Sm. stem, leaf and inflorescence are done.

Table 1: Preliminary Phytochemical analysis of *Leucas urticifolia* (Vahl) R. Br. ex Sm.- Stem, Leaf and Inflorescence powder

Class of compound	Stem			Leaf			Inflorescence		
	Aqueous	Ethyl acetate	Methanol	Aqueous	Ethyl acetate	Methanol	Aqueous	Ethyl acetate	Methanol
Alkaloids	-	++	+	+	++	+	-	++	+
Phenolics	++	+++	+++	-	+++	++	+	++	++
Flavonoids	--	+	+	-	++	+	-	-	+
Tannins	-	++	+	-	+++	++	-	+	++
Glycosides	-	+	+	-	++	+++	+	-	+
Terpenoids	+	+++	++	+	+++	++	+	+	+
Steroids	+	++	+	+	++	+	+	+	++
Saponins	+	+	+	++	++	+++	-	-	++

(+ indicates presence in sample, ++ or +++ indicates strong positive in sample & - indicates absence or not detected in sample)

Quantitative analysis of major phytochemicals

The quantitative analysis was done only in methanolic leaf extracts. It was observed that plant has significant level of tested phytochemicals. The average values of the content of respective phytochemical are presented in Table-2.

Table 2: Quantitative analysis of alkaloids, phenolics & flavonoids (mg/g sample)

S. No.	Phytochemicals present	Quantity
1	Alkaloids	0.79 ± 0.20
2	Phenolics	1.75± 0.25
3	Flavonoids	0.93± 0.15

Thin Layer Chromatography (TLC) analysis

The powdered extract of leaves of *Leucas urticifolia* (Vahl) R. Br. ex Sm. was used to identify the available phytochemicals using Thin Layer Chromatography (TLC). The analytic mode was designed to identify alkaloids, phenolics, flavonoids & saponins using suitable solvent systems and spraying reagents or visualizer.

Table 3: Thin Layer Chromatography analysis of leaf powder of *Leucas urticifolia* (Vahl) R. Br. ex Sm

Class of Compounds	Solvent system	Powdered Extract	Spraying reagent/ Visualizer	Total Bands observed	R_f values
Alkaloids	Methanol: Conc. NH_4OH (200:3)	Aqueous	Dragendroff's reagent	00	--
		Ethyl acetate		01	0.15
		Methanol		01	0.18
Phenolics	Chloroform: Methanol (27:0.3)	Aqueous	Ferric chloride reagent	00	--
		Ethyl acetate		01	0.58
		Methanol		01	0.27
Flavonoids	Chloroform: Methanol (19:1)	Aqueous	UV- light	01	0.59
		Ethyl acetate		01	0.58
		Methanol		02	0.52, 0.56

Saponins	Chloroform: Glacial acetic acid: Methanol: Water (64:34:12:8)	Aqueous	Iodine vapour	01	0.72
		Ethyl acetate		02	0.85, 0.98
		Methanol		02	0.52, 0.94

High Performance Thin Layer Chromatography (HPTLC)

The HPTLC analysis of methanolic leaf extracts of sample shows the presence of various phytochemicals in both the solvent systems. They are shown in the tables below-

(A) Analysis using solvent systems - Petroleum ether: Acetone: Formic acid (35:10:05)

Table 4: HPTLC data of *Leucas urticifolia* (Vahl) R. Br.ex Sm. methanolic leaf extract at 366 nm.

Peak	Start *Rf*	Start Height	Max *Rf*	Max Height	Max %	End *Rf*	End Height	Area	Area %	Assigned compounds
1	0.01	0.2	0.05	252.3	17.68	0.06	241.7	4674.9	9.69	1. Unidentified
2	0.06	244.4	0.07	299.7	21.01	0.14	67.0	9679.5	20.06	**2.Chlorogenic acid**
3	0.24	33.5	0.25	55.5	3.89	0.30	22.7	1860.5	3.85	3. Unidentified
4	0.30	23.7	0.30	23.7	1.66	0.35	14.8	790.3	1.64	**4. Gallic acid**
5	0.38	12.1	0.40	40.8	2.86	0.41	12.1	383.0	0.79	5. Unidentified
6	0.46	16.3	0.49	27.2	1.91	0.53	6.6	1039.8	2.15	6. Unidentified
7	0.71	0.5	0.74	13.9	0.97	0.76	0.3	303.7	0.63	**7. Ferulic acid**
8	0.81	0.2	0.83	40.1	2.81	0.84	21.8	611.6	1.27	8. Unidentified
9	0.85	15.6	0.87	305.9	21.44	0.88	287.8	5640.4	11.69	**9. Kaempferol**
10	0.89	287.8	0.93	367.5	25.76	0.98	167.3	23279.7	48.23	**10. P-Coumarin acid**

Table 5: HPTLC data of *Leucas urticifolia* (Vahl) R. Br. ex Sm. methanolic leaf extract at 540 nm

Peak	Start *Rf*	Start Height	Max *Rf*	Max Height	Max %	End *Rf*	End Height	Area	Area %	Assigned compounds
1	0.02	5.1	0.04	18.5	3.37	0.05	11.4	381.0	4.30	1. Unidentified
2	0.05	12.3	0.06	24.7	4.50	0.06	24.4	237.6	2.68	2. Unidentified
3	0.07	22.6	0.07	30.9	5.63	0.09	8.8	389.8	4.39	**3. Chlorogenic acid**
4	0.09	10.0	0.10	24.2	4.42	0.11	6.9	315.9	3.56	4. Unidentified
5	0.11	7.8	0.12	13.3	2.43	0.13	0.0	162.2	1.83	5. Unidentified
6	0.14	1.8	0.16	22.2	4.04	0.17	1.1	396.4	4.47	6. Unidentified
7	0.19	5.3	0.21	25.1	4.58	0.22	19.8	459.6	5.18	7. Unidentified
8	0.22	19.9	0.23	20.6	3.75	0.24	10.6	275.5	3.11	8. Unidentified
9	0.25	0.1	0.27	23.0	4.19	0.28	3.8	313.1	3.53	9. Unidentified
10	0.30	2.5	0.31	14.6	2.66	0.32	0.5	172.2	1.94	**10. Gallic acid**
11	0.33	4.3	0.35	13.7	2.50	0.35	11.0	173.3	1.95	11. Unidentified
12	0.35	11.5	0.36	15.9	2.90	0.37	4.1	123.5	1.39	12. Unidentified
13	0.37	5.3	0.39	17.8	3.24	0.39	15.2	255.1	2.88	**13. Catechin**
14	0.39	13.1	0.41	28.9	5.27	0.43	17.8	542.4	6.12	14. Unidentified
15	0.44	16.2	0.45	26.5	4.83	0.45	19.0	321.2	3.62	15. Unidentified
16	0.51	8.0	0.52	24.4	4.45	0.52	19.3	196.4	2.21	**16. Myricetin**
17	0.63	16.0	0.65	26.6	4.84	0.66	3.8	481.3	5.43	**17.Quercetin**
18	0.68	13.7	0.70	25.1	4.58	0.71	10.2	574.5	6.48	18. Unidentified
19	0.72	10.5	0.73	29.8	5.44	0.74	20.8	430.5	4.85	**19. Vanillic acid**
20	0.77	26.6	0.78	33.2	6.05	0.81	19.2	766.4	8.64	20. Unidentified
21	0.83	19.2	0.84	24.4	4.45	0.87	10.0	529.1	5.96	21. Unidentified
22	0.89	11.1	0.90	29.4	5.36	0.92	12.8	483.5	5.45	**22. Apigenin**
23	0.92	14.4	0.95	35.9	6.55	0.97	0.7	889.3	10.03	23. Unidentified

(B) Analysis using solvent systems- Chloroform: Methanol (7.5:2.5)

Table 6: HPTLC data of *Leucas urticifolia* (Vahl) R. Br.ex Sm. methanolic leaf extract at 366 nm.

Peak	Start *Rf*	Start Height	Max *Rf*	Max Height	Max %	End *Rf*	End Height	Area	Area %	Assigned compounds
1	0.01	0.2	0.05	252.3	17.68	0.06	241.7	4674.9	9.69	1. Unidentified
2	0.06	244.4	0.07	299.7	21.01	0.14	67.0	9679.5	20.06	**2.Chlorogenic acid**
3	0.24	33.5	0.25	55.5	3.89	0.30	22.7	1860.5	3.85	3. Unidentified
4	0.30	23.7	0.30	23.7	1.66	0.35	14.8	790.3	1.64	**4. Gallic acid**
5	0.38	12.1	0.40	40.8	2.86	0.41	12.1	383.0	0.79	5. Unidentified
6	0.46	16.3	0.49	27.2	1.91	0.53	6.6	1039.8	2.15	6. Unidentified
7	0.71	0.5	0.74	13.9	0.97	0.76	0.3	303.7	0.63	**7. Ferulic acid**
8	0.81	0.2	0.83	40.1	2.81	0.84	21.8	611.6	1.27	8. Unidentified
9	0.85	15.6	0.87	305.9	21.44	0.88	287.8	5640.4	11.69	**9. Kaempferol**
10	0.89	287.8	0.93	367.5	25.76	0.98	167.3	23279.7	48.23	**10. P-Coumarin acid**

Table 7: HPTLC data of *Leucas urticifolia* (Vahl) R. Br. ex Sm. methanolic leaf extract at 540 nm

Peak	Start *Rf*	Start Height	Max *Rf*	Max Height	Max %	End *Rf*	End Height	Area	Area %	Assigned compounds
1	0.02	5.1	0.04	18.5	3.37	0.05	11.4	381.0	4.30	1. Unidentified
2	0.05	12.3	0.06	24.7	4.50	0.06	24.4	237.6	2.68	2. Unidentified
3	0.07	22.6	0.07	30.9	5.63	0.09	8.8	389.8	4.39	**3. Chlorogenic acid**
4	0.09	10.0	0.10	24.2	4.42	0.11	6.9	315.9	3.56	4. Unidentified
5	0.11	7.8	0.12	13.3	2.43	0.13	0.0	162.2	1.83	5. Unidentified
6	0.14	1.8	0.16	22.2	4.04	0.17	1.1	396.4	4.47	6. Unidentified
7	0.19	5.3	0.21	25.1	4.58	0.22	19.8	459.6	5.18	7. Unidentified
8	0.22	19.9	0.23	20.6	3.75	0.24	10.6	275.5	3.11	8. Unidentified
9	0.25	0.1	0.27	23.0	4.19	0.28	3.8	313.1	3.53	9. Unidentified
10	0.30	2.5	0.31	14.6	2.66	0.32	0.5	172.2	1.94	**10. Gallic acid**
11	0.33	4.3	0.35	13.7	2.50	0.35	11.0	173.3	1.95	11. Unidentified
12	0.35	11.5	0.36	15.9	2.90	0.37	4.1	123.5	1.39	12. Unidentified
13	0.37	5.3	0.39	17.8	3.24	0.39	15.2	255.1	2.88	**13. Catechin**
14	0.39	13.1	0.41	28.9	5.27	0.43	17.8	542.4	6.12	14. Unidentified
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21	0.83	19.2	0.84	24.4	4.45	0.87	10.0	529.1	5.96	21. Unidentified
22	0.89	11.1	0.90	29.4	5.36	0.92	12.8	483.5	5.45	**22. Apigenin**
23	0.92	14.4	0.95	35.9	6.55	0.97	0.7	889.3	10.03	23. Unidentified

Results

It is found that plant is rich in phytochemical composition. However, the leaf parts are found to have more concentration of phytochemicals analyzed. (Table-1) Further, most of the phytochemicals are found to be extractable in ethyl acetate solvent followed by methanol. Aqueous extracts show availability of minimum number of phytochemicals. Ethyl acetate extracts of plant shows more amount of available phenolics and terpenoids; similar results are obtained in case of methanolic extracts, but the color intensity of the tests are less in methanolic extracts corresponding to the level of phytochemical compounds. The least content of the phytochemicals is found in inflorescence which also follow more phytochemical extractability in methanol.

In case of *Leucas urticifolia* (Vahl) R. Br. ex Sm., aqueous stem extract of stem shows presence of phenolics, terpenoids, steroids and saponins while rest are absent. Its aqueous extract of leaf and inflorescence indicates presence of alkaloids,

terpenoids, steroids, saponins and phenolics (Table-2). The overall qualitative analysis suggests that the ethyl acetate might be useful in extracting available phytochemicals but the diversity analysis, methanol is most suitable solvent.

Discussion

The ethnomedicinal observation reveals that *Leucas urticifolia* (Vahl) R. Br. ex Sm. is used ethno medicinally as astringent, on skin diseases, on diarrhoea and dysentery, fever, typhoid, respiratory disorders, urinary infections & inflammation**,** mental disorders and as abortifacient. The Ethno medicinal properties of plant can be attributed to the chemical compounds present in them.

The studies reveal that 11 phytochemical constituents were isolated from the methanolic leaf extract of the plant. All the above properties can be attributed to the Pharmacognostic findings of various researchers. The phytochemicals acting as anti-inflammatory agent are Ferulic acid (Mancuso & Santangelo, 2014) [8], Gallic acid (Hsiang *et al.* 2013 & Chang *et al.* 2016) [6, 2], p-Caumarin (Venugopala, 2013) [17], Vanillic acid (Cassia *et al.* 2015, Stanely *et al.* 2011 [1, 16] and Kaempferol (Melo *et al.* 2009 & Hamalainen *et al.* 2007) [9, 3] isolated from this plant. Gallic acid also exhibits effect against respiratory disorders like allergies, asthma, rhinitis, sinusitis etc and as anxiolytic agent. (Chang *et al.* 2006) [6].

The phytochemicals effective on stomach and abdominal disorders are Ferulic acid as antimicrobial agent (Ou & Kwok 2004) [11]; Apigenin as gastric myorelaxant (Wang *et al.* 2014 & Rotondo *et al.* 2009) [18] & p-Caumarin as antibacterial agent (Venugopal *et al.* 2013) [17].

The phytochemical p-Caumarin shows the antiviral property (Pereira *et al.* 2018 & Venugopal *et al.* 2013) [12, 17]. Antiplatelet aggregation activity has been expressed by Ferulic acid (Anti- thrombosis) (Ou & Kwok 2004) [11] and p-Caumarin (Anticoagulant) (Venugopala *et al.* 2013) [17].

Hence these drugs hold a good promise for managing variety of ailments. The Chromatographic profile of plant is also helpful in identification of drugs. The presence of tannin is responsible for the reasons that the animal does not graze these plants.

Medicinal and aromatic plants are playing a vital role in economic, social, cultural and ecological aspects of local communities especially in developing countries world over. These are the botanicals that provide people with medicines - to prevent, diseases, maintain health or cure ailments. In one form or another, they benefit virtually everyone on this planet. Medicinal and aromatic plants grow in almost all terrestrial and some aquatic ecosystems around the world. However increasing demand of these plants, their habitats are threatening and many species harvested from the wild leading the threat of extinction of several species.

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