

PHYTOCHEMICAL STUDIES ON *LAURENCIA OBTUSA* (HUDSON) LAMOURUX

Johnson M*, Babu A, Janakiraman N, Renisheya Joy Jeba Malar T

Centre for Plant Biotechnology, Department of Plant Biology and Plant Biotechnology,
St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India.

E-mail of Corresponding Author: ptcjohnson@gmail.com

Abstract

Aim: The present study was aimed to explore the phytochemical constituents present in *Laurencia obtusa* collected from Tamil Nadu, South India.

Methods: Phytochemical screening of different extracts was carried out using the standard procedure and the results were illustrated.

Results: Steroids show the maximum (4/6) presence in acetone, benzene, chloroform and petroleum ether extracts followed by alkaloids (3/6) in ethanol, acetone and chloroform extracts. The UV-Vis phytochemical profile of various extracts of *L. obtusa* was tabulated. The qualitative HPLC fingerprint profile of ethanolic extract of *L. obtusa* was selected at a wavelength of 254 nm due to sharpness of the peaks and proper baseline. The profile displayed one prominent peak at a retention time of 1.953 min and some moderate peaks were observed at a retention time of 3.000, 2.570 and 2.467 min respectively.

Conclusion: The present studies on *L. obtusa* produced novel phytochemical markers in standardization as useful analytical tools to check not only the quality of the powder but also the presence of adulterants in ayurvedic drugs.

Keywords: Phytochemistry, Seaweeds, UV-Vis, HPLC.

1. Introduction

Marine algae serve as important resources for bioactive natural products and they possess number of valuable biological activities such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. From the literature, it is observed that the edible seaweeds contain a significant amount of the protein, vitamins and minerals essential for the human nutrition¹. Most of the bioactive substances isolated from marine algae are chemically classified as brominated, aromatics, nitrogen-heterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated polysaccharides. On other hand, the algae are also used as food stuff, animal fodder, fertilizer, industrial material such as agar and minor medicines².

To date, over 2400 natural products have been isolated from seaweeds (mainly from the divisions Rhodophyta, Phaeophyta, and Chlorophyta), the majority of which come from subtropical and tropical populations³⁻⁴. In general, lipophilic nonpolar compounds such as terpenoids, acetogenins, and compounds of mixed biosynthesis occur in relatively low concentrations ranging from 0.2% to 2% of algal

dry weight but polar polyphenolics can occur in concentrations as high as 15% of algal dry mass⁵⁻⁶. The greatest variety of natural products is probably found among Rhodophyta, in which all known classes of compounds are represented, many of which are halogenated⁴. As an example of Rhodophyta's broad chemical variety, members of the genus *Laurencia* produce an amazing array of complex terpenoids and acetogenins, possibly making it the world's most chemically complex genus.

Red algae of the genus *Laurencia* J. V. Lamour (Rhodomelaceae) are found in tropical and subtropical regions throughout the world and are an extremely rich source of secondary metabolites with diverse structural features, mainly halogenated sesquiterpenes and C15-acetogenins⁷⁻⁸. It is suggested that these metabolites function as a chemical defense against herbivores⁹, fouling organisms, and pathogens¹⁰. These compounds have been shown to possess some interesting pharmacological activities, such as antitumor¹¹, antibacterial¹², antifungal¹³, and antiviral activities¹⁴. However, information is lacking on the seasonal and geographic variations in the specific metabolites of seaweeds from South India. Thus the present study was aimed to explore the phytochemical

constituents present in *Laurencia obtusa*, medicinally important red algae.

2. Materials and Methods

2.1 Collection of Materials: *Laurencia obtusa* was collected by handpicking at Rasthacaud coastal waters. The collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. It was then thoroughly washed with tap water followed by distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread out at room temperature in shade. The shade dried samples were grounded to fine powder using tissue blender. The powdered samples were then stored in refrigerator for further use.

2.2 Preparation of extracts: 10 g of air dried powder was extracted with 60 mL of solvents viz., petroleum ether, benzene, chloroform, acetone, ethanol and aqueous. The sample was kept in dark for 72 h with intermittent shaking. After incubation, the solution was filtered through filter paper and the filtrate was collected (crude extracts).

2.3 Phytochemical and fluorescence analysis: Phytochemical screening of the extracts was carried out according to the standard methods¹⁵. Fine powder and their extracts obtained using various solvents viz., petroleum ether, benzene, chloroform, acetone, ethanol and aqueous were examined under visible and UV light. The powdered materials were also treated with various reagents such as 50% nitric acid, 50% sulphuric acid, 1N HCl, 1N NaOH and changes in colour were recorded¹⁶.

2.4 UV-Vis and HPLC analysis: The different extracts of *L. obtusa* were centrifuged at 3000 rpm for 10 min and then filtered through Whatmann No.1 filter paper using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvents. The crude extracts containing the bioactive compound was analysed spectroscopically for further confirmation. They

were scanned in the wavelength ranging from 200-1100 nm using Shimadzu spectrophotometer and characteristic peaks were detected.

HPLC method was performed on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UV-Vis detector SPD-10AT, Rheodyne injector fitted with a 20 μ L loop and auto injector SIL-10AT. A Hypersil BDS C-18 column (4.6 $\times$ 250 mm, 5 μ m size) with a C-18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1 mL min⁻¹ at ambient temperature (25-28°C). The mobile phase was consisted of 0.1% v/v ethanol (Solvent A) and water (Solvent B). The mobile phase was prepared daily, filtered through a 0.45 μ m and sonicated before use. Total running time was 15 min. The sample injection volume was 20 μ L while the wavelength of the UV-Vis detector was set at 254 nm^{17,18}.

3. Results

The preliminary phytochemical screening of different chemical compounds was tested in six different extracts of *L. obtusa* and the results were illustrated in Table 1. Thus out of (1 $\times$ 6 $\times$ 7 = 42) tests for the presence or absence of the above compounds, 13 tests gave positive results and the remaining 29 gave negative results. Steroids show the maximum (4/6) presence in acetone, benzene, chloroform and petroleum ether extracts followed by alkaloids (3/6) in ethanol, acetone and chloroform extracts. Saponins and tannins showed their occurrence (2/6) in aqueous and ethanol extracts. Among the six different extracts, ethanol extracts showed the presence of maximum number (5) of compounds. Next to that, chloroform, acetone and aqueous extracts show the presence of 2 compounds. The results of the fluorescence analysis of the seaweed extracts and raw powder are recorded in Table 2.

Table 1: Preliminary phytochemical analysis of different extracts of *L. obtusa*

Tests	Aqueous	Ethanol	Acetone	Benzene	Chloroform	Petroleum ether
Alkaloids	-	+	+	-	+	-
Phenolics	-	+	-	-	-	-
Steroids	-	-	+	+	+	+
Saponin	+	+	-	-	-	-
Flavonoids	-	+	-	-	-	-
Tannins	+	+	-	-	-	-

Table 2: Proximate analysis of *L. obtusa*

Solvents	<i>L. obtusa</i> (extracts)		<i>L. obtusa</i> (raw powder)	
	Fluorescent light	UV light	Fluorescent light	UV light
Aqueous	Green	Black	Black	Turbid
Ethanol	Dark green	Light green	Light green	Green
Acetone	Dark brown	Ash colour	Dark green	Sandal
Benzene	Brown	Ash colour	Dark yellow	Sand
Chloroform	Dark brown	Ash colour	Brown	Sandal
Pet. ether	Dark brown	Ash colour	Light yellow	Light green
50% HNO ₃	Pale yellow	Light yellow	Light yellow	Light yellow
1N HCl	Dark brown	Black	Light brown	Green
1N NaOH	Greenish brown	Brown	Dark brown	Yellow
1N H ₂ SO ₄	Black	Black	Sandal	Brownish green

The UV-Vis phytochemical profile of various extracts of *L. obtusa* was displayed in Table 3. The qualitative UV-VIS fingerprint profile of petroleum ether extract of *L. obtusa* was selected at the wavelength of 300 nm to 700 nm due to sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 360, 430, 440, 450, 460, 480, 500, 530, 650, 660, 670 and 680 with the absorption 1.762, 1.300, 0.795, 0.652, 0.535, 0.464, 0.450, 0.280, 0.302, 0.868, 1.710 and 0.457 respectively (Fig. 1 A). The qualitative UV-VIS fingerprint profile of chloroform extract of *L. obtusa* was selected at a wavelength of 300 nm to 700 nm due to sharpness of the peaks and proper baseline. The profile displayed the compounds separated at the nm of 310, 440, 450, 460, 480, 500, 510, 610, 650, 660 and 680 with the absorption 1.965, 1.265, 0.869, 0.716, 0.580, 0.689, 0.676, 0.465, 0.573, 1.462 and 0.923 respectively (Fig. 1 E). The qualitative UV-VIS fingerprint profile of acetone extract of *L. obtusa* was selected at a wavelength of 400 nm to 700 nm due to sharpness of the peaks and proper baseline. The profile demonstrated the compounds separated at the nm of 440, 480, 500, 510, 520, 530, 600, 610, 640, 680 and 690 with the absorption 3.263, 1.293, 1.303, 1.094, 0.825, 0.976, 1.013, 1.209, 1.344, 1.369 and 1.609 respectively (Fig. 1 C). The qualitative UV-VIS fingerprint profile of aqueous extract of *L. obtusa* was selected at a wavelength of 400 nm to 700 nm due to

sharpness of the peaks and proper baseline. The profile illustrated the compounds separated at the nm of 410, 420, 440, 510, 520, 640, 650, 660 and 680 with the absorption 1.216, 1.112, 0.939, 0.579, 0.547, 0.330, 0.318, 0.306 and 0.283 respectively (Fig. 1 B). The qualitative UV-VIS fingerprint profile of benzene extract of *L. obtusa* was selected at a wavelength of 400 nm to 700 nm due to sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 450, 460, 470, 500, 510, 610, 630 and 680 with the absorption 1.583, 1.441, 1.251, 1.261, 1.133, 0.675, 0.453 and 1.709 (Fig. 1 F). The qualitative UV-VIS fingerprint profile of ethanol extract of *L. obtusa* was selected at a wavelength of 400 nm to 700 nm due to sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm 440, 470, 480, 500, 520, 650, 660 and 670 with the absorption 1.618, 0.922, 0.778, 0.581, 0.401, 0.747, 0.864 and 0.659 respectively (Fig. 1 D).

The qualitative HPLC fingerprint profile of ethanolic extracts of *L. obtusa* was selected at a wavelength of 254 nm due to sharpness of the peaks and proper baseline. Ethanolic extract prepared by cold extraction was subjected to HPLC for the separation and identification of constituents present in *L. obtusa*. Three compounds were separated at different retention time 1.953, 2.570 and 3.000 respectively. The qualitative HPLC fingerprint profile of ethanolic

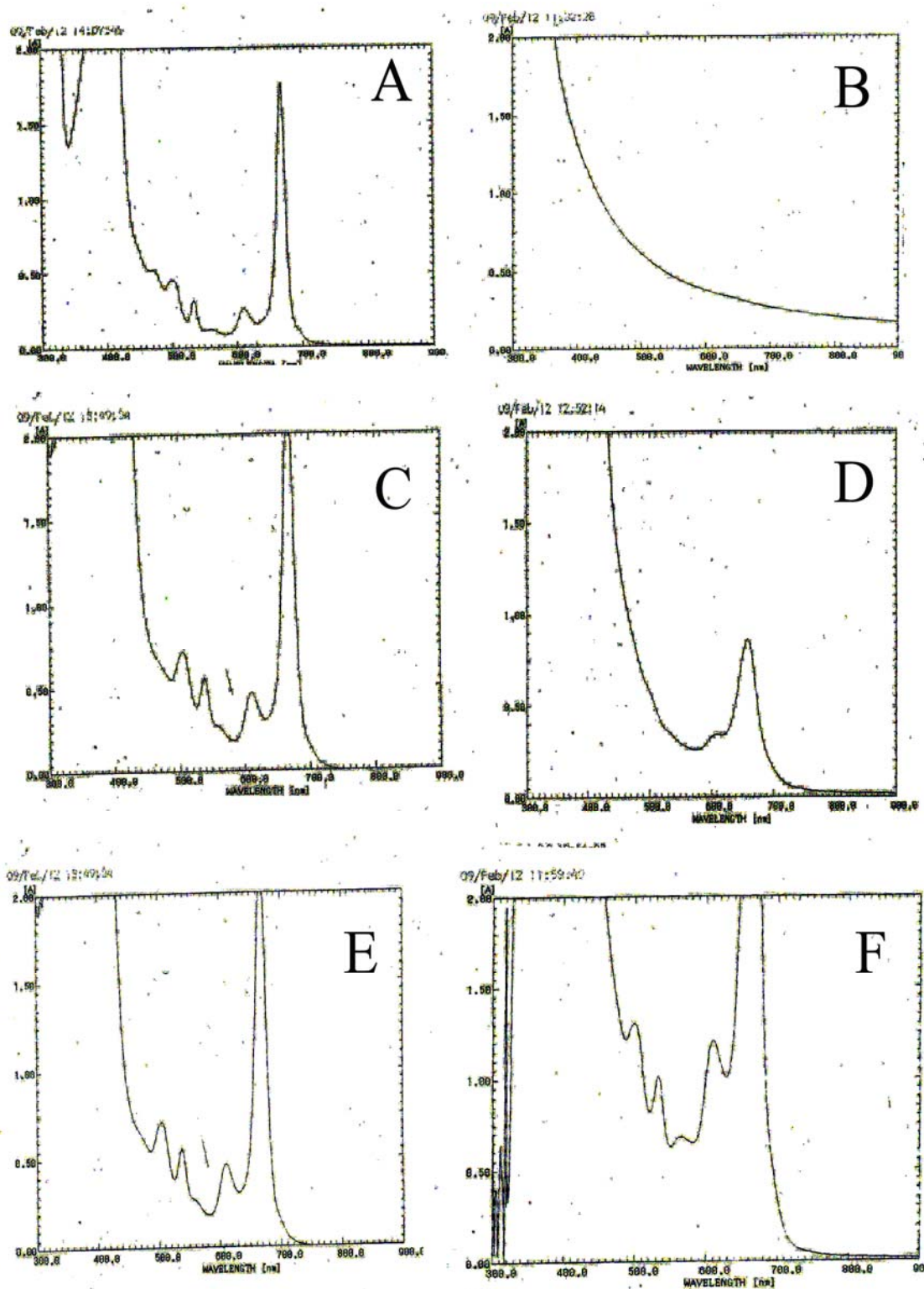


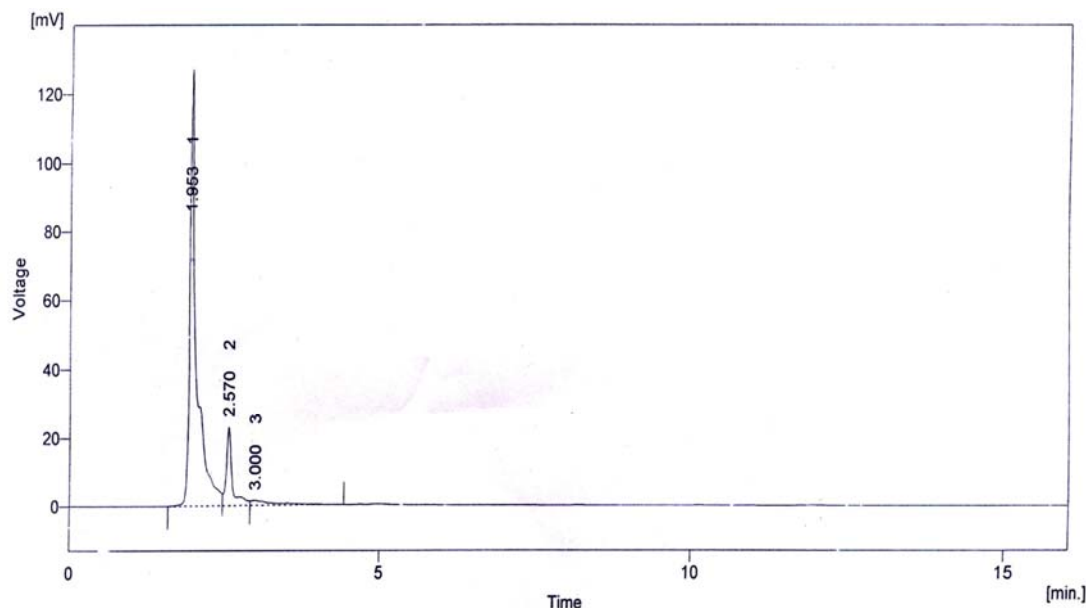
Fig. 1: UV- Vis Spectrum of *Laurencia obtusa* (Hudson) Lamourux

A. Petroleum Ether cold extract; B. Aqueous cold extract; C. Acetone cold extract; D. Ethanol cold extract; E. Chloroform cold extract; F. Benzene cold extract

Table 3: UV-VIS peak values of different extracts of *L. obtusa*

Aqueous		Ethanol		Acetone		Chloroform		Benzene		Petroleum ether	
nm	Abs	nm	Abs	nm	Abs	nm	Abs	nm	Abs	nm	Abs
410	1.216	440	1.618	440	3.263	310	1.965	450	1.583	360	1.762
420	1.112	470	0.922	480	1.293	440	1.265	460	1.441	430	1.300
440	0.939	480	0.778	500	1.303	450	0.869	470	1.251	440	0.795
510	0.579	500	0.581	510	1.094	460	0.716	500	1.261	450	0.652
520	0.547	520	0.401	520	0.825	480	0.580	510	1.133	460	0.535
640	0.330	650	0.747	530	0.976	500	0.689	610	0.675	480	0.464
650	0.318	660	0.864	600	1.013	510	0.676	630	0.453	500	0.450
660	0.306	670	0.659	610	1.209	610	0.465	680	1.709	530	0.280
680	0.283			640	1.344	650	0.573			650	0.302
				680	1.369	660	1.462			660	0.868
				690	1.609	680	0.923			670	1.710
										680	0.457

extract of *L. obtusa* was selected at a wavelength of 254 nm due to sharpness of the peaks and proper baseline. The profile displayed one prominent peak at a retention time of 1.953 min and some moderate peaks were observed at a retention time of 3.000, 2.570 and 2.467 min respectively (Fig. 2).

Fig. 2: HPLC Profile of *Laurencia obtusa* (Hudson) Lamourux – ethanolic cold extract

4. Discussion

Seaweeds are considered as rich source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, anthelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae^{19,20}. It is a real fact that the importance of marine organisms as a source of new substances is growing. With

marine species comprising approximately a half of the total global biodiversity, the sea offers an enormous resource for novel compounds, and it has been classified as the largest remaining reservoir of natural molecules to be evaluated for drug activity. A very different kind of substances have been obtained from marine organisms among other reasons because they are living in a very exigent, competitive and aggressive surrounding very different in many aspects from the terrestrial environment, a

situation that demands the production of quite specific and potent active molecules¹.

Phenolic compounds are commonly found in plants, including seaweeds, and have been reported to have a wide range of biological activities including antioxidant properties²¹⁻²³. Reports have revealed that phenolic compounds are one of the most effective antioxidants in brown algae²⁴. In the present study also, the phenolics presence was confirmed by the qualitative analysis in the crude extracts of *L. obtusa*. Flavonoids, the largest groups of phenolic compounds are known to contain a broad spectrum of chemical and biological activities including antioxidant and free radical scavenging properties²⁵. Flavonoids include flavonols, flavones, catechins, proanthocyanidins, anthocyanidins and isoflavonoids²⁶. Flavonoids are important in plant defense mechanisms against invading bacteria and other types of environmental stress, such as wounding and excessive light or ultraviolet (UV) radiation^{27,28}. In the present study also, flavonoids were observed only in ethanolic extracts of *L. obtusa*.

Tannins are defined as naturally occurring plant polyphenolic compounds and are widespread among terrestrial and marine plants²⁹. Vegetable tannins are secondary plant metabolites subdivided into condensed and hydrolysable compounds. Hydrolyzable tannins are gallic or egallic acid which easily hydrolyze in acidic media, and condensed tannins are polymeric flavonoids³⁰. In contrast to terrestrial tannins, phlorotannins are tannin compounds, which have been found only in marine algae^{29,31}. Phlorotannin purified from several brown algae have been reported to possess strong antioxidant activity which may be associated with their unique molecular skeleton³². They are more potent free radical scavenger than other polyphenols derived from terrestrial plants³³. Tannins has been also found to have antiviral, antibacterial, antiparasitic effects, anti-inflammatory, antiulcer and antioxidant property for possible therapeutic applications^{34,35}. The present study results confirm the tannin presence in *L. obtusa*. It suggests that the selected seaweeds can be used as antiviral, antibacterial, antiparasitic agents and exercise to treat the diseases like ulcer, gonnorrhoea, leucorrhoea after clinical screening.

Alkaloids are commonly found to have antimicrobial properties³⁶ against both Gram-positive and Gram-negative bacteria³⁷. In the

present study also, alkaloids presence were confirmed in chloroform, acetone and ethanolic extracts by the qualitative analysis described by Harborne. Saponins possess specific physical, chemical and biological activities that make them useful as drugs. Some of these biological properties include antimicrobial, anti-inflammatory, anti-feedent, and hemolytic effects³⁸. In the present investigation, saponins showed its presence in aqueous and ethanolic extracts of *L. obtusa*. Steroids may serve as an intermediate for the biosynthesis of downstream secondary natural products and it is believed to be a biosynthetic precursor for cardenolides in plants³⁹. Independent of their function, the presence of steroids in practically every organism suggests that they have a powerful role in chemosystematics⁴⁰.

Seaweeds known as medicinal, are rich in secondary metabolites which includes alkaloids, glycosides, phenols, flavonoids, tannins, saponins, steroids, related active metabolites are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Recently, a number of studies have been reported on the phytochemistry of seaweeds across the world⁴¹⁻⁴³. In the present investigation also, *L. obtusa* have been selected from Tamil Nadu, India for phytochemical screening on the basis of traditional and pharmaceutical uses. The present phytochemical study confirmed the presence of phenols, alkaloids, tannins, steroids, saponins and flavonoids with varied degree.

Conclusion

The present studies on *L. obtusa* produced novel phytochemical markers in standardization as useful analytical tools to check not only the quality of the powder but also the presence of adulterants in ayurvedic drugs. Fluorescence, UV-VIS and HPLC analysis can be used as effective markers in identifying authentic from its adulterants. It also suggested that *L. obtusa* may be rich sources of phytochemicals which can be isolated and further screened for different kinds of biological activities depending on their reported therapeutic uses. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

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