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Some quality standards for *Dronapushpi Panchanga* (*Leucas aspera* Spreng) Powder

Research Article

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Abstract

Background: *Dronapushpi* (*Leucas aspera* Spreng) of Lamiaceae is found as a weed throughout the country. It is described in various *Nighantus*. To maintain the effectiveness of crude drugs, the standardisation and proper identification of the plant is very important. Fresh juice of *Dronapushpi* is efficacious in malarial fever, collyrium prepared from the juice is used in jaundice, the decoction of the herb is used to ulcer as wash liquid, also applied externally to poisonous insect bites and also useful in worm infestation, inflammation, bronchitis, asthma and cough. In this study *Dronapushpi panchanga* (whole plant) was studied systematically to evaluate its quality standards. Methods: Macro-microscopic features, physico-chemical and phytochemical investigation were performed as per Pharmacopoeial procedures. Results: The pharmacognostic study revealed the macroscopic characters, physicochemical constants and phytochemical nature of *Donapushpi*. The extracts which were screened for its phytochemicals showed the presence of constituents like coumarin, flavonoids, carbohydrates, phenols and tannin in both the ethanolic and aqueous extracts. HPTLC profile was developed as fingerprint of extracts. Conclusion: Results of the present investigation *L. aspera* plant will help in validation of this crude drug.

Key Words: Diaphoretic, Extraction, HPTLC, Inflammation, Jaundice.

Introduction

Ayurveda is an oldest system of medicine bestowed on humanity by great *Rishis* (sages) of India, which has influenced all other system of medicines either directly or indirectly. (1).

Dronapushpi (*Leucas aspera* Spreng) of Lamiaceae is found as a weed throughout the country. It is easily available and described in various *Nighantus* (lexicons). Various pharmacological actions like *Shopha* (swelling or inflammation), *Kamala* (jaundice), *Tamakashwasa* (bronchial asthma) and *Kasajit* (alleviates cough) are mentioned in *Kaiyadeva Nighantu* and *Bhavaparkasha Nighantu* (lexicon) (2-4).

Every year about 20,000 deaths happen due to liver disorders. The plants as sources of medicines have shown to preserve the standard functional level of the liver (5). Eighty per cent of population in the world depends on plant origin traditional medicine (6).

L. aspera is found throughout India from Himalaya to Ceylon as weed. In case of chronic rheumatism, chronic skin eruptions of psoriasis leaves are found to be helpful and also various parts of plant are utilised as an antipyretic, insecticide, stimulant, emmenagogue, expectorant, aperient and diaphoretic. External application of are found to be useful in snake bite (2,7).

In *Brhat trayi* there is no description about *Dronapushpi*. Even in *Dhanvantari nighantu* we do not come across *Dronapushpi. Sodhala* indicated it in *Kamala* (liver disease), *Krimi* (worm infestation) and *Sopha* (inflammation). In one context he emphasized its use in *Pakshaghata* (hemiplegia). *Adhadamalla* commented *Dronapushpi* as *Nahula* or *Guma*. This plant is known as *Guma* in North India (8).

Drug evaluation is done for authentication of its identity and confirmation of its quality and purity and identification adulteration if any. Over the recent years, systemic changes took place in the analysis of crude drugs. Besides its morphological and microscopic evaluation, active constituents of the crude drugs are also estimated by chemical evaluation of the crude drugs. Both qualitative and quantitative evaluation can be performed with the development of separation techniques and instrumental analysis (9). In the present study, *Dronapushpi panchanga* (*L. aspera* whole plant) was screened for its macro-microscopic characters, physico-chemical and phytochemicals examination for deriving some quality standards for the drug.

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Materials and methods

Collection and authentication

The test drug *Dronapushpi Panchanga* was procured from the natural habitat from Hassan district of Karnataka and authenticated at SDM centre for Research in Ayurveda and Allied science, Udupi (10). The standard procedure was followed to prepare the *Churna* (powder) at SDMCAH, Hassan, Karnatak's *Rasashastra* and *Bhaishajya Kalpana* Department (11). The plant material was shade dried before powdering (Fig. 1A).

Macroscopic study

Photographs of plant parts from natural habitat were taken. Macroscopic characters of *Panchanga* (whole plant) of *L. aspera* were analysed systematically and made a note of its size, shape etc morphological characters.

Powder microscopic study

With chloral hydrate solution, the powder sample was heated and mounted, using glycerine, on a microscopic slide. The diagnostic features recorded and photographed using Zeiss's AXIO Trinocular microscope which is attached with Zeiss AxioCam camera under bright field light. Scale- bars indicated the magnifications of the figures.

Physicochemical evaluation

As per the Pharmacopoeial protocol, physicochemical examinations like loss on drying (moisture content) at 105^{0}C (LOD), water and ethanol soluble and successive extractive values, total ash and acid insoluble ash values were ascertained (12).

Qualitative chemical tests

Basic phytochemical examination was performed to find out different types of phytochemicals present in the plant using the established color tests(13).

HPTLC fingerprinting

With the help of 10 ml of ethanol 1 gram of powder was extracted. Using Linomat 5 TLC applicator 5, 10µl of the above extract was transferred on a pre-coated aluminium plates with silica gel F254 to a band width of 7mm using a Hamilton syringe. The plate was developed in Toluene: Ethyl acetate: Formic acid 10: 2.5: 0.5 and were visualized in UV 254, 366, under white light and then derivatised with vanillin sulphuric acid and scanned under UV 254 and 366 nm. R_f, colour of the spots and densitometric scan were recorded (14).

Results

Macroscopic study

Whole plant powder is fine, slightly fibrous, greenish yellow in colour (Fig. 1B) with faint distinctive odour and moderately bitter taste.

Powder microscopy

Powder of whole plant showed presence of bundle of trichomes (Fig. 2A), trichomes with striation (Fig. 2B), multicellular covering trichomes (Fig. 2C), glandular trichomes (Fig. 2D), fragments of simple trichomes (Fig. 2E), pitted parenchyma (Fig. 2F), fragments of epidermis in surface view (Fig. 2G), sclereids (Fig. 2H), fragments of vessels (Fig. 2I), bundle of pitted fibres (Fig. 2J), thin-walled fibres (Fig. 2K) and pitted tracheids (Fig. 2L)

Figure 1. Macroscopy of *Panchanga* (whole plant) of *Leucas aspera*

Fig 1A. Fresh whole pnat

Fig 1B. Powder

Physicochemical tests

Physicochemical parameters such as loss on drying (moisture content), total ash, acid insoluble and water soluble ash, alcohol and water soluble extractive standard were ascertained (Table 1).

Table 1. Physico-chemical parameters of *Panchanga* (whole plant) of *Leucas aspera*

Parameter	% w/w
Loss on drying at 105^{o}C	8.81
Total ash	7.71
Acid insoluble ash	0.97
Water soluble ash	3.07
Water soluble extractive	10.77
Alcohol soluble extractive	8.78

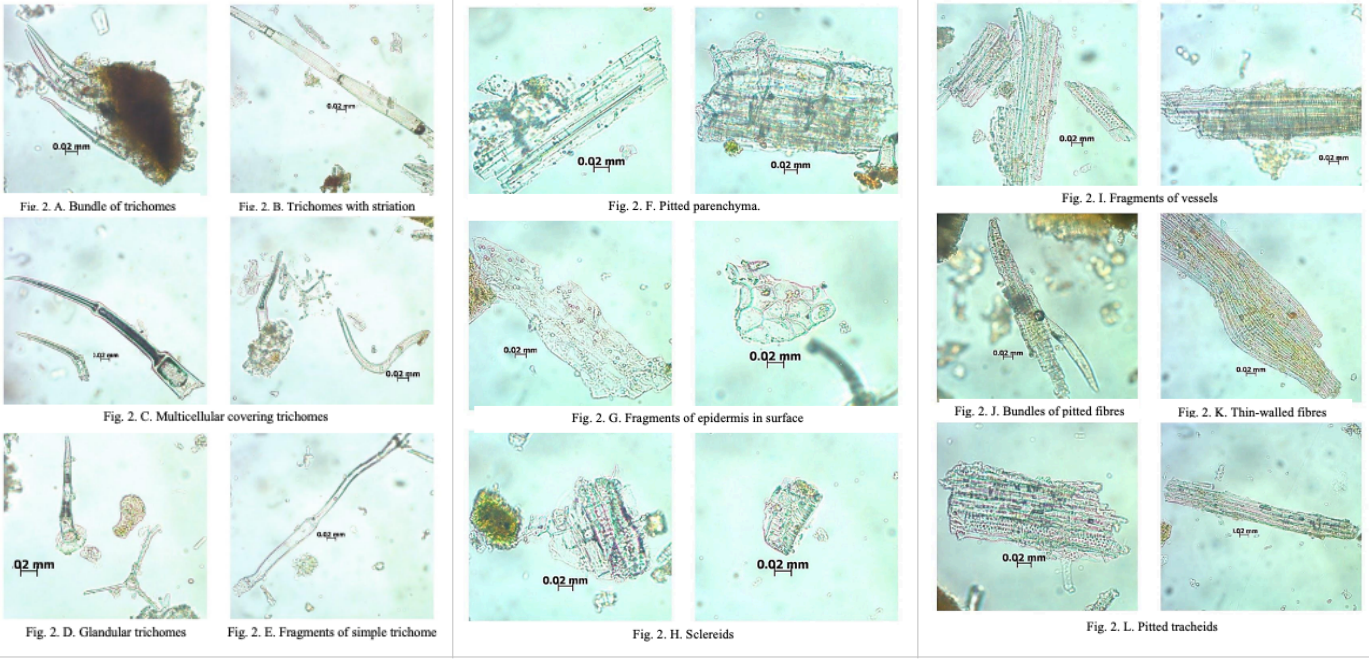
Phytochemical analysis

Qualitative screening of present study was performed for ethanolic extract showed the appearance of phytochemicals like coumarin, flavonoids, carbohydrates, steroids, phenols and tannin (Table 2).

Table 2. Preliminary phytochemical test results of alcoholic and water extracts of *Panchanga* (whole plant) of *Leucas aspera*

Test	Ethanol
Alkaloid	-
Amino acids	-
Coumarin	+
Flavonoids	+
Carbohydrate/ glycoside	+
Steroid	+
Phenol	+
Tannin	+
Terpenoid	-
Resins/ Wax	-
Saponins	-
Quinone	-

Figure 2. Microscopy of powder of *Panchanga* (whole plant) of *Leucas aspera*



HPTLC fingerprinting

Fingerprint of chloroform fraction of total ethanol extract of *L. aspera* was carried out by making use of toluene: ethyl acetate: formic acid (10:2.5: 0.5) as solvent system. Under short UV it showed 9 spots; under long UV there were 11 spots; under white light it showed 7 spots and under white light post-derivatisation, there were 15 spots (Table 3, Fig. 3).

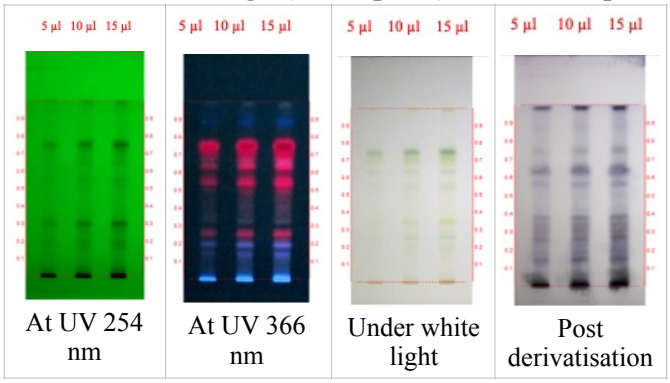
On densitometric scan of the plate at UV 254 nm, 13 peaks were detected with 3 of them were major accounting to 18 to 33 % area (Fig. 4) and even at 366nm, 13 peaks were detected, 4 of them were major accounting to 10 to 17 % area (Fig. 5) and at 540 nm, 9 peaks were detected, one with R_f 0.68 was the major peak with area % of 43.52 (Fig. 6).

Table 3. R_f value of alcohol extract of *Panchanga* (whole plant) of *Leucas aspera*

Under short	Under long	Under white	Post –
0.05 L Green	-	-	0.05 Blue
0.14 L Green	0.14 F Blue	-	-
-	-	-	0.16 Violet
0.19 L Green	0.19 F Blue	0.19 L Yellow	0.19 Violet
	-	0.24 L Green	0.24 Violet
0.27 L Green	0.27F Red	-	0.27 Brown
0.30 Green	-	-	0.30 Brown
-	0.32 F L Blue	-	-
-	-	0.34 Yellow	0.34 Brown
-	-	-	0.38 Brown
-	0.41 F L Pink	-	-
-	-	-	0.43 L Blue
-	0.50 F Pink	-	0.50 L Blue
0.55 L Green	0.55 F Red	0.55 Green	0.55Violet
0.64 L Green	0.64 F Red	0.64 Green	0.64 Violet
-	-	0.71 L Green	-
0.75 Green	0.75 F Red	0.75 Green	0.75 L Green
-	0.89 F Blue	-	0.89 L Blue
0.98 L Green	0.98 F Green	0.98 Yellow	0.98 Blue

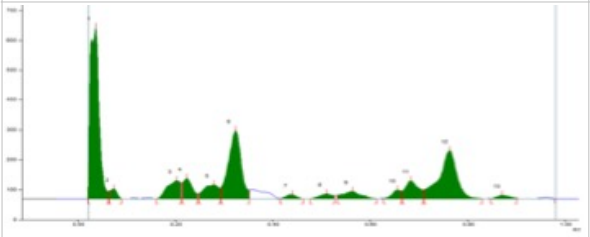
L - Light, F- Fluorescent

Figure. 3. TLC Photo documentation of alcohol extract of *Panchanga* (whole plant) of *Leucas aspera*



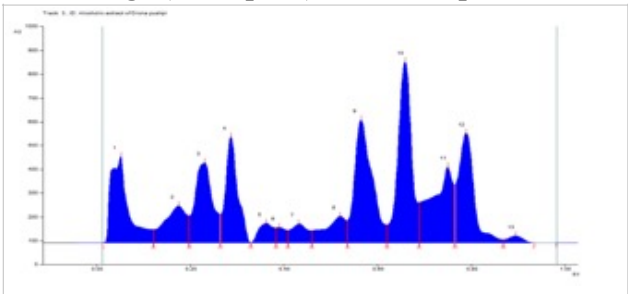
Solvent system – Toluene: Ethyl acetate: Formic acid (10: 2.5: 0.5)

Figure 4. HPTLC Densitometric scan of alcohol extract of *Panchanga* (whole plant) of *Leucas aspera* at 254 nm



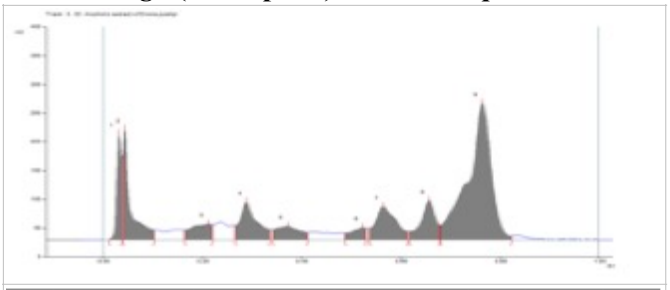
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.0 AU	0.04 Rf	572.8 AU	43.16 %	0.06 Rf	25.2 AU	8435.6 AU	33.04 %
2	0.06 Rf	25.9 AU	0.07 Rf	33.3 AU	2.51 %	0.09 Rf	0.6 AU	407.1 AU	1.59 %
3	0.16 Rf	0.3 AU	0.20 Rf	61.0 AU	4.59 %	0.21 Rf	52.8 AU	1363.6 AU	5.34 %
4	0.21 Rf	52.8 AU	0.22 Rf	68.1 AU	5.13 %	0.25 Rf	17.3 AU	1109.0 AU	4.34 %
5	0.25 Rf	18.0 AU	0.28 Rf	46.1 AU	3.48 %	0.29 Rf	36.3 AU	1194.8 AU	4.68 %
6	0.29 Rf	37.5 AU	0.32 Rf	227.2 AU	17.12 %	0.35 Rf	29.0 AU	4854.2 AU	19.01 %
7	0.42 Rf	0.7 AU	0.44 Rf	14.6 AU	1.10 %	0.46 Rf	0.3 AU	253.2 AU	0.99 %
8	0.48 Rf	0.6 AU	0.51 Rf	16.6 AU	1.25 %	0.53 Rf	10.9 AU	357.6 AU	1.40 %
9	0.53 Rf	12.7 AU	0.56 Rf	25.0 AU	1.88 %	0.61 Rf	0.1 AU	778.8 AU	3.05 %
10	0.63 Rf	0.8 AU	0.66 Rf	29.5 AU	2.22 %	0.67 Rf	26.4 AU	445.8 AU	1.75 %
11	0.67 Rf	26.8 AU	0.68 Rf	60.3 AU	4.54 %	0.71 Rf	30.2 AU	1325.5 AU	5.19 %
12	0.71 Rf	30.6 AU	0.76 Rf	160.6 AU	12.10 %	0.83 Rf	0.1 AU	4753.3 AU	18.62 %
13	0.85 Rf	0.6 AU	0.87 Rf	12.2 AU	0.92 %	0.90 Rf	2.3 AU	256.1 AU	1.00 %

Figure 5. HPTLC Densitometric scan of alcohol extract of *Panchanga* (whole plant) of *Leucas aspera* at 366 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.3 AU	0.05 Rf	362.3 AU	9.73 %	0.12 Rf	56.0 AU	11123.4 AU	10.62 %
2	0.12 Rf	56.0 AU	0.18 Rf	154.7 AU	4.15 %	0.20 Rf	13.3 AU	5811.7 AU	5.55 %
3	0.20 Rf	113.3 AU	0.23 Rf	335.6 AU	9.01 %	0.26 Rf	19.2 AU	10283.4 AU	9.82 %
4	0.27 Rf	119.3 AU	0.29 Rf	446.0 AU	11.97 %	0.33 Rf	1.2 AU	9444.5 AU	9.02 %
5	0.33 Rf	0.2 AU	0.36 Rf	82.2 AU	2.21 %	0.38 Rf	63.2 AU	2116.8 AU	2.02 %
6	0.38 Rf	63.4 AU	0.39 Rf	64.9 AU	1.74 %	0.41 Rf	52.7 AU	1084.3 AU	1.04 %
7	0.41 Rf	52.9 AU	0.43 Rf	79.7 AU	2.14 %	0.46 Rf	51.3 AU	2313.3 AU	2.21 %
8	0.46 Rf	51.4 AU	0.52 Rf	111.6 AU	2.99 %	0.54 Rf	92.9 AU	4043.5 AU	3.86 %
9	0.54 Rf	93.7 AU	0.57 Rf	518.4 AU	13.92 %	0.62 Rf	75.6 AU	15751.7 AU	15.04 %
10	0.62 Rf	75.8 AU	0.66 Rf	760.5 AU	20.41 %	0.69 Rf	68.4 AU	17847.0 AU	17.04 %
11	0.69 Rf	168.9 AU	0.75 Rf	318.3 AU	8.54 %	0.77 Rf	41.4 AU	11841.5 AU	11.31 %
12	0.77 Rf	243.1 AU	0.79 Rf	462.0 AU	12.40 %	0.87 Rf	13.9 AU	12311.1 AU	11.76 %
13	0.87 Rf	13.9 AU	0.90 Rf	29.0 AU	0.78 %	0.94 Rf	0.0 AU	755.2 AU	0.72 %

Figure 6. HPTLC Densitometric scan of alcohol extract of *Panchanga* (whole plant) of *Leucas aspera* at 540 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.1 AU	0.03 Rf	185.5 AU	21.03 %	0.04 Rf	40.6 AU	1489.4 AU	7.10 %
2	0.04 Rf	148.8 AU	0.04 Rf	192.9 AU	21.87 %	0.10 Rf	17.5 AU	2455.3 AU	11.70 %
3	0.16 Rf	17.6 AU	0.21 Rf	27.8 AU	3.16 %	0.22 Rf	26.6 AU	980.0 AU	4.67 %
4	0.27 Rf	24.9 AU	0.29 Rf	66.0 AU	7.48 %	0.34 Rf	18.2 AU	1836.4 AU	8.75 %
5	0.34 Rf	18.3 AU	0.37 Rf	23.8 AU	2.69 %	0.41 Rf	12.7 AU	959.3 AU	4.57 %
6	0.49 Rf	11.6 AU	0.52 Rf	21.8 AU	2.47 %	0.53 Rf	20.5 AU	528.3 AU	2.52 %
7	0.54 Rf	19.7 AU	0.57 Rf	57.8 AU	6.55 %	0.61 Rf	15.0 AU	1948.1 AU	9.28 %
8	0.62 Rf	14.7 AU	0.66 Rf	68.7 AU	7.78 %	0.68 Rf	25.7 AU	1657.4 AU	7.90 %
9	0.68 Rf	25.8 AU	0.77 Rf	237.9 AU	26.97 %	0.82 Rf	6.7 AU	9135.6 AU	43.52 %

Discussion and Conclusion

To maintain the efficacy of the plant derived raw drugs, standardisation of a raw drug and right identification of the same is vital. The medicinal plant in fresh form can easily be identified but the same in dried state become difficult since most of the identifying features will change. Botanical source of the drug and adulterants or substitutes can be identified by macro-microscopy. TLC is a fundamental of every herbal monograph. By investigating standardisation parameters like macroscopic, microscopic, physicochemical, phytochemical and HPTLC fingerprinting the quality of a plant medicine can be ensured along with adulteration and substitution, if any (14,15).

Many powdered materials are pushed into market to mask its true identity. Addition of foreign matter also goes undetected when a raw drug is marketed as powder. An organoleptic and microscopic analysis can identify any such practices. The *panchanga churna* (whole plant) powder is slightly smooth, fibrous and colour is pale greenish with faint distinctive odour and moderately bitter and sweet in taste. Bundle of trichomes, trichome with striation, multicellular covering trichomes, glandular trichome, fragment of simple trichome, parenchyma, pitted parenchyma, epidermal fragments in surface view, sclereids, fragment of vessels, bundle of pitted fibres, thin walled fibres, pitted tracheids were observed in powder microscopy.

Examination of physico-chemical composition as per international standards using pharmacopeia procedures is a productive technique in developing quality standards of herbal drugs (14, 15). Deterioration and shelf life of the drug depends on its water content, even the preservation of the drugs will also be affected if the moisture content is very high. The loss on drying (moisture content) at 105° C was 8.805 % w/w indicating moisture and volatile matter. Total ash value (7.71 % w/w) is suggestive of the total inorganic content after incineration in drug. Acid insoluble ash (AIA) value 0.974 % w/w revealed the presence of siliceous substance, minimum AIA value means less content of siliceous matter resulting from proper washing of raw drugs, especially underground raw drugs. Water soluble ash (3.07 % w/w) indicated the amount of ash which is readily soluble in water. Water (10.77%) and alcohol soluble (8.78%) extractive values indicated active constituents soluble in water and ethanol respectively (15-17).

Chemical composition from the secondary metabolite of plant drugs is responsible for the action of drugs on target disease. Various organic functional groups can be detected by the phyto-chemical tests that indicate the group of phytochemicals occurring in the plant. These tests show the presence discrete class of components present in the extract (18-20). The phyto-chemical analysis on the alcoholic and water extract of *L. aspera* revealed presence of coumarin, flavanoids, carbohydrate/glycoside, steroids, phenol and tannins in alcoholic extract of *Dronapushpi Panchaga curna.*

For micro-analytical separation and determination of natural products, TLC and HPTLC techniques are important analytical tools. HPTLC is a finest evolution of TLC principle which is useful in qualitative and quantitative analysis, that requires short time and has better resolution (9). Fingerprint of chloroform fraction of total ethanol extract of *L. aspera* was obtained by using suitable solvent system. Under short UV it showed 9 spots; under long UV there were 11 spots; under white light it showed 7 spots and under white light post- derivatisation, revealed 15 spots. On densitometric scan of the plate at UV 254 nm, 13 peaks were detected with 3 of them were major accounting to 18 to 33 % area and even at 366 nm, 13 peaks were detected, 4 of them were major accounting to 10 to 17 % area and at 540 nm, 9 peaks were detected, one with Rf 0.68 was the major peak with area % of 43.52.

The HPTLC finger print profile of ethanol extract of *L. aspera showed maximum compounds* under white

light post- derivatisation. On densitometric scan of *L. aspera* ethanol extract, showed the maximum peaks at 366 nm. The HPTLC fingerprint developed in this study will be an effective identity test for chemical identification of this plant.

Previous reports on powder microscopic evaluation of *Leucas aspera* showed the presence of fragments of epidermal cells of leaf, stem, petal and sepals, patches of collenchyma, sessile and stalked glandular trichomes, single celled and multicelled non-glandular trichomes, stomata vessels, tracheids, hairs of sepal, palisade cells, pollen grains, fragments of fibers and seed (21) and in comparison to previous work, present study revealed the presence of bundles of trichomes, trichome with striation, multicellular covering trichomes, fragment of simple trichome, parenchyma, pitted parenchyma, epidermal fragments in surface view, sclereids, fragment of vessels, bundle of pitted fibres, thin walled fibres, and pitted tracheids. Earlier reports on preliminary chemical examination of *L. aspera* showed the presence of triterpenoids in whole plant and the aerial parts of the plant showed the presence of phytochemicals such as alkaloids, carbohydrates, reducing sugars, proteins, phenolics, tannins, steroids, flavonoids and glycosides. In addition to above phytochemicals like flavonoids, carbogydrate, steroid, phenol, tannins; the whole plants showed positive result for coumrin in alcoholic extract of whole plant in this study (22-33).

Results of these investigations are useful for authentication of *L. aspera* plant powder and the data will help in monograph preparation and can be utilised as source guide for this plant.

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Conflicts of Interest of each author/contributors: Nil

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