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ANALYTICAL STUDY OF SOUTH INDIA MARKET SAMPLES OF BHARANGI (*CLERODENDRUM SERRATUM* LINN.)

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ABSTRACT

Bharangi (*Clerodendrum serratum* Linn. Family - Verbenaceae) root is one of the most common herbs in Ayurvedic formulations used to treat respiratory disorders. It is identified with different names in different places. The issue in the present era is the roots of Bharangi are being adulterated with *Clerodendrum infortunatum* Linn. *Clerodendrum phlomodies* Linn, and Quassia bark in the market. Hence, a humble effort has been made to identify and evaluate a genuine market sample of Bharangi root. Bharangi root samples were collected from different markets in South India: Chennai, Vijayawada, Hubli, Pune and Thiruvananthapuram. The qualitative and quantitative evaluation was done using physicochemical analysis, phytochemical tests, TLC and HPTLC to know the genuine samples of Bharangi root in different markets, followed by comparing these samples with the standards of API. Among the five samples, four were genuine since their analytical values were nearer to the standards of API and one sample was found adulterated since it differed from the standard analytical values.

Keywords: Bharangi, Physicochemical, Phytochemical, TLC, HPTLC

INTRODUCTION

Plants have been a significant source of medicine since ancient times, and about 80% of the world's population uses herbal medicine to treat different ailments.[1] In recent years, the demand for plant-derived products in developed countries has increased. These products are increasingly sought as medicinal products, nutraceuticals, and cosmetics.[2] It is necessary to maintain the purity and quality of herbal drugs in the commercial market and to have a quality product. However, the drugs do not comply with the standards prescribed for authentic drugs as they are frequently adulterated for commercial purposes.[3]

Bharangi (Figures 1 and 2) is also known as Bhargavi, Padma and Angaravalli. It is ruksha (dry), katu (pungent), tikta (bitter), kashayarasayukta (astringent), ushna (hot n potency), easily digestible, increases digestive fire, cures Gulma (disease characterised by pain), raktavikara (diseases caused by vitiation of blood), Shotha (swelling), Kasa (cough), Shwasa (breathlessness), nasal catarrh, Jwara (fever) and mitigates Kapha and Vata.[4]

Bharangi is identified as *Clerodendrum serratum* Linn. It is a small shrub with quadrangular stems. Leaves are opposite or in whorls of three elliptic with serrated margins. Flowers are in terminal panicles. Fruits purple drupes.[5] Bharangi is mentioned in the treatment of Swasa and Kasa (respiratory ailments) as a constituent of various medicines. *C. serratum* is a traditional medicine to treat asthma, infectious disorders and inflammatory conditions.[6]

According to Vaidyakashabdasindu, there are two varieties of Bharangi viz.
1.Shwetapushpa, *Clerodendrum indicum* Kuntze.
2.Neelapushpa, *Clerodendrum serratum* Linn.

Sometimes, *Clerodendrum infortunatum* Linn. is also used as Bharangi [7].

Clerodendrum serratum Linn. (Verbenaceae) and *Clerodendrum indicum* Kuntze are used as Bharangi (Verbenaceae). Quassia bark and Agnimantha *(Clerodendrum phlomodies* Linn) are also substituted for Bharangi.[8]

In this study, we have identified and evaluated a genuine market sample of Bharangi root. Bharangi root samples from different markets of South India: Chennai (Figure 3), Vijayawada (Figure 4), Hubli (Figure 5), Pune (Figure 6), and Thiruvananthapuram (Figure 7). The qualitative and quantitative evaluation was done using physicochemical analysis, phytochemical tests, TLC and HPTLC to know the genuine samples of Bharangi root in different markets, followed by comparing these samples with the standards of API [9].

MATERIALS AND METHODS

Bharangi root samples (Figure 3-7) were randomly collected from different South India markets like Chennai (S1), Vijayawada (S2), Hubli (S3), Pune (S4) and Thiruvananthapuram (S5). The samples were divided into two parts: one was kept for macroscopic and microscopic study, and another was made into coarse powder. [10]

The macroscopic and microscopic studies were carried out and compared with the description of the drug in API.

The samples were tested for ash values (total ash, acid-insoluble and water-soluble) and moisture content.

Extraction of the samples was done with four solvents: petroleum ether at 60-80 ^{o}C, chloroform at 60-70 ^{o}C, ethyl alcohol at 65-95

°C, and water at 90-100 °C, which were from non-polar to polar and maintained at the specific temperatures.

The test for alkaloids. carbohydrates, monosaccharides, proteins, amino acids, steroids, tannins, glycosides, fixed oils and fats were done.

TLC (Thin Layer Chromatography)

The TLC chamber was thoroughly cleaned and dried. The solvent system, i.e., n-Butanol: Acetic acid: Water (4:1:5), was poured into the chamber, and the lid was closed. The chamber was kept undisturbed for about an hour for saturation. The TLC plates were prepared with silica gel (1:2) and were kept in a hot air oven for 15 minutes at a temperature not exceeding 100 °C to make it devoid of moisture. The alcoholic extract was dissolved in alcohol. The plates were spotted with the help of capillary tubes 2 cm away from the base. A distance of 1 cm was maintained between each spot. The spotted plate was gently immersed in the TLC chamber containing the saturated solvent system so that the solvent had linear contact with the plate. The solvent was allowed to rise to the required distance. The plate was removed, and the solvent front (distance travelled by the solvent) was immediately marked with a pencil line. Plates were visualised under UV, and Rf values were noted. The plates were sprayed with suitable detecting agents and dried in a hot air oven for a few minutes, and the spots were observed.

The Rf values of the spots were calculated by using the formula,
Rf = Distance travelled by the solute / Distance travelled by the solvent front.

High-performance thin-layer chromatography (HPTLC)

HPTLC study was carried out at UWIN Life Sciences, Bangalore, Karnataka, India.

Sample: 2 gm of each sample was extracted using 30 ml chloroform for 6 hours under reflux. The extracts were then filtered through ordinary filter paper. Each extract was then concentrated to 10 ml to get the 200 mg/ml concentration. The extract was then passed through sodium sulphate to remove aqueous matter. The solutions were then used for the HPTLC spotting.

Mobile phase: Toluene: Ethyl acetate: Formic acid (5:5:1)
Stationary phase: Silica gel 60 F254 TLC plates.
Derivatisation agent: Anisaldehyde-sulphuric acid
Spotting volume: 5 µl

RESULTS

Macroscopic features S1, S2, S3 and S5 samples showed hard, woody and cylindrical dark, brown-coloured roots with acrid taste and characteristic odour and externally elongated lenticels. S4 differed from the above samples, with a greyish-brown colour, bitter taste and no characteristic odour, and rough outer surface with fissures and cracks.

Microscopic features of S1, S2, S3 and S5 showed stratified cork composed of thin walled, tangentially elongated cells. Secondary cortex wide, inner cells are polyhedral, almost ellipsoidal with intercellular spaces. Stone cells, acicular calcium oxalate crystals, and brown colouring matter were observed. The secondary phloem consists of sieve elements and parenchymatous cells modified into stone cells similar to the secondary cortex. Secondary xylem diffused porous consisting of vessels, tracheid, fibres and xylem parenchyma transverse by xylem rays. Acicular crystals and abundant simple and compound starch grains are present in several cells throughout the region. The microscopic study of sample 4 showed a wide cork of thin–walled, rectangular cells arranged in radial rows. The secondary cortex was of thin–walled oval and tangentially elongated parenchymatous cells towards the outer side and rounded cells towards the inner side. Several stone cells in singles and groups have highly thickened walls with distinct pits. Prismatic and cluster crystals of calcium oxalate are present. Starch grains are simple and scarcely present. The secondary phloem was wide, consisting of sieve elements, phloem parenchyma, phloem fibres and stone cells. Medullary rays are mostly uniseriate, composed of rectangular cells having brown colouring matter in some cells.

Physicochemical analyses of different Bharangi root powder market samples are mentioned in Table 1.

Extraction values: Alcoholic extract was more in S1 and significantly less in S2. S4 had the highest water-soluble extract. Petroleum ether extract was more in S1, and chloroform extract was more in S3. The extraction values of different samples in different solvents are shown in Table 2.

Phytochemicals identified in different market samples of Bharangi are shown in Table 3.

The TLC of samples is shown in Table 4.
Rf values of alcoholic extraction of different market samples of Bharangi. 3 spots under 365 nm and 4 spots after spray were observed in S1. S2, S3, and S4 had 3 spots under UV and 3 after spray. 4 spots were observed after spray in S5 (Figure 8).

The results of HPTLC are shown in Table 5. It shows the presence of four common substances in all samples. S1, S2, S3 had 6 substances. S5 had only three substances (Figure 9).

Figure 1: Bharangi, *Clerodendrum serratum* Linn.

Figure 2: Roots of Bharangi

Figure 3: S1 (Chennai sample)

Figure 4: S2 (Vijayawada sample)

Figure 5: S3 (Hubli sample)

Figure 6: S4 (Pune sample)

Figure 7: S5 (Thiruvananthapuram sample)

Figure 8: TLC of Samples

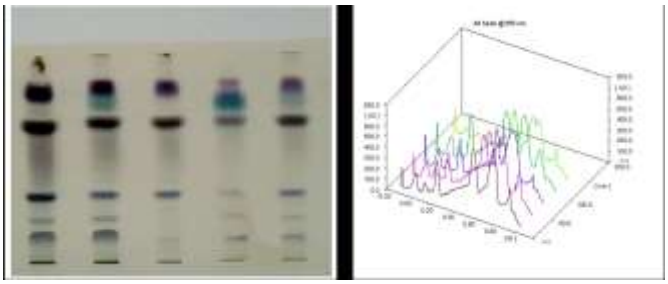

Figure 9: HPTLC of Samples

Table 1: Comparative physicochemical analysis of different market samples of Bharangi root powder

Parameter	S 1	S 2	S 3	S 4	S 5	**Standard values** (Ref. API)
Total ash value	8.75%	3.5%	6.25%	3.828%	1.58%	<11%
Acid insoluble ash	0.92%	1.21%	1.59%	1.453%	0.598%	<1%
Water soluble ash	3.65%	2.34%	2.16%	3.775%	1.367%	NA
Moisture content %	1.31%	0.9%	1.04%	2.25%	1.7%	NA
Total % of foreign matter	Nil	34%	0.5%	Nil	0.409%	<2%

Table 2: Comparative extractive values of different market samples of Bharangi root powder

Name of the test	S 1	S 2	S 3	S 4	S 5	**Standard values** (Ref. API)
Alcohol soluble extract value	6.74%	2.21%	5.05%	3.94%	3.473%	>6%
Water soluble extract value	20.02%	17.02%	15.91%	21.286%	3.58%	>12%
P.E extract value	2.02%	1.1%	0.782%	0.453%	0.292%	NA
Chloroform extract value	4.4%	2.96%	4.892%	0.707%	0.605%	NA

Table 3: Results of phytochemical tests of different market samples of Bharangi root

Test for	S1	S2	S3	S4	S5
Carbohydrates	+	+	+	+	+
Reducing sugars	+	+	+	+	+
Monosaccharides	--	--	--	--	--
Non reducing sugars	--	--	--	--	--
Proteins	+	+	+	--	+
Amino acids	+	+	+	--	+
Fats	--	--	--	--	--
Fixed oils	--	--	--	--	--
Volatile oil	--	--	--	--	--
Steroids	+	+	+	+	+
Glycosides	--	--	--	--	--
Saponins	+	+	+	+	+
Alkaloids	+	+	+	+	+
Tannins	+	+	+	+	+

Table 4: Rf values in TLC of different market samples of Bharangi root

Samples	365 nm	With spray agent
Sample 1	0.6, 0.76, 0.9 (3 spots)	0.5, 0.6, 0.8, 0.9 (4 spots)
Sample 2	0.8, 0.9 (2 spots)	0.8, 0.9, 0.5 (3 spots)
Sample 3	0.78, 0.9 (2 spots)	0.8, 0.9, 0.52 (3 spots)
Sample 4	0.5, 0.9 (2 spots)	0.6, 0.8, 0.9 (3 spots)
Sample 5	0.8, 0.9 (2 spots)	0.2, 0.5 0.8, 0.9 (4 spots)

Table 5: Rf values in HPTLC of different market samples of Bharangi root

Samples	Rf values	Colour of the Band
Sample 1	0.11	Greenish blue
	0.21	Blue
	0.34	Dark blue
	0.69	Dark Green
	0.76	Blue
	0.82	Dark Violet
Sample 2	0.11	Greenish blue
	0.21	Blue
	0.34	Dark blue
	0.69	Dark Green
	0.76	Blue
	0.82	Dark Violet
Sample 3	0.11	Greenish blue
	0.21	Blue
	0.34	Dark blue
	0.69	Dark Green
	0.76	Blue
	0.82	Dark Violet
Sample 4	0.21	Blue
	0.34	Dark blue
	0.69	Dark Green
	0.76	Blue
	0.82	Dark Violet
Sample 5	0.34	Dark blue
	0.69	Dark Green
	0.82	Dark Violet

DISCUSSIONS

Clerodendrum serratum Linn. is a member of Verbenaceae distributed in the deciduous forests of the Western Ghats of India. As per the traditional claim, roots are the potential drug source for various medicinal trials. The plant species are decreasing due to the destruction of natural habitat, over-exploitation of the drug, poor seed setting and poor seed germination.[12]

Bharangi is a common herb that is used in formulations which are specially indicated for respiratory disorders. Charaka mentioned the leaves of Bharangi in shaka (vegetables) varga[11]. Sushruta explained under Rodradi gana, Arkadi gana and Surasadi gana, which are indicated in Kaphaja vikara and Shwasa-Kasa vikara (respiratory diseases)[13].

Many synonyms are described in the literature, which explains the morphological characteristics of leaves like Kharapatra Kharashaka[14], and flowers like Brahmasuvarcala and Padma. There are no synonyms indicating the morphological characters of the root of Bharangi.

Bharang*i* is included under a controversial drug. In different regions of India, about nine drugs are used as Bharangi. Finally, Bharangi is considered *Clerodendrum serratum* Linn. belonging to the Verbenaceae family [8].

Among five market samples, S4, collected from Pune, was bark, and others were root samples. Macroscopic and microscopic differences are seen as bark and root samples. In S2, 34% of unwanted substances like stones, dust, faecal matter of rats, plastic threads and pieces of some other drugs were seen. It shows the adulteration of the drug to increase the weight.

All the samples had saponin, the main constituent of the Bharangi, steroids, carbohydrates, tannins, proteins and alkaloids. Proteins and alkaloids were absent in S4.

TLC and HPTLC have shown 3-5 substances. It may be due to the presence of exhausted materials in the sample.

CONCLUSION

The root is used as a medicinal part in Bharangi (*Clerodendrum serratum* Linn.*).* An analytical study of Bharangi market samples was done with five samples collected from different markets in South India. The results were compared to the description in The Ayurvedic Pharmacopoeia, considered standard in this study. Among five samples, four were similar in morphological characters, but the S4, collected from the Pune market, was different in its morphological structure. It shows the adulteration of the whole drug. From the results of the analytical study of five market samples of Bharangi, the sample collected from Chennai was nearer to the standards mentioned in API. The samples collected from Thiruvananthapuram, Vijayawada and Hubli were

partially close to standard values. This study shows that the adulteration of herbal products is commonly practised in the market. It is essential to give awareness about adulteration to the public, which may reduce the efficacy of herbal products and show the harmful effects on health.

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