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PHARMACOGNOSTIC AND HPTLC COMPARISON OF KERALA MARKET SAMPLES OF BHARANGI (CHERUTHEK) WITH GENUINE SOURCE PLANT *ROTHECA SERRATA* (L.) STEANE & MABB

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Bhāraṅgī, (*Rotheca serrata (L.)* Steane & Mabb, Pharmacognosy, Market study, Adulteration, *Ayurveda*

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ABSTRACT: Background: *Bhāraṅgī,* herbal drug used in *Ayurveda* as *Kapha-Vata samaka* and indicated in *Swasa*, *Kasa*, *Vrana*, *Shotha etc*. It is an important ingredient in many of the preparations like *Kantakaryavaleha, Kanakasava, Dasamularista, Lodhrasavam, Sudarsanasavam*. As per API the accepted source of *Bhāraṅgī* is *Rotheca serrata (L.)* Steane & Mabb belongs to family *Lamiaceae.* There were ambient studies in which it was found that roots of *Bhāraṅgī* had been adulterated with stem of the same in the market. But there were no published works indicating the presence of adulterants in the Kerala market, hence this study was proposed to assess the genuineness of raw drug available in the name of *Cheruthek* (*Bhāraṅgī)* in different Kerala markets with the root of genuine source plant *R. serrata*. **Materials and Methods:** Fresh root of *Bhāraṅgī* (*Rotheca serrata (L.)* Steane & Mabb) are collected from the natural habitat. The market samples of *Cheruthek* were collected from six different regions in Kerala. All samples were screened for pharmacognostic parameters- macroscopic, microscopic, histo-chemical and powder microscopic characterization along with HPTLC profile and compared with the genuine drug. **Result & Conclusion:** After the pharmacognostic comparison and HPTLC profiling of six market samples of the drug *Cheruthek* collected from selected districts of Kerala, the Kozhikode sample had similar characters of the *R. serrata;* Thiruvananthapuram sample was having characters of the plant *Premna herbaceae* Roxb. All other samples were adulterated with various plant parts.

INTRODUCTION: *Ayurveda* is increasingly gaining recognition as one of the medical sciences worldwide in the last few decades. Plant materials were used throughout the world as home remedies, over-the-counter drug products and raw materials for the pharmaceutical industry, and represent a substantial proportion of the global drug market. Commercialization in the medical system leads to substituting or adulterating herbal drugs to withstand scarcity.

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Using commercially available inferior-quality drugs had compromised the therapeutic efficacy of *Ayurveda* medicine. Hence, the quality standardization of drugs had got greater importance in *Ayurveda*. As per CCRAS, a market sample survey has been found to be one of the priority areas for drug research [1].

Bhārangī, a potent Ayurvedic drug used in respiratory disorders, which acts as *Kapha Vata Samaka*. In classics the roots were considered as the part used along with root bark which is used in many of the Ayurvedic formulations like *Bharangyadi kashaya, Bharangyadi churna, Kantakaryavaleha, Bhārangī Guda, Kanakasava, Lodhrasava etc*. As per Ayurvedic Pharmacopeia of India and Quality Standards of Indian Medicinal

Plants, states that *Rotheca serrata* (L.) Steane & Mabb belongs to the family *Lamiaceae* is the botanical source of *Bhāraṅgī* and the official part used is dried roots [2, 3]. Due to increased demand and over-exploitation, there is a significant reduction in the availability of genuine drug; hence chances for adulteration with other spurious, inferior or useless parts of same or different plants is very high. Some previous studies found that the roots of *Bhāraṅgī* are adulterated with the stem, in many markets. From a previous study conducted on the market samples of Bhāraṅgī, it was found that all the samples were adulterated and none of the samples were genuine (Singh *et. al.* 2015) [4]. The most of the samples obtained in South Indian are observed to be the roots and root nodules of *Pygmaeopremna herbacea* (syn. *Premna herbacea*) (Vasudevan *et. al.* 1971) [5]. Though *Brihattrayis* and *Nighantukara* have described only one variety of *Bhāraṅgī,* we come across two types of *Bhāraṅgī* in *Vaidyaka Sabda Sindhu*, the white flowered and blue flowered. This description has become a source for controversy. The latter authors clarified that the white-flowered variety is identified as *Clerodendrum indicum* (L.) Kuntze and the blue flowered one as *Rotheca serrata* (L.) Steane & Mabb [6, 7]. There were no published works are available about the genuinity of *Bhāraṅgī* in Kerala market samples. Hence in this study, mainly aimed to compare the different market samples of *Bhāraṅgī (Cheruthek)* by Pharmacognostic and High-performance thin layer chromatography (HPTLC) profiling with the root of genuine source plant *Rotheca serrata (L.) Steane & Mabb*.

MATERIALS AND METHODS: The root and stem of genuine sample *of Bhāraṅgī (Cheruthek)* were collected from Herbal Garden of Vaidyaratnam P. S. Varrier *Ayurveda* College, Kottakkal and Vrindavanodyanam, Thanal Mathrusadanam, Mayanoor, Thrissur. The samples were authenticated and specimens were deposited at the Herbarium of Center for Medicinal Plants Research (CMPR), Arya Vaidya Sala, Kottakkal, Kerala. (Herbarium Voucher No: 10047). The different market sample of *Bhāraṅgī (Cheruthek)* were procured from six different markets of Kerala. (Thiruvananthapuram, Idukki, Palakkad, Kozhikode, Wayanad and Kasargod) The genuine sample of *Rotheca serrata (L.)* Steane & Mabbwere compared with the market samples, on basis of pharmacognostic parameters along with HPTLC profile.

Samples were named as follows:

Samples 1: Roots and stem of *Rotheca serrata (L.)* Steane & Mabb

Sample 2: Sample collected from Thiruvananthapuram market.

Sample 3: Sample collected from Idukki market.

Sample 4: Sample collected from Palakkad market.

Sample 5: Sample collected from Kozhikode market.

Sample 6: Sample collected from Wayanad market.

Sample 7: Sample collected from Kasargod market.

Sample 8: Root of *Clerodendrum indicum.*

Methodology: External characters were studied and documented per standard API protocol. The materials for anatomical study were processed as per the standard protocol. The powder analysis and maceration study were done. Observations were done under Leica DM 1000 LED microscope and photographs were taken using Leica DFC 295 in-built camera. For HPTLC profiling of the water extracts of the samples were done under the solvent system - Ethyl acetate: methanol: water (9:1:1). Observed under UV light at 254, 366nm and after derivatization with ANS reagent, recorded the R_f value. Densitometric scanning of plates was done using Camag TLC scanner 3 at 254, 366 and 550nm.

RESULTS: The genuine sample of *Bhāraṅgī* and its six market samples were compared by its pharmacognostic parameters using macroscopic, microscopic, histochemical, powder microscopic characters along with HPTLC profile.

Macroscopic Comparison: Organoleptic characters of the Samples of *Bhāraṅgī* were shown in **Table 1** and **Fig. 1**. Samples showed almost identical characteristics such as colour, fractures, external markings and cut surfaces. The main differences observed between the samples were in

thickness. The sample 1; root and stem had extremely different texture compared to market samples. Sample 6 showed extremely different macroscopy when compared to others.

TABLE 1: MACROSCOPIC COMPARISON OF THE SAMPLES OF *BHĀRAṅGĪ*

		Colour	Shape	Size	Surface	Fractures	Odour	Taste
Sample 1	*R. serrata*– Root	Outer surface brown in colour, wood portion	Cylindrical	Diameter upto 2.5cm	Rough, longitudinal striations & lenticels	Short	Characteristic odour	Acrid
	R. serrata– Stem	creamish white	Cylindrical	Diameter upto 3cm	Rough, longitudinal striations & lenticels	short	No Characteristic odour	Acrid
Sample 2	Root	Dark to light muddy brown colour, Wood portion is	Cylindrical long slender; stoloniferous roots	Diameter 0.5-1cm	Rough; longitudinal striations	Short, brittle	Characteristic odour	Acrid
	Stolon	creamy brown	Cylindrical long slender; stoloniferous roots	Diameter 2-3cm	Rough; longitudinal striations	Short, brittle	Characteristic odour	Acrid
	Tuber		Cylindrical long slender; stoloniferous roots	Diameter 2-3cm	Rough; longitudinal striations	Short, brittle	Characteristic odour	Acrid
Sample 3	Root	Dark to light muddy brown colour, Wood portion is	Cylindrical, lateral roots-long slender;	root pieces-1-1.5cm in diameter; 6-8cm long;	Rough; longitudinal striations	Short, brittle	Characteristic odour	Acrid
	Tuber	creamy brown	stoloniferous roots, subglobous	Root nodules-Diameter 2.5-3cm;	Rough; longitudinal striations	Short, brittle	Characteristic odour	Acrid
Sample 4	Root	Brown in colour; Wood portion is creamy brown	Cylindrical,Lateral roots were long slender;	0.5-1cm in diameter, 3-4cm long pieces	Rough; longitudinal striations;minute lenticels	Short	Characteristic odour	Acrid
	Stem	Brown in colour	stout to slender, cylindrical	diameter of 2-2.5 cm	Rough; longitudinal striations; minute lenticels	Short	Characteristic odour	Acrid
Sample 5	Root	Brown in colour; Wood portion is creamy brown	Cylindrical, Lateral roots were long slender	1-1.5cm in diameter, 5-7cm long pieces	Rough, longitudinal striations	Short	Characteristic odour	Acrid
	Stem	Brown in colour	Stout to slender, cylindrical	Diameter of 2-2.5cm	smooth;longitudinal striations, minute lenticels	Short	Characteristic odour	Acrid
Sample 6		Dark- light brown in colour,	Fibrous	1.5-2cm long pieces	Rough -smooth		No Characteristic odour	
Sample 7	Root	Brown in colour, wood creamy brown	cylindrical; Lateral roots were long slender;	0.5-1cm in diameter,1-2cm long pieces	Rough longitudinal striations	Short	Characteristic odour	Acrid
	Stem	Brown in colour	stout to slender, cylindrical& branched	Diameter of 1-2cm	Smooth, longitudinal striations	Short	Characteristic odour	Acrid
C. indicum - Root		Yellowish brown in colour; wood portion creamish white	Cylindrical,	1cm in diameter	Smooth; longitudinal striations	Short	Characteristic odour	Acrid

Microscopic Comparison:

Microscopy of Sample 1 (Root and Stem):

Root: TS of the root was almost circular in outline with outer layers of cork ruptured at many places followed by a wide cortex, phloem and well-developed xylem region. Detailed TS showed a well-developed cork region with two distinct zones - outer zone consisting of up to 15 layers of comparatively thick walled rectangular to tangentially elongated cells which often peeled off at many places. Inner zone composed of more than 15 layers of tangentially elongated radially arranged cells. Cortex was composed of 10 - 15 layers of oval or elongated loosely arranged parenchymatous cells with inter-cellular spaces. Simple and compound starch grains measuring upto 10-15µm in diameter are scattered throughout the cortical region along with acicular and rod-shaped calcium oxalate crystals.

Solitary and groups of fibers and stone cells present throughout the cortical region. Phloem is comparatively narrow composed of compactly arranged compressed cells often alternating with medullary ray cells at many places. Simple starch grains and acicular and rod-shaped crystals of calcium oxalate were present at some places.

Centre portion occupied by xylem elements such as vessels, fibers, parenchyma and medullary rays. Xylem vessels were round to oval in shape, 30-100µm diameter usually in solitary and rarely grouped. Medullary rays 2-3 seriated composed of radially elongated pitted parenchymatous cells often filled with starch grains and acicular crystals of calcium oxalate. Starch grains also observed in xylem elements including parenchyma cells and fibers. The presence of growth rings in mature roots was observed in **Table 2** and **Fig. 2.**

Stem: TS of the stem was almost circular in outline with narrow cork layers which were ruptured at many places followed by wide cortex, narrow phloem and well-developed xylem region. Detailed TS showed narrow cork region with 3-5 layers of rectangular to tangentially elongated cells. Cortex were composed of 10-15 layers of oval to elongated loosely arranged parenchymatous cells with intercellular spaces. Pericyclic fibers seen in cortical region in discontinuous patches alternating with solitary or group of stone cells. Starch grains of diameter 10-15µm and acicular crystals present in some cortical cells. Phloem narrow composed of compactly arranged compressed cells often alternating with medullary ray cells. Xylem region composed of vessels, fibers and medullary rays. Xylem vessels round to oval in shape, 40-150µm diameter usually solitary and rarely in grouped and scattered al-throughout the xylem region.

Tyloses present. Xylem fibers thick walled and possess major part of xylem region. Medullary ray uni-bi seriated composed of radially elongated pitted parenchyma cells. Pith is comparatively large and occupies the central region, composed of parenchymatous cells filled with starch grains and acicular and rod-shaped crystals of calcium oxalate at some regions **Table 2** and **Fig. 2.**

Microscopic Comparison with Market Samples: The TS of the Thiruvananthapuram sample and Idukki sample had dissimilar microscopic characteristics with that of genuine sample. The presence of orange-brown deposition at the inner layers of cork cells were evidently seen in the Thiruvananthapuram and Idukki samples.

The presence of stone cells was very few in the cortical region in the Thiruvananthapuram sample and Idukki sample. While its abundant in case of genuine sample. The presence of cortical fibers were found in the root samples of Thiruvananthapuram sample and Idukki sample. Starch grains and acicular crystals were present comparatively less with genuine sample. Presence of oil globules in case of the Thiruvananthapuram sample and Idukki sample. Palakkad and Kozhikode samples had similar microscopic characteristics to genuine samples.

The presence of stone cells and fibers was even in both samples, starch grains and acicular crystals of calcium oxalate. Wayanad and Kasargod samples did not resemble any of the microscopic characters of the genuine sample. The Wayanad sample prominently showed the vascular region which is dissimilar with that of genuine. The Kasargod sample showed different TS of root and stem pieces. It possesses uni-bi seriated medullary rays. Presence oil globules were seen in both samples **Table 2**, **Fig. 3.**

TABLE 2: MICROSCOPIC COMPARISON OF THE SAMPLES OF BHĀRANGĪ

		Cork	Cortex	Phloem	Xylem
Sample 1	*Rotheca serrata* – *Root*	Two distinct zones- tangentially elongated – peeled off at places. radially arranged; tannin depositions	Wide; round to oval, loosely arranged parenchyma; Rounded starch grains; acicular and rod-shaped crystals; scattered fibres; stone cells and sclereids; tannin deposition	Narrow zone; compactly arranged cells; Rounded starch grains; acicular and rod shaped crystals; parenchyma alternating with ray cells	Round to oval vessels; Multiseriate medullary rays, rarely uni to bi seriate; Starch grains in medullary rays and xylem fibres; acicular and rod shaped crystals in medullary rays. Bigger sized vessels in outer and centre portion of the wood
	Rotheca serrata – *Stem*	Narrow; tangentially elongated cells; tannin depositions	Wide; oval to elongated loosely arranged parenchymatous cells; discontinuous ring of pericyclic fibres; stone cells; starch grains and acicular crystals; tannin depositions	Narrow zone; compactly arranged cells; parenchyma alternating with ray cells	Major portion occupied by fibres; round to oval vessels; uni to bi seriate medullary rays; Widepith polygonal parenchymatous with starch grains, acicular and rod shaped crystals
Sample 2	Root	2-5 layers of tangentially elongated irregular cells;	10-12 layers; loosely arranged parenchymatous cells; cortical fiber patches; prismatic crystals;	Narrow and compressed; 3-5 layers; phloem fibers	round shaped vessels; Bigger sized vessels located at outer portion of wood, uni-bi seriate medullary rays;
	Stolon	tangentially elongated cells; inner layer of cells contains orange brown depositions;	5 - 8 layers of elongated parenchyma cells; stone cells; tannin depositions	5 - 10 layers of closely packed cells	In radial patches; vessel diameter; alternating with multiseriate medullary rays; polygonal parenchymatous pith
	Tuber	20 - 25 layers of thick walled, tangentially elongated cells; inner layer of cells contains orange brown depositions	oval to round tangentially elongated thin-walled parenchymatous cells; stone cells; sclereids; acicular crystals; tannin depositions	round to oval tangentially elongated cells; parenchyma alternating with ray cells; acicular crystals	multi seriate medullary rays; acicular crystals and starch grains; rounded parenchymatous pith with starch grains
Sample 3	Root	10 - 15 layers, narrow compressed cells; inner layers contain orange brown depositions; oil globules	oval to rectangular elongated parenchymatous cells; Stones cells and sclereids prismatic and acicular crystals of calcium oxalate;	narrow and compressed round to oval cells; parenchyma alternating with ray cells; acicular crystals of calcium oxalate, presence of oil globules	Vessels less in number multi-seriated medullary rays; starch grains
	Tuber	tangentially elongated cells; inner layer of cells contains orange brown depositions; presence of oil globules	oval to round tangentially elongated cells; stone cells; sclereids; starch grains, oil globules	Narrow and compressed, compactly arranged cells	Vessels less in number; uni - bi seriated medullary rays; pith loosely arranged parenchymatous cells, starch grains present in xylem and pith
Sample 4	Root	rectangular to tangentially elongated cells	Narrow and compressed layers of oval or elongated cells; Acicular, prismatic and rod-shaped crystals; fibers, stone cells scattered	narrow and compressed, compactly arranged cells	Vessels round to oval in shape; Tyloses present; multi - seriated medullary rays
	Stem	rectangular to tangentially elongated cells; Tannin	oval to elongated loosely arranged cells; Fibres, sclereids; stone	Narrow; compactly arranged compressed cells alternating with	Xylem vessels large, round to oval in shape; multi seriated medullary rays; pith is large

		depositions	cells; Granular masses and acicular crystals	medullary ray cells	
Sample 5	Root	two distinct zones; outer zones- 8-10 layers; rectangular to tangentially elongated cells; – peeled off at places; Inner zone - more than 5 - 8 layers; tangentially elongated cells.; tannin depositions	20-30 layers; oval or elongated loosely arranged parenchymatous cells; Simple and compound starch grains; acicular and rod-shaped crystals; stone cells and fibers; tannin depositions	Narrow; compactly arranged compressed cells alternating with medullary ray cells; starch grains; acicular and rod-shaped crystals	Xylem vessels were round to oval in shape, Tyloses present; multi- seriate medullary rays; starch grains and acicular crystals;
	Stem	Narrow; 8 - 10 layers rectangular to tangentially elongated cells; tannin depositions	15 - 20 layers of oval to elongated loosely arranged parenchymatous cells; pericyclic fibers, stone cells; Granular masses and acicular crystals; tannin depositions	Narrow; compactly arranged compressed cells; cells alternating with medullary ray cells.	vessels were round to oval in shape; Tyloses present; major portion occupied by xylem fibers; uni - bi seriated medullary rays; acicular crystals; pith large; starch grains, prismatic, rod and acicular crystals
Sample 6		Two distinct zones; reddish brown depositions	Narrow, elongated irregular cells	Wide, compressed ells; with brown depositions; alternating with ray cells; fibres scattered throughout	Well-developed xylem resembling a heart wood. Vessel's solitary with diameter. Medullary raysuni– multi seriate
Sample 7	Root	rectangular to tangentially elongated cells; peeled off at many places	oval or elongated loosely arranged parenchymatous cells; acicular and rod-shaped crystals	Narrow; compactly arranged compressed cells; fibres in solitary and in groups; acicular crystals	vessels were round to oval in shape; uni-bi seriated medullary rays; starch grains
	Stem	Narrow; rectangular to tangentially elongated cells;	oval to elongated loosely arranged parenchymatous cells; Pericyclic fibers in patches; stone cells; Starch grains & acicular crystals	Narrow; compactly arranged compressed cells	vessels were round to oval; uni-bi seriated medullary rays; acicular crystals; starch grains
Sample 8	*C. indicum* - *Root*	Two distinct zones; outer zone - upto 5 layers- thick walled rectangular to tangentially elongated cells; peeled off at many places; Inner zone- 5 layers; tangentially elongated cells; tannin depositions	10-12 layers; oval or elongated loosely arranged parenchymatous cells; prismatic crystals; stone cells; starch grains;	Wide, compactly arranged compressed cells, cells alternating with medullary ray cells; starch grains; prismatic crystals;	vessels were round to oval; uni-bi seriated medullary rays; starch grains

HPTLC Profile: HPTLC chromatograms and densitometric profiles of 8 samples at 254nm, 366nm and 550 nm were recorded. **Fig. 4** the Rf values of separated compounds of each sample were noted. **Table 3** At 254 nm, sample 5 has maximum number of peaks (10 peaks), sample 8 with 8 peaks, and sample 4 with 7 peaks. Rf values of many peaks of these samples were similar indicating same chemical constituents. *Rotheca serrata (L.)* Steane & Mabb. root sample (sample 1R) had only seven peaks and its stem (sample 1S) with 8 peaks. Sample 6 shows very less peaks. Peak intensities were varied in case sample 2 and sample 3. Sample 5 showed some unique peaks that were observed in the genuine stem and root.

TABLE 3: Rf VALUE OF SAMPLES

Wave-length- of Samples																										
UV 254 nm									UV 366 nm									UV 550nm								
1R	**1S**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**1R**	**1S**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**1R**	**1S**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
0.02	0.07	0.16	0.07	0.07	0.02	0.29	0.08	0.06	0.02	0.02	0.04	0.07	0.03	0.02	0.25	0.05	0.02	0.03	0.04	0.03	0.07	0.04	0.03	0.02	0.03	0.04
0.07	0.16	0.18	0.15	0.14	0.07	0.44	0.17	0.00	0.00	0.07	0.19	0.16	0.07	0.07	0.28	0.00	0.00	0.00	0.09	0.07	0.10	0.08	0.07	0.25	0.00	0.08
0.19	0.19	0.27	0.18	0.18	0.15		0.28	0.15	0.19	0.19	0.30	0.18	0.15	0.15	0.59	0.18	0.08	0.15	0.14	0.14	0.19	0.11	0.14	0.29	0.12	0.14
0.31	0.27	0.49	0.26	0.25	0.18		0.48	0.20	0.31	0.27	0.33	0.30	0.18	0.18	0.93	0.29	0.14	0.20	0.19	0.19	0.27	0.14	0.18	0.35	0.18	0.23
0.40	0.31	0.56	0.30	0.29	0.25			0.27	0.41	0.31	0.49	0.40	0.25	0.25		0.93	0.26	0.30	0.31	0.28	0.31	0.19	0.26	0.41	0.25	0.25
0.49	0.41		0.91	0.47	0.30			0.39	0.54	0.50		0.54	0.29	0.30			0.51	0.52	0.50	0.40	0.41	0.40	0.40	0.49	0.33	0.33
0.54	0.50			0.52	0.39			0.51		0.57		0.53	0.48	0.39			0.56	0.75	0.71	0.50	0.54	0.45	0.40	0.49	0.31	0.45
	0.55				0.47			0.57		0.54			0.52	0.47				0.79	0.82	0.55	0.59	0.47	0.53	0.78	0.35	0.57
					0.53					0.95			0.94	0.52				0.82	0.80	0.81	0.91	0.72	0.53	0.81	0.79	0.82
					0.93									0.93				0.97	0.82	0.81	0.94	0.78	0.78	0.93	0.93	0.94
																			0.84	0.87		0.80	0.80			
																			0.95	0.94		0.93	0.93			

DISCUSSION: Quality and Safety are fundamental principles in the provision of herbal medicines for health care. Scientific studies estimate herbal product adulteration as 40-60% in India, the most of Indian herbal medicinal products are essentially mixed with one or a few other herbs that could lessen the therapeutic activity of the main ingredients [8]. Only a few studies are available on quality standards of some market samples.

A study conducted in South Indian Market samples of *Bhāraṅgī*, it was noted that the roots of *C. serratum* was the accepted species for the drug. As per previous study other species coming as *Bhāraṅgī* were G*ardenia latifolia*, *G. resenifera, G. turgid*, *Premna intergrifolia* and *Picrasma quassioides*. However, it has been observed that in South Indian Pharmacies, the root and root nodules of *Pygmacopremna herbacea* were used as *Bhāraṅgī* in their preparations (K. Vasudevan Nair et.al 1982)[5]. In a study conducted on the four Indian market samples of *Bhāraṅgī,* it was found that all samples were adulterated and none of the market sample was found as genuine drug *Clerodendrum serratum* [3, 4, 9]. Another study showed that the roots of *Bhāraṅgī* were adulterated with the stem of *Clerodendrum serratum*, mostly in South Indian markets [10, 11]. Eventually there were no particular studies conducted on *Bhāraṅgī* in Kerala markets. This study thus aimed to conclude the exact identity of the market sample of *Bhāraṅgī* with special regards to Kerala markets. After undergoing macroscopic examination, morphologically sample 4, 5 & 7 had stout, cylindrical roots similar to genuine root; even then, sample 4 & 7 had a mixture of stem and roots. Sample 2 and 3 possess stoloniferous roots that don't match the original sample ie. Sample 1 which was collected from Mayyanoor, Thrissur.

There were differences in the dimensions and thickness. Sample 5 had similar characters as that of sample 1. Sample 6 was having extremely different morphological characters when compared with the genuine root. Hence, the chances of genuine roots of *Bhāraṅgī* coming in the market is comparatively less based on macroscopical observations. Sample 2 & 3 had similar morphological characteristics of that of roots of *Premna herbacea* Roxb. In a previous study also stated that sample collected from Southern Kerala can of roots of *Premna herbacea* Roxb [4]. In-order to compare the microscopic character of Thiruvananthapuram sample, previous study in which the pharmacognostic evaluation of roots of *Pygmaeopremna herbacea* (syn. *Premna herbacea*.) discusses the macroscopic, microscopic characteristics along with HPTLC profile of the *Premna herbacea* Roxbwere noted (Rastogi. S *et. al.* 2005) [12]. None of the samples had similarities with the sample 8 *C. indicum* [7].

TS of genuine root of *R. serrata* was compared with that of 6 market samples. There was extreme difference in case of the Thiruvananthapuram sample (sample 2) and Idukki sample (sample 3) with that of genuine one. Presence of cortical fibers in patches rather than bundle of stone cells. Absence of starch in root samples and the presence of oil globules in both the samples. There was presence of orange-brown colour depositions in the inner cork layers unlike original sample. The Palakkad sample (Sample 4) and Kozhikode sample (sample 5) possess similar microscopic characteristics of that of genuine sample. Palakkad sample possess the stem pieces which did not have the microscopic characteristics of the stem of *R. serrata.* Presence of oil globules were noted in both the samples. Starch grains were present but less abundant as compared to genuine sample. Wayanad sample (sample 6) and Kasargod sample (Sample7) had no significant similarity with genuine sample. Wayanad sample had well developed xylem resembling a heart wood. Phloem was wide, compressed with brown depositions, fibers are scattered. In Kasargod sample, one of the root pieces showed similar characters of genuine one other sample are different hence it's a mixture of plant parts. None of the samples had similarities with the sample 8 *C. indicum.*

The HPTLC comparison of water extracts of different samples were carried out. HPTLC chromatograms and densitometric profiles of nine samples at 254nm, 366nm, and 550nm were recorded. The Rf values of the separated compounds of each sample were noted. At 254nm, root of *R. serrata* had only seven peaks and the stem had eight peaks. The peak intensities were different for each sample, the peak intensities of sample 5 had similarity with that of genuine sample i.e. sample 1. Sample 4 also show some similar peaks but less. Similar Rf values uniformly present in all the samples studied indicate the presence of similar phyto-constituents. Similarly, at 366nm, root of *R. serrata* had only seven peaks and the stem had nine peaks. Sample 5 shows more similar bands with the sample 1 when compared with others, indicating the presence of a greater number of chemical constituents than the original one. There were no previously published comparable works to conclude these differences in the number of peaks of different samples. After analyzing the result in Table 4, it was clear that the raw drugs available in the market in the name of Cheruthek (Bharangi) were not derived from a single botanical source, revealing the practice of adulteration and substitution in the raw drug industry.

TABLE 4: INTERPRETATION OF MARKET SAMPLES

Sl. no.	Samples	Remarks
1	Sample 2- Thiruvananthapuram	Stoloniferous roots- sample had similar characters of roots of *Premna herbacea*
2	Sample 3- Idukki	Root along with other plant parts it was mostly a mixture. Sample were mixture of roots
3	Sample 4- Palakkad	The samples were mixture of various plant parts
4	Sample 5- Kozhikode	The sample had characters of *Rotheca serrata*
5	Sample 6- Wayanad	The sample was adulterant
6	Sample 7- Kasargod	The sample was a mixture of various plant parts- Adulterant

CONCLUSION: The samples of the drug *Bhāraṅgī* (*Cheruthek*) collected from 6 different markets from different districts of Kerala were pharmacognostically different, except the sample collected from Kozhikode. The organoleptic and microscopic evaluation of the market samples revealed that only Kozhikode sample had similar characteristics with that of the genuine sample of *Bhāraṅgī- Rotheca serrata* (L.) Steane & Mabb. Even though the genuine part used was root, Kozhikode sample possess both the stem and root. HPTLC profiling also revealed that similar peaks with different band thickness may be due to differences in maturity, soil, climate, and time of collection of drugs. From the previous reports, the Thiruvananthapuram sample can be the roots of *Premna herbacea* one of the adulterants of the drug *Bharangi*. The Idukki sample too contains roots of *Premna herbacea* along with other mixtures. The Palakkad sample contains the roots and stems of R. serrata and other mixtures. The Wayanad and Kasargod samples were completely different and had no similarity with the source plants *R. serrata & C. indicum.* Hence, it was clear that none of the market samples were only root but a mixture of root and stem.

FIG. 1: SAMPLES OF BHARANGI ALONG WITH SAMPLES FROM DIFFERENT MARKETS IN KERALA. A-B. Genuine sample bharngi; a. root of rotheca. b. stem of *Rotheca serrata*; c. root of *Clerodendrum indicum*; d. Thiruvananthapuram market sample; e. Idukki market sample; f. Palakkad market sample g. Kozhikod market sample, h. Wayanad maekwt sample; i. Kasargod market samole.

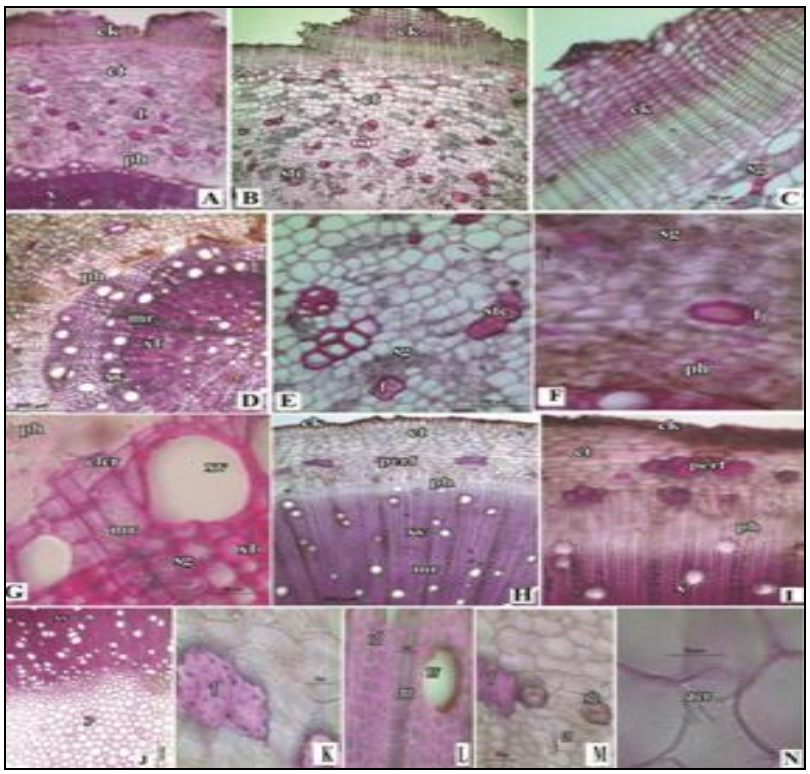

FIG. 2: MICROSCOPIC CHARACTERS OF THE ROOT AND STEM OF R. SERRATA. A-G. TS OF ROOT. A. TS of root portion enlarged; b. TS portion inlarge showing outer portion; c. TS cork region enlarged; d. ts showing stelar region; e. and f. enlarged view of cortical region; g. xylum region portion enlarged; h-n. TS of stem; h. t sod stem portion enlarged; i. TS portion; k. pericycle fibers l. xylume vessels m. enlarged cortical region n. parenchyma cells of pith ck, cork; clcr, crystals of calcium oxalte; ct, cortex; f, fiber; mr. modularly rays; ph phloem; sg starch grains; stc, stone cells; x, xylem; xf, xylem fibers; xv, xylem vessels

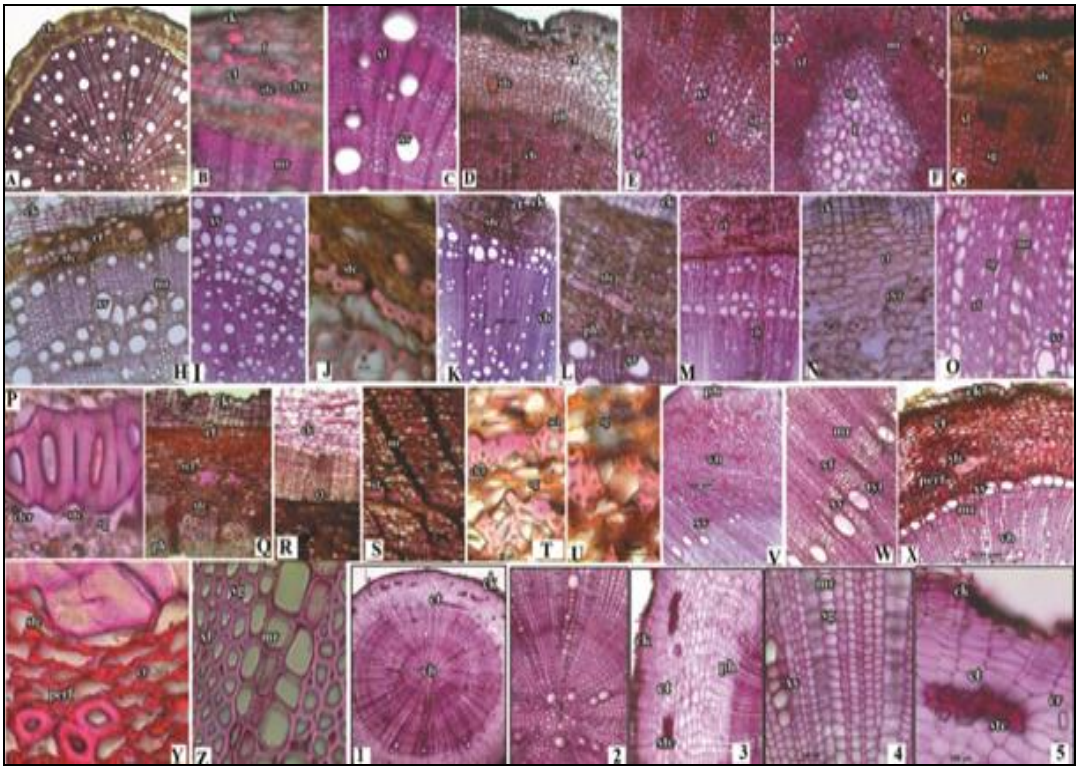

FIG. 3: A-Z: MICROSCOPY OF MARKET SAMPLE OF BHARANGI. A-C. TS of thiruvanthapuram market sample; D-G. TS of Idukki market sample; H-L. TS of palakkad markets saple; M-Q. TS of Kozhikode market sample; R-W. TS of wayanad sample; X-Z. TS of kasargod market sample; 1-5 microscopy *of Clerodendrum indicum.*

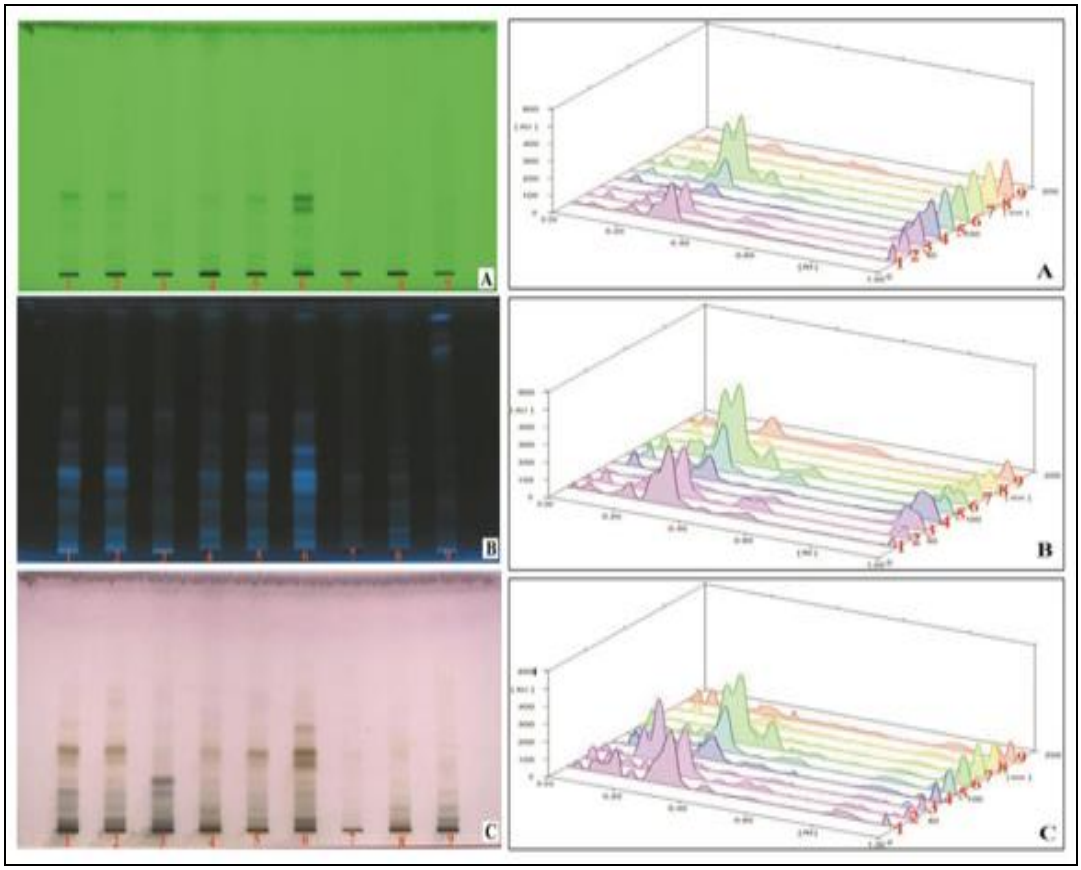

FIG. 4: HPTLC COMPARISON OF GENUINE AND MARKET SAMPLES OF BHARANGI. A-c. TLC profile of sample at 254nm, 366nm after derivatisation; d-f; denitometeric profile at 254nm 366nm and after derivatisation. 1. *Rotheca serrata* rot, 2.Rotheca serrata stem, 3. Thiruvananthapuram sample, 4. Idikki sample, 5. Palakkad sample, 6. Kozhikode sample, 7. Wayanad sample, 8. Kasargod sample, 9. Root of *Clerodendrum indium.*

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REFERENCES:

1. Sharma PC, Yelne MB and Dennis TJ: Database on Medicinal Plants Used in *Ayurveda*. Vol New Delhi. Central Council for Research in *Ayurveda* & Siddha 73-75.
2. Anonymous: The Ayurvedic Pharmacopoeia of India. 1st ed. Part 1, Volume III. Delhi: the Controller of Publications Civil Lines, Delhi 2001; 25-26.
3. D CD, R KY and B VP: Abhava pratinidhi dravya: A Comparative Phytochemical Study of Bharangi and Kantakari. IJAM [Internet]. 2021 Sep. 29 [cited 2022 Sep. 30]; 12(3): 588-92. Available from: https://ijam.co.in/index.php/ijam/article/view/2070
4. Singh RK, Singh A, Nathani S and Murthy AR: Cardinal Identification Features of Bharangi and its Market Samples. Int J of Ayur and Herb Med 2015; 5(2): 1689-94.
5. Vasudevan Nair K, Yogasimhan SN, Keshavamurthy KR and Mart Z: Studies onsome South Indian market samples of Ayurvedic drugs-I. Ancient Sci Life 1971; 2: 71-78.
6. Shastri JLN: Textbook of Dravyaguna Vigyanana. Reprint 2010. Vol. 2.Varanasi: Chaukhamba Orientalia, Varanasi. 2010; 422.
7. Lahari Sidde, Malathi S, Swethalatha S and Rajani K: A Breif Review on *Clerodendrum indicum*. International Journal of Indigenous Herbs & drugs 2018; 3(5): 1-4.
8. Shanmughanandhan D, Raghupathy S, Newmaster SG, Mohanasundaram S and Sathishkumar R: Estimating herbal product authentication and adulteration in India using vouchered DNA based biological reference material library. Drug Saf 2016; 39: 1211-27.
9. Gadade Jayashree and Patil Swaroopa A: *Rotheca serrata*: an Overview. Int J of Life Scie Special 2018; 10: 185-190.
10. Narayanan N, Thirugnanasambantham P, Viswananthan S, Sukumar E and Jeyaraman P: Pharmacognostical Studies on the Roots of *Clerodendrum serratum*. Pharmaceutical Biology 2002; 40(5): 362-368, DOI:10.1076/phbi.40.5.362.8454.
11. D CD, R KY and B VP: Abhava pratinidhi dravya: A Comparative Phytochemical Study of Bharangi and Kantakari. IJAM [Internet]. 2021 Sep. 29 [cited 2022 Sep. 30]; 12(3):588-92. Available from: https://ijam.co.in/index.php/ijam/article/view/2070
12. Rastogi Subha, Madan Mohan, Kumar K, Rawat AKS and Pushpangadan P: Pharmacognostical Evaluation of Roots of *Pygmaeopremna herbacea* (Roxb.) Mold. Naturals Product Sciences 2005; 11(2); 58-62.

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