

A COMPARATIVE STUDY TO EVALUATE DIFFERENT COMMERCIAL SAMPLES OF
RUBIA CORDIFOLIA L. THROUGH OUT INDIA

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Article Received on 27/04/2017

Article Revised on 18/05/2017

Article Accepted on 07/06/2017

ABSTRACT

Ayurveda is not a science dealing only with drugs it is more a “way of life” and describes methods for promotion, prolongation and maintenance of positive health. *Manjistha* is a commonly and popularly used drug in many formulations from the time of *Puranas* to till date. As per classical literature available in Ayurveda, it is evident that drug *Manjistha* is having much significant and importance being extensively used for its various benefits and it has been screened for many pharmacological activities. *Acharya Charak* described it as *Varnya*, *Vishaghna* and *Jvarahara* while *Acharya Susruta* mentioned it as *Pittashamaka*. It acts as *Raktadoshara*, *Vranaropana*, *Varnya*, *Jwarhara*, *Vishaghna*. **Aims and Objectives:** To compare the genuine sample of *Manjistha* (*Rubia cordifolia* Linn.) with different market samples on the basis of pharmacognostic and phytochemical study, to compile and compare the data generated during the study to find out whether there is any significant differences exist in pharmacognostical and phytochemical profiles of different market samples. **Materials and Methods:** The quantitative phytochemical studies of the drug samples were carried out by studying the percentage of ash, extractive values and qualitative screening was carried out by TLC, HPTLC and different biochemical tests. Thus, the present work aims in forming certain parameters for identification of drug with the help of various phytochemical observations. **Conclusion:** All H.P.T.L.C results of market samples compared with genuine, only sample2 (Gujarat) shows maximum similarity in constituents.

KEYWORDS: *Rubia cordifolia*, commercial samples, roots.

INTRODUCTION

“*Manjistha*” *Rubia cordifolia*, L. (family-Rubiaceae), is an important herbal drug used in Indian system of medicine. The root of the plant is commonly known as *Manjistha* and sold in the market under the commercial name *Manjith*. It is famous as *Varnya* in the traditional medicine.^[1] Plant drug has number of vernacular names like *Aruna*, *Bhandi*, *Bhandiralatik* in Sanskrit, *Mandar*, *Manjit* in Hindi. It is used as antidysentric, antipyretic, analgesic, anthelmintic, improves the voice, the complexion and cures the *Kapha*, the inflammation diseases of the uterus, the vagina, the eye, the ear and the blood. It is also used in the cure of leucoderma, ulcers, urinary discharges, jaundice, and piles. The Ruberythric acid is one of the major constituents of the root and is widely used as phytotherapeutic drug in the treatment of calcium containing stones in the urinary tract. *Rubia cordifolia* is used in Ayurveda as an ingredient of popular formulations like *Candana asava*, *Asvagandhadhi Arista*, *Jatyadi Ghrita*, *Phala Ghrita*, *Brhanmajisthadi Kvatha*, *Curna*, *Pinda Taila*, *Manjisthadi Taila*. The drug according to Ayurveda is *Guru* and *Ruksa* in *Guna*, *Kasaya*, *Tikta* and *Madhura* in *Rasa*, *Katu* in *Vipaka* and *Usna* in *Veerya*.^[2] Thus, due to

its high medicinal value, the present work was carried out to study the root of authentic sample with the commercial samples from Haryana, Gujrat, Assam and Karnataka with genuine sample from Western Ghats of Karnataka. *Rubia tinctorum* Linn. reported to be sold as *innManjistha* (*Rubia cordifolia* L.). Stems of *Manjistha* are also used as substitute of roots. It is also adulterated with roots of *Rubia tinctorum*, roots of *Oldenlandia umbellata* linn. or *Hedyotis umbellata* and sometimes roots of *kirattikta*.^[3]

MATERIALS AND METHODS

The study was designed under two headings.

- Pharmacognostic study including macroscopic and microscopic study
- Analytical study

Pharmacognostic study materials drug – *rubia cordifolia* linn. Plant identification

The plant *manjistha* (*rubia cordifolia* linn.) was identified on the basis of its:

1. Synonyms given in classics of ayurveda.
2. Morphology and family characters of the plant.

Authentication

Genuine sample was procured from the madikeri forests after proper identification.

Collection

Literary data was collected from the library of K.V.G Ayurvedic Medical College and Hospital, Sullia and internet, collection of the samples from markets of Haryana, Gujarat, Karnataka and Assam was done.

These samples were coded as:

Sample S1 – Karnataka

Sample S2 - Gujarat

Sample S3 – Haryana

Sample S4 – Assam

Analytical Study

Materials

- Hand lenses were used for the detection of foreign matter.
- For quantitative extraction (Aqueous, Acetone, Alcoholic, Chloroform and Petroleum ether) of all samples, water bath, conical flasks etc. are used.
- Total ash, acid insoluble ash, and moisture content, are determined by using silica crucible, oven, desiccator, electronic balance & muffle furnace.
- All chemicals and reagents used were of A.R. grade for the qualitative analysis of extracts.

Physico-Chemical Study

Methodology

In physical methods quantitative standards like total ash, acid insoluble ash, water-soluble ash, moisture content, extractives, foreign matter and pH are determined.^[4]

Preliminary Phyto-chemical screening

Procedures

Preliminary phytochemical tests: are used to detect the presence of various organic functional groups, which is the indicative of type of phytochemicals present in the plant. These tests indicate the presence of different class of constituents present in the extract. Tests were performed as per the methodology mentioned by Harborne JB, 1973 (Phytochemical Methods. Jackman H. (Ed.), London, p. 70.^[5] The following tests have been carried out for Acetone, Petroleum ether, Chloroform, Alcoholic and Aqueous extracts.

Tests for alkaloids

Dragendroff's test: To a few mg of extract dissolved in alcohol, a few drops of acetic acid and Dragendroff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

Wagners's test: To a few mg of extract dissolved in acetic acid, a few drops of Wagner's reagent was added. A reddish brown precipitate formed indicates the presence of alkaloids.

Mayer's test: To a few mg of extract dissolved in acetic acid, a few drops of Mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.

Hager's test: To a few mg of extract dissolved in acetic acid, 3 ml of Hager's reagent was added, the formation of yellow precipitate indicates the presence of alkaloids.

Tests for carbohydrates

Molisch's test: To the extract, 1 ml of α -naphthol solution and conc. sulphuric acid were added along the sides of test tube. Violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.

Fehling's test: A few mg of extract was mixed with equal quantities of Fehling's solution A and B. The mixture was warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.

Benedict's test: To 5 ml of Benedict's reagent, a few mg of extract was added, and boiled for two minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates.

Anthrone-sulphuric acid test: A few mg of the extract was mixed with equal quantity of anthrone and treated with two drops of conc. sulphuric acid. It was then heated gently on a water bath. Dark green colour formed indicates the presence of sugar/glycoside.

Test for steroids

Liebermann-Burchard test: To the extract dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and cooled. Few drops of conc. Sulphuric acid was added along the sides of the test tube. Appearance of bluish green colour indicates the presence of steroids.

Salkowski test: The extract was dissolved in chloroform and equal volume of conc. Sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

Test for saponins: To a few mg of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponins.

Test for tannins: To the extract, a few drops of dilute solution of ferric chloride was added, formation of dark blue colour shows the presence of tannins.

Test for flavonoids

Shinoda's test: To the extract in alcohol, a few magnesium turnings and few drops of conc. hydrochloric acid were added and heated on a water bath. Formation of red to pink colour indicates the presence of flavonoids.

Test for phenol

To the extract in alcohol, added two drops of alcoholic ferric chloride. Formation of blue to blue black indicates the presence of phenol.

Test for coumarins

To the extract in alcohol, a few drops of 2 N sodium hydroxide solution was added. Dark yellow colour formation indicates the presence of coumarins.

Test for triterpenoids

The extract was warmed with tin bits and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

Test for carboxylic acid

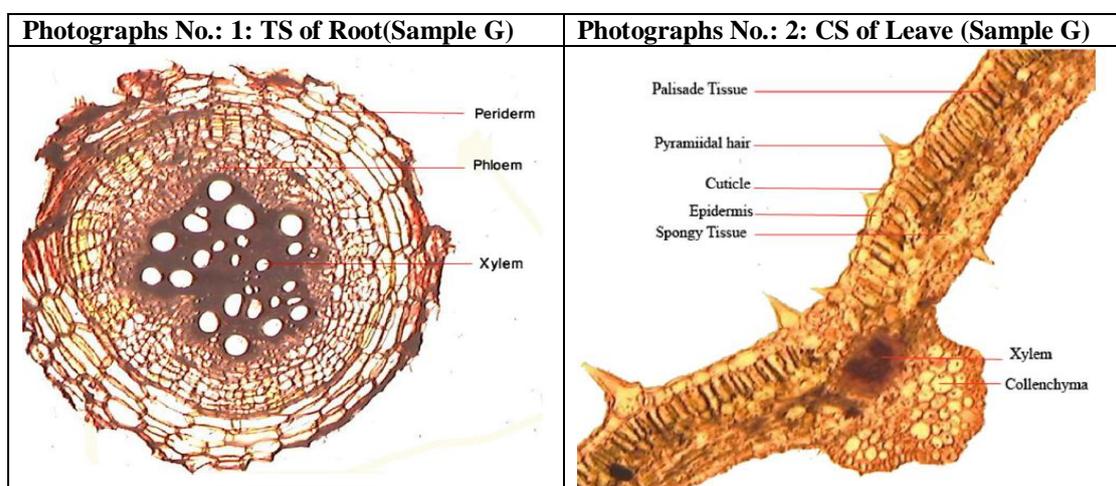
Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

Tests for aminoacids

Extract dissolved in alcohol treated with few drops of ninhydrin solution. Violet colour indicates the presence of amino acids.

OBSERVATION AND RESULTS**Organoleptic study****Table No. 1 Comparative macroscopic features of different market samples of *Manjistha*.**

Characters	Karnataka S1	Gujarat S2	Haryana S3	Assam S4	Sample G
Physical appearance	Roots and stems	Roots and stems	Roots and stems	Roots and stems	Roots
Colour	Dark Brown	Brown	Light Brown	Brown	Dark Brown
Odour	Distinct	Distinct	Distinct	Distinct	Distinct
Taste	Sweet	Sweet	Sweet	Sweet	Sweet
Size	6-10cm	5-8cm	6-10cm	3-6cm	5-8cm
Fracture	Short & Irregular	Short & Irregular	Short & Irregular	Short & Irregular	Short&Smooth

Table 2: Photographs of TS of Root and CS of Leave of *Rubia cordifolia*.^[6]**Identity, Purity and Strength****Table 3: Comparative physiochemical analysis of different market samples of *Manjistha*.**

Parameter	Karnataka S1	Gujarat S2	Haryana S3	Assam S4	Sample G
Total ash value	6.95	11.5	15.5	9.51	13.7
Acid insoluble ash	0.91	2.29	1.25	1.46	2.15
Water soluble ash	3.75	6.35	6.17	5.02	6.49
Alcohol soluble extractive	3.36	1.03	2.5	4.24	1.45
Water soluble extractive	2.53	3.54	4.60	3.17	3.71
Ether soluble extractive	0.28	0.25	0.07	0.21	0.10
Moisture Content	2.94	2.21	3.39	1.83	1.53
Foreign matter	NIL	0.4%	0.61%	0.28%	NIL
Ph	4.11	4.2	3.97	3.67	4.48
Volatile oil	Nil	Nil	Nil	Nil	Nil

Phytochemical Analysis of Manjistha

Table 4: Phytochemical analysis of Aqueous extract of different market samples of Manjistha.

Parameters	Tests	S1	S2	S3	S4	Sample G
Reducing sugars	Fehlings test	+ve	+ve	+ve	+ve	+ve
	Benedict's test	+ve	+ve	+ve	+ve	+ve
Alkaloids	Hager's test	-ve	-ve	-ve	-ve	-ve
Sterols	Salkowski reaction	-ve	-ve	-ve	-ve	-ve
Tannins	Ferric chloride Test	+ve	+ve	+ve	+ve	+ve
Glycosides	Borntrager's test	-ve	-ve	-ve	-ve	-ve
Amino acids	Ninhydrin Test	-ve	-ve	-ve	-ve	-ve
Saponnins	Iodine Test	-ve	-ve	-ve	-ve	+ve
Phenols	Ferric chloride Test	+ve	+ve	+ve	+ve	+ve
Proteins	Biuret test	-ve	+ve	+ve	-ve	+ve

Inorganic Constituents

Table 5: Showing the results of the tests for Inorganic constituents.

Constituents	Haryana S1	Gujarat S2	Karnataka S3	Assam S4	Sample G
Calcium	+	+	+	+	+
Magnesium	+	+	+	+	+
Phosphate	-	-	-	-	-
Sulphate	=	-	-	-	=
Carbonate	=	-	=	=	-
Sodium	+	+	+	+	+
Iron	+	+	+	+	+

Table No 6. Showing HPTLC results of alcoholic extracts of different samples.

At 254 nm

Visualised Under	Karnataka S1	Gujarat S2	Haryana S3	Assam S4	Sample G
At 254 nm	10 spots are found at the Rf of 0.20,0.25,0.36, 0.41,0.45,0.52, 0.67,0.84,0.90, 0.95	11 spots are found at the Rf of 0.11,0.19,0.20, 0.27,0.31,0.39, 0.44,0.66,0.82, 0.89,0.95	10spots are found at the Rf of 0.13,0.21,0.27, 0.39,0.47,0.55, 0.69,0.85,0.90, 0.94	10 spots are Found at the Rf of 0.19,0.24, 0.30,0.33, 0.43,0.49, 0.64,0.82, 0.89,0.95	12 spots are found at the Rf of 0.12,0.21, 0.27,0.35, 0.41,0.44, 0.57,0.70, 0.83,0.86, 0.91,0.96

DISCUSSION

In the present study, four different market samples were collected from different parts of India. Assam, Haryana, Gujarat and Karnataka and the original sample was collected personally from Madikeri after proper identification. *Manjistha* is mainly found in hilly areas. In API, standards of roots are not available, only stem standards are given, so all the market samples are compared with genuine sample. All the five samples were tested by the modern procedures like Macroscopic study, Microscopic study, Physicochemical tests, Phytochemical tests and Chromatographic study.

High performance thin layer chromatography

At 254 nm (Table No. 6)

- In alcoholic extracts of sample-G and 1(Karnataka), 12 and 10 constituents were separated respectively, in that 1 same constituent is observed at Rf 0.41.

- In sample-G and 2(Gujarat), 12 and 11 constituents were separated respectively, in that 2 same constituents are observed at Rf 0.27 and 0.44
- In sample-G and 3(Haryana), 12 and 10 constituents were separated respectively, in that 2 same constituents are observed at Rf 0.21 and 0.27.
- In sample-G and 4(Assam), 12 and 10 constituents were separated respectively, in that no any same constituent is observed.

At 366nm

- In alcoholic extracts of sample-G and 1(Karnataka), 8 and 11 constituents were separated respectively, in that 1 same constituent is observed at Rf 0.20
- In sample -G and 2(Gujarat), 8 and 11 constituents were separated respectively, in that 3 same constituents are observed at Rf 0.20,0.27,0.50.

- In sample-G and 3(Haryana), 8 and 10 constituents were separated respectively, in that 2 same constituents are observed at Rf 0.20, and 0.85.
- In sample-G and 4(Assam), both were having same number of Rf spots ie.8, but no same constituent is observed.
- All these findings of H.P.T.L.C. the sample 2(Gujarat) is nearer to genuine sample.

CONCLUSION

- The *Manjistha* is found in *Vedas*, *Puranas* and *Samhitas* and it has been used in various medicinal preparations. From ancient times, it is used mainly as natural coloring agent in the fabrics industry. *Manjistha* has *madhur*, *tikta* and *kashaya rasa*, *Guru guna*, *Ushna veerya* and *Katu vipaka*. Wide range of therapeutic utility of *Manjistha* has been mentioned in the classics and it is mainly indicated in *rakta vikaras*, *varna vikaras* etc. Foreign matter was observed nil in the genuine, Karnataka and Gujarat sample. In sample 3(Haryana) and Sample 4(Assam), unwanted substances like stones, dust were seen. In Physico-chemical analysis, In case of market samples, only the Sample-2(Gujarat) complies with Genuine sample standards. The results of Pharmacognostical studies of sample 4 (Assam) shows it's variance with the genuine sample. Further study should be conducted on this behalf. Reducing sugars, proteins, Sterols, Tannins, Glycosides and anthraquinones are present in equal amount in all the samples in all the extractions. All H.P.T.L.C results of market samples compared with genuine, only sample2 (Gujarat) shows maximum similarity in constituents.
- From the results of pharmacognostic and analytical study of four market samples of *Manjistha* its can be graded as

Sample 2 Gujarat	}	- Grade A
Sample 3 Haryana		- Grade B
Sample 1 Karnataka		- Grade C
Sample 4 Assam		

Scope for further study

- Detailed Pharmacognostic and Physico-chemical study of market samples should be compared with different species of *Rubia*.
- All available species of *Manjistha* should be tested clinically.

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