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Pharmacognostic studies on Identity of Manjishtha [Rubiacordifolia Linn.]- An Ayurvedic plant.

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ABSTRACT : *Manjishtha [Rubia cordifolia Linn] from Rubiaceae family is a useful medicinal plant used in the treatment of shotha (inflammation), udar, amavat, skin disorder. In market Deshi- indigenous and Irani manjishtha is available. This study is aimed at assessing the scientific evaluation of deshi and irani Rubia cordifolia in the course of pharmacognostical and phytochemical analysis, which mainly covered the macroscopic and microscopic features of the roots and stem and Phytochemical parameters such as pH, total ash value, water-soluble extract values were assessed in the preliminary physicochemical screening. Thin layer chromatography (TLC) and fluorescence analysis were carried out for the separation of components.*

KEY WORDS- *pharmacognosy, phytochemistry, TLC , Rubia cordifolia, Rubia tintorum*

I. INTRODUCTION

Manjishtha is one of the most commonly used plants in Ayurvedic pharmacopeia for skin and other mainly inflammatory diseases like odema- *sotha*, rheumatism- *amavata*, acites- *udar* and such others [1]. Its anti inflammatory activity and clinical use in Eczema are established by one of the authors [2] [3]. *Rubia cordifolia L. [Rubiaceae family]* is normally considered as Manjishtha. This is a is a very variable, scandent, perennial creeper or climber, upto 10 meters long found throughout India in hilly districts climbing on bushes or creeping at the edges of forests and along pathways ascending to an altitude of 3750 ft. to 8000 [4] [5]. It is major ingredient of several Ayurvedic formulations like Manjishthadi Kwath, an aqueous decoction, Manjishthadi Churna, a powder and Manjishthadi Malahar, an ointment [6]. It was found that Manjishtha offered in Mumbai market were of two types, one called *deshi*- indigenous [*Rubia cordifolia L*.] and other *Irani* claimed to be of Iranian origin [Rubia tinctorium]. However, on further inquiry it was found that there was no actual import from Iran but mostly collected from north- western regions of India. It was therefore considered appropriate to undertake pharamcognostic studies on this plant to establish its identity.

II. MATERIAL AND METHODS

2.1 Plant materials-

The plant being available at high attitude the taxonomically identified authentic plants and plant parts of Rubia cordifolia, Linn were collected over visits from forest areas of Mahabaleshwar to cover seasonal variations including flowering and fruiting times for confirmed taxonomical identification. [Figure 1, 2]
The market samples of both, claimed to *deshi*- ingenuous variety and Iranian variety were collected from local market and other sources.

2.2 Instrumentation and techniques-

Detailed macroscopic on different samples of *Manjishtha* were undertaken and documented. [7]

2.3 Macroscopic study-

The macroscopic of different samples were studied as per standard procedures. All the samples were studied carefully whenever necessary with a hand lens or a dissecting microscope. Observations giving full descriptions and identifying characters were recorded. Characters such as size, shape, colour, odour, taste, external markings, fracture, etc. were noted. [8]

2.4 Microscopic study

Roots were processed as per the standard procedures for histological examinations, and microscopic characters were drawn with prism type of camera lucida. Resistant tissues such as vessels and fibers in roots, stems and market samples were studied by using macerated material. Microscopic examination was conducted by mounting the macerated material in Farrant's medium.

2.5 Physico-chemical studies-

The physico-chemical parameters such as Moisture contain and extractive values (water-soluble alcohol soluble) were determined. These parameters were analyzed in accordance with the Ayurvedic Pharmacopeia of India.[9]

2.6 Preliminary qualitative tests

The extracts were analyzed for the presence sugars, carbohydrates, tannins, steroids, flavonoids and saponins using standard protocol.

2.7 Chromatographic study

Thin layer chromatography [TLC] of aqueous extract was carried outfor the normal phase separation of components [10].

2.8 Fluorescence analysis

Fluorescence analysis of extracts was carried out as per procedure given by Chase, C. R. Jr. and Pratt, R.J. Rideley A.A. and Grant, J and Randley, J.A.[11]

III. OBSERVATION AND DISCUSSION

3.1 Morphological study

The morphological characteristics of the Rubia cordifolia Linn – Authentic sample, market sample Indian and *Irani* are shown in [Table 1].

Table– 1Morphological characteristics of crude drug of *Rubia cordifolia Linn*.-

Observations	**Authentic sample from Mahabaleshwar**	**Market sample (Indian Origin)**	**Market sample (Iranian origin)**
Physical state	Solid	solid	Solid
Condition	Freshly dried whole as well as un peeled	Dried cut few partly peeled	Dried cut un peeled
Morphological nature	Roots and stems/stolons in equal quantity when separated	Roots and stems /stolons mixed 25:75	Roots and stems/stolons mixed 75:25
Origin	Roots and stems of naturally growing plants collected personally from Mahabaleshwar	Unknown; said to have been originated from North East India	Unknown; said to have been imported from Iran
Size and shape	Cut into cylindrical pieces as required	Shape cylindrical and straight size variable usually the pieces are from 2" to 6" in length	Shape cylindrical and twisted length variable from 1" to 4"
Colour	Purple brown roots Bright purple brown	Purple brown	Dusty brown roots, Dark brown
Odour	Characteristic	Characteristic	Characteristic and little pleasant
Surface characters	Surface longitudinally furrowed scars present in few	Surface longitudinally furrowed	Surface longitudinally deeply grooved
Texture and fracture	Brittle, hard and splintery roots hard to break. External bark gets peeled off easily	Brittle, hard splintery roots, hard to break. External bark gets peeled easily	Flexible, soft fibrous. Roots very hard. It is difficult to peels off the outer bark.
Inner surface	Bright purple furrowed	Bright purple furrowed	Bright orange brown deeply furrowed
Taste	Bitter and astringent	Bitter and astringent	Bitter and astringent
Solubility in water	purplish colour	purplish colour	Orange red

3.2 Microscopy section of Stem-

***3.2.1 Rubiacordifolia Linn*. Self collected sample- [Figure 3]**

The transverse section of the stem is circular and smooth in outline. The periderm is well developed with 4 to 11 layers of thin walled, compactly arranged, rectangularcells. The periderm is followed by 8 to 12 layers of flattened compactly arranged parenchymatous cells. The size of the cells varies from15 x 35 to 35 x 105. This layer is comparatively thin and in some sections xylem vessels are also seen. The cortex cells are longitudinally flattened, angular in outline and have intercellular spaces. This differs from Indian market stem in not havingthick walled and much compactly arranged cells. Primary xylem is present towards the center characterized by small vessels with xylem parenchyma cells. Perimedullary sheath is narrow and made up of round or irregular thin-walled cells.The secondary xylem forms a complete cylinder and is diffused porous. The xylem vessels are uniformly distributed with larger vessels towards the periphery and smaller towards the inner boundary. The medullary rays are unisereate. Central cylinder is represented by pith which is much smaller in comparison to the stellar region. The pith when present consists of irregular, thin-walled, isodiametric, small parenchymatous cells which are compactly arranged without intercellular spaces. Raphide crystals are present in the pith cells.

3.2.2 Rubiacordifolia Linn-Manjishtha- Iranian Variety [Figure 5]

A transverse section of the stem of the Iranian market sample is circular in outline with deep indentations. The larger sectionsare lobed in character, divided into five lobes. The lobes aredivided by layer of much pigmented cells running from periphery to the center.Regular periderm is absent. The outermost region consists of 5to 12 layers of thin-walled radically flattened irregularly shaped compactly arranged suberised cells. In some sections it is absent or one celled. Periderm is followed by 15 to 25 layers of compactly arranged parenchymatous cells without intercellular spaces with xylem vessels in between. Secondary phloem is poorly developed and narrow. Phloem cells vary in size from 30 x 45 to 75 x 120. Cortex is well presented by compactly arranged 6 to 18 layers of suberised cells and it consists of xylem vessels and parenchyma. Secondary xylem is divided into wedge shaped masses by broad rays of inter fascicular parenchyma and weak growth rings. In some sections there are alternate circular bands of light and dark colors. The xylem vessels are numerous, smaller in diameter and grouped. Larger vessels are found towards the periphery and smaller towards the center. Medullary rays are uniseriate. Thin walled fibers are present. Pith in the center is equal to or more than the stealer region. It is hollowed in the center or represented by marginal paramedulary sheath of thin-walled cells.

3.2.3 Rubiacordifolia Linn Manjishtha - Indian Market Sample [Figure 7]

A transverse section of the stem of Indian market sample is circular and smooth inoutline. The periderm is well developed with 2 to 15 layers of thin-walled square or rectangularcompactly arranged cells. Phloem parenchyma region with 40 to 30 layers, the cells are rectangular, sometimes spindle shaped, very compactly arranged. The cortex is 12 to 80 layered of longitudinally stressed, flattened parenchymatous cells.
The secondary phloem is well developed, made up of 6-7 layers. The secondary xylem forms a complete cylinder. It is diffused porouswith numerousscattered, round and uniformly distributed vessels. The medullar rays are unisereate, parenchymatous and apotracheal. The growth rings are not much distinct. Primary xylem ispresent towards the center region with small vessels and xylem parachyma cells. Pith in the center is much smaller in comparison to the stellar region. The cells are compactly arranged without intercellular spaces. Raphide crystals are present in the pith cell.

3.3 Microscopic study of Root

***3.3.1 Rubiacordifolia Linn*. Self collected sample [Figure 4]**

The transverse section of the root of *RubiacordifoliaLinn* is circular but wavy in outline and without any deep indentations. The periderm consists of 2 to 10 layers of thin walled rectangular or squares cells varies from 35 x 45.4 to 50 x 90.4. which are irregular in outline. Thick narrow cortex of l to 8 layers consisting of compactly arranged radically flattened, oval or angular in shape, irregular in outline, suppressed parenchymatous cells and spindle shaped cells in between parenchymatous cells are found. The size of the cortical cells varies from 8 x 35 to 17 x 120 contain clusters of rod- shaped structures resemblingmycorrhiza. The cortex is characterized by the presence of acicular raphides. The Phloem is made up of 2 to 3 layers of thin walled parenchyma cells. The phellogen consists of 1 to 3 layers of cork cambium cells which are rectangular and thick-walled. The wood region consists of secondary xylem which forms thecentral cylinder. Wood parenchyma cells are apotrachealand consisted of small compactly arranged cells. The size of the xylem parenchyma cells varies from 12 x 18 to 100 x 114.

3.3.2 Rubia cordifolia Linn-Manjishtha- Iranian Variety [Figure 6]

The transverse section of the root of the Iranian market sample is more or less circularin outline with numerous pitslike indentations. The outermost covering is much dark in colour and indistinct. The typical periderm is absent. In younger sections it is present in the form of 2 to 5 layers of compactly arranged thick-walled, slightly lobed subseries cells. Mature sections have 3 to 9 layers of irregularly outlined rectangular, pigmented cells. The cells vary in size from 8 x 18 to 17 x 85. The wood region consists of parenchymatous cells which is generally paratracheal and smaller in amount. The primary xylem in the center is generally tetrarch and secondary xylem vessels are well - developed uniformly scattered, numerous, variable in size and with larger vessels towards the center.Large and smaller xylem vessels are found distributed alternately in circular rows. Medullary rays are uniseriateand Growth rings are much conspicuous. The central cylinder in younger sections consists of rectangular parenchymatouscells with scatter xylem vessels. In mature sections, the central cylinder has small xylem vessels, which are compactly arranged in the centre.

3.3.3 Rubiacordifolia Linn Manjishtha - Indian Market Sample [Figure 8]

The transverse section of the root of the Indian Market Sample is more or less circular in outline without any deep indentations. The diagnosticmicroscopically characters as seen from periphery tothe centre can be distinguished as the periderm consisting of cork/phloem and phellogen cells followed by phrllodermand the vascular tissues. The periderm consists of 2 to 9 layers of thin-walled ruptured parenchyma, either round, squares or rectangular the cells. The cork is comparativelynarrower than in the Iranian variety. The phellogen consists of 1 to 3 layers of cork cambium cells which are thick-walled and rectangular. Inner to the periderm is the cortex which is entirely parenchymatous, narrow and of 2 to 12 layers. The cells are radiallyflattened, spindle shaped or angular and compactly arranged. Some of the cortical cells contain clusters of rod shaped structures. The cortex also contains bundles of asciculararaphides. The primary phloem is crushed while the secondary phloem is muchless and is found nearer to the inner cortical boundry. The phloem cells size varies from 17 x 35 to 30 x 95. Bark fibres are absent in this region. The outermost layer of vessels is slightly smaller but otherwise the vessels from periphery to centre are larger tosmaller in size. The thickening is bordered pitted. The medullar rays are present in wood region which are not distinct and are not continuous.

3.4 Phyto chemical analysis

In physical evaluation, moisture content, and extractive values viz., water, alcohol, ether, acetone and benzene soluble extractive values were determined.

Table 2: Physico-chemical parameters of *Rubia Cordifolia Linn*

Parameters	**Indian market**	**Irani market**	**Rubiacordifolia root**
Moisture content	11.2773 %	15.320	15.406
Water Extractive values	2.2975 wt	3.4560	1.8550
Alcohol Extractive values	1.1585	2.4425	1.0055
Ether Extractive values	0.6340	0.8295	0.610
Acetone Extractive values	0.434	0.5575	0.696
Chlorophorm Extractive values	0.316	0.4025	0.5625
Benzene Extractive values	0.3915	0.8225	0.4905

Table 3: Qualitative analysis of Alcohol extract of *Rubia cordifolia*

Chemical constituents	**Indian market**	**Irani market**	**Rubia cordifolia root**
Alkaloids	Absent	Absent	Absent
Glycosides	Present	Present	Present
Volatile oils	Absent	Absent	Absent
Flavanoids	Absent	Present	Absent
Phenols	Absent	Absent	Absent
Proteins	Absent	Absent	Absent
Quinines	Absent	Absent	Absent
Reducing Sugar	Absent	Absent	Absent
Saponins	Present	Present	Present
Anthraquinone	Present	Present	Present

3.5 Thin Layer Chromatography-

The TLC studies of root shows that alizarin is more than purpurin in Rubia cordifolia self collected and Irani sample, while Alizarin is slightly less than that of purpurin in market sample

3.6 Fluorescence Analysis:

Table 4 Observation under Ultra Violet Light

Samples	Powder	Alcoholic	Aqueous	Benzene	Chloroform	Ether
Indian	Orange	yellow	Dark green	Dark brown	Fern Green	Purple
Iranian	Brown	orange	Dark green	yellow	Dark green	Purple
Self collected	yellow	Green	Dark green	orange	Green	Purple

IV DISCUSSION

Irani and Indian roots of Manjishtha has been sold in crude drug markets and also use in many Ayurvedic and Siddha formulations. The authentic and market samples were analyzed for pharamacognostical evaluation. Under the name of **Manjishtha,** even though, all the Ayurvedic Texts, mention *R. cordifolia L. (Rubiaceae),* as an official drug, samples collected from market were authenticated as of *R. tinctorum L. (Rubiaceae),* which is usually sold in the Indian market under the name of **Irani Majith.** *R. cordifolia* roots known as **Deshi Majith** are not available in sufficient quantity, to meet the commercial demand of the Indian market, *R .tinctorum* is used under the name of *R. cordifolia.* The most remarkable morphological characters of *R. cordifolia* which distinguishes it from *R. tinctorum,* is its enlarged crown, dark reddish colour and the comparatively smooth surface of the root and stem pieces. Similarly, the bigger size of the xylem vessels and the greater area occupied by the xylem region, forms an important histological characters to distinguish *R. cordifolia* from *R. tinctorum.* Histological, the root and stems of the Indian and Iranian samples differ with following characters-

Table 5: Differences between the transverse sections the roots of Indian and Iranian Manjishtha

Indian	Iranian
Outline smooth	Outline wavy
Periderm of thin walled cells	Periderm of thick walled cells (In larger sections it is absent but presented by 5-8 layers of cells).
Cortex is narrow	Cortex is broader
Cortical cells regular in outline	Cortical cells sinuate in outline.
Mycorrhizal contents found in some sections	Mycorrhizal contents totally lacking
Raphides present.	Raphides not seen.
Distribution of vessels is not uniform. Larger outside and smaller inside	Vessels distributed uniformly.
Wood parenchyma apotracheal	Wood parenchyma paratracheal

Table 6: Differences between the transverse sections the stems of Indian and Iranian Manjishtha

Indian	Iranian
Outline circular	Outline with deep indentations.
Periderm well developed	Regular periderm absent
Cortex is narrow	Cortex well developed with compactly arranged cells.
Inter-fascicular parenchyma absent	Inter-fascicular parenchymay present
Secondary phloem well developed	Secondary phloem poorly developed
Secondary xylem forming a cylinder	Secondary xylem divided into a wedge shaped manner
Pith is narrow (small)	Pith is large

In moisture content, the drug seems to absorb and retain a high percentage of moisture and is therefore subject to mould attack. It is therefore necessary to store it in a very dry place where the percentage if moisture is less than 5%. In extractive value the difference was seen in the extractive values of alcohol and benzene. Phytochemically, the two species differ in the present of **flavonoids** in *R. tinctorum* only.
The TLC profile remain same in self collected and Irani manjishtha sample where slightly difference seen in Indian market sample.

In fluorescence analysis Powder, Alcoholic, benzene and chloroform extracts showed different color pattern under UV light while Aqueous and ether extracts showed similar color pattern.

V. CONCLUSION

Detailed macro-microscopic characters and phytochemical analysis were laid down for Rubia cordifolia roots and stems which will serve as diagnostic tools for its Identification.

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FIGURES

Figure 1. Rubia cordifolia plant

Figure 2. Rubia tinctorum plant

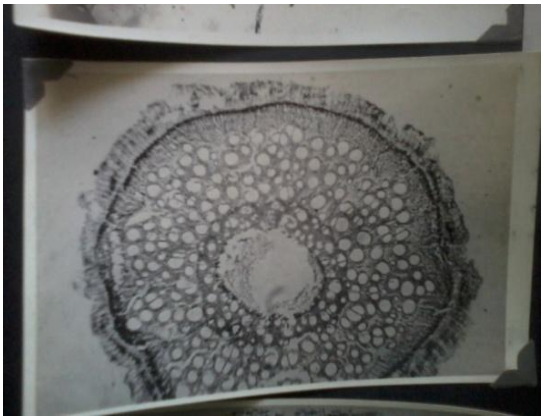

Figure 3. Microscopic of Self collected stem

Figure 4. Microscopic of Self collected root

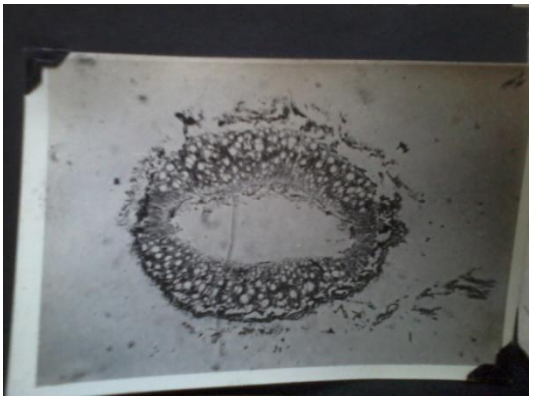

Figure 5. Microscopic of Irani stem

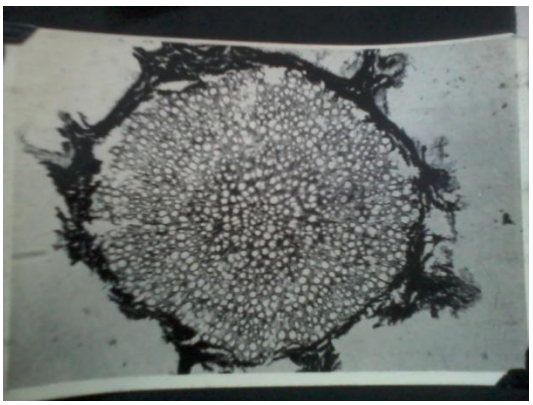

Figure 6. Microscopic of Irani root

Figure 7. Microscopic of Indian market sample stem sampleroot

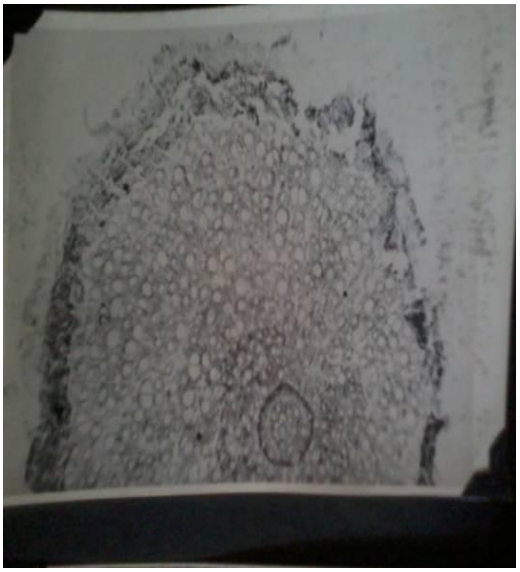

Figure 8. Microscopic of Indian market