

## Quality control parameters to detect adulteration of market samples of Indian *Manjistha* by Irani *Manjistha*

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The stems of *Manjistha* are official in Ayurvedic Pharmacopoeia of India and equated with *Rubia cordifolia* L. They are traditionally used for curing blood disorders, skin diseases, as anti-inflammatory, haemostatic, urolithotriptic, antipyretic, analgesic, anthelmintic and for improving complexion. They are found in several important traditional medicinal formulations and hold a great export potential as well. However, stems of *Rubia tinctorum* L. are also available in the Indian market, which are not official and have been found to contain lucidin, a genotoxic compound. The present investigation deals with comparative pharmacognostical study of stems of both species and establishment of various quality parameters. Morphologically, the stems of both the species vary a lot. Transverse section of both stems showed the presence of reddish dye in cortex and acicular crystals of calcium oxalate; however, the width of cork, cortex, stele and pith varied. Powder microscopy revealed the presence of annular and pitted xylem vessels, xylem fibers and acicular crystals in singular and bundles; however their sizes varied in both species. From the phytochemical screening, the detected phyto-constituents were estimated by their respective procedures, which also varied in both species. Thus, the present work can serve as a very useful botanical and phytochemical tool in the identification, authentication and standardization of the raw material and prepared formulations of *R. cordifolia* L. and distinguishing it from of *R. tinctorum* L., which will consequently benefit the herbal industries and traditional medicinal healers.

**Keywords:** Blood disorders, *Manjistha*, *Rubia cordifolia* L., *Rubia tinctorum* L., Skin diseases, Traditional medicine

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*Manjistha* (Indian *Manjistha*, *Desi Majith*, Indian Madder) is official in Ayurvedic Pharmacopoeia of India and equated with *Rubia cordifolia* L. (Family Rubiaceae). It is traditionally used in blood disorders, skin diseases, inflammation, kidney stones, fever, pain, improving skin complexion and for curing *Kapha* disorders. *Dashmoolarishta*, *Ashwagandharishta*, *Khadiradi Gutika*, *Irimedadi taila*, *Manjisthadi taila* and *Manjisthadi Kwatha* are some of its important Indian traditional medicinal formulations<sup>1,2</sup>. However, *Rubia tinctorum* L. (*Alizari*, *Irani Majith*, European Madder)<sup>3</sup> is also available in the market but not official in Ayurvedic Pharmacopoeia of India. It has also been found to contain lucidin, which is known for its genotoxicity<sup>4</sup>. This makes a detailed study of the two species necessary. The present investigation deals with comparative study of stems of both species and establishment of various qualitative and quantitative parameters, so that adulteration in raw

material and finished preparations of *Manjistha* can be detected.

### Methodology

Dried stems of *R. cordifolia* L. and *R. tinctorum* L. were purchased from Yucca (Mumbai) and voucher specimens [RK/COG/2012/RC] and [RK/COG/2012/RT] of both the respective species were deposited in the college laboratory. The stems were compared morphologically and used for transverse section study. The stems of *R. cordifolia* are slender, cylindrical, slightly flattened, smooth with no distinct striations, wiry, upto 0.3 cm thick, light reddish brown with distinct nodes possibly having scaly leaves. The stems of *R. tinctorum* are stout, cylindrical, rough with distinct longitudinal striations, upto 0.8 cm thick, dark reddish-brown with no distinct nodes or scaly leaves (Figs. 1 & 2)<sup>5,6</sup>.

The stems were powdered, stored in airtight containers and used for quantitative microscopy and phytochemical studies. For microscopical studies,

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Fig. 1—*Rubia tinctorum* (Right) and *Rubia cordifolia* (Left).

safranin was used for staining. Photomicrography of the transverse sections and the powdered drug was performed using camera. Quantitative microscopic study was performed using camera lucida and stage micrometer slide (Table 1)<sup>7</sup>.

Phytochemical screening of both the species was performed using the appropriate extract (aqueous and alcoholic) and a battery of chemical tests (Table 2)<sup>8-12</sup>. Anthraquinone glycosides<sup>13</sup> and tannins present in both the samples were also estimated (Table 3)<sup>14</sup>.

Thin layer chromatography of alcoholic extract of the two samples was performed on pre-coated silica gel G 60 plates (Merck) using the mobile phase n-Butanol: Acetic acid: Water (4:1:5). The developed plates were observed in natural light, UV (366nm) and derivatized using iodine vapors as well as 5% methanol-sulfuric acid reagent followed by heating at 110°C for 10 min<sup>15</sup>.

## Results

### Microscopy: Transverse Section

Stems of both species show exfoliating cork forming dome shaped structure, consisting of squarish and tangentially elongated, thin walled cells, though the cork of *R. cordifolia* is wider. Cortex consists of tangentially elongated, thin walled cells, some of which contain acicular calcium oxalate crystals as isolated or in bundles, which are present more in *R. cordifolia*. Secondary phloem is wider in *R. tinctorum* and has cells filled with reddish color, composed of sieve tubes and phloem parenchyma. Secondary xylem is wider in *R. cordifolia* and consists of xylem vessels, xylem fibres and pitted xylem parenchyma. Pith is wider in *R. tinctorum* and lies in the centre consisting of thin walled, parenchymatous cells, having microsphenoidal crystals of calcium oxalate (Figs. 3-5).



Fig. 2—Stems of: *Rubia tinctorum* (Left) and *Rubia cordifolia* (Right).

Table 1—Quantitative microscopy

Number of observations = 25

Parameters	Measured value (µm)	
	<i>Rubia cordifolia</i> L.	<i>Rubia tinctorum</i> L.
Length of xylem fibers	92.5	105
Length of xylem vessels	132.5	115
Length of acicular crystals	3.2	3.9

Table 2—Phytochemical screening

Phytoconstituent	Test	Results	
		<i>Rubia cordifolia</i>	<i>Rubia tinctorum</i>
Alkaloids	Dragendorff's test	-ve	-ve
	Wagner's test	-ve	-ve
	Mayer's test	-ve	-ve
	Hager's test	-ve	-ve
Flavonoids	Shinoda test	-ve	-ve
	Lead acetate test	-ve	-ve
Sterols	Salkowski test	+ve	+ve
	Liebermann	+ve	+ve
	Buchardt test	+ve	+ve
	Legal's test	+ve	+ve
Cardiac glycosides	Baljet test	+ve	+ve
	Keller Killiani test	-ve	-ve
Anthraquinone glycosides	Modified	+ve	+ve
	Borntrager test		
Saponins	Foam test	-ve	-ve
Tannins	Ferric chloride test	+ve	+ve
Phenolics	Folin ciocalteu test	+ve	+ve
Sugars	Fehling's test	-ve	-ve
	Molisch test	+ve	+ve
Gums	Ruthenium red test	-ve	-ve

Table 3—Estimation of phytoconstituents

PHYTOCONSTITUENTS	% w/w± SD	
	<i>Rubia cordifolia</i>	<i>Rubia tinctorum</i>
Anthraquinone glycosides	4.02±0.6	2.58±0.4
Tannins	20±0.7	25±0.8
Number of observations = 3		

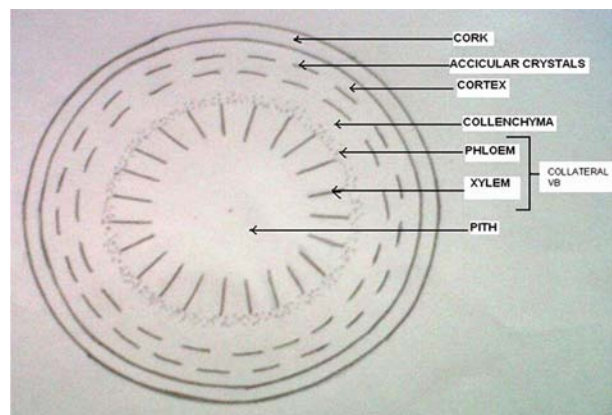
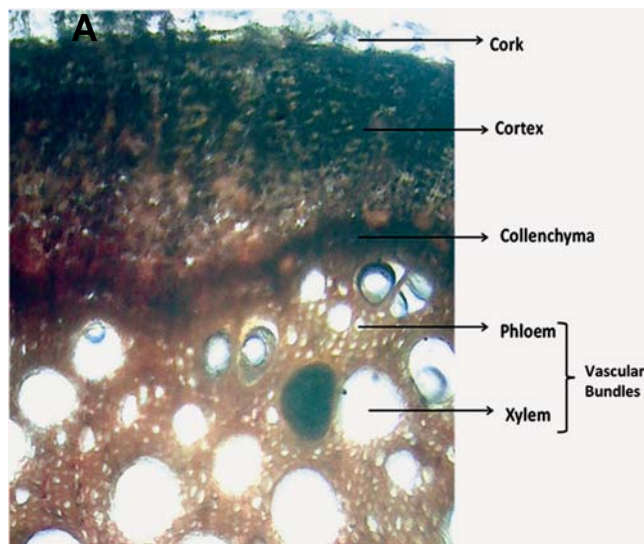
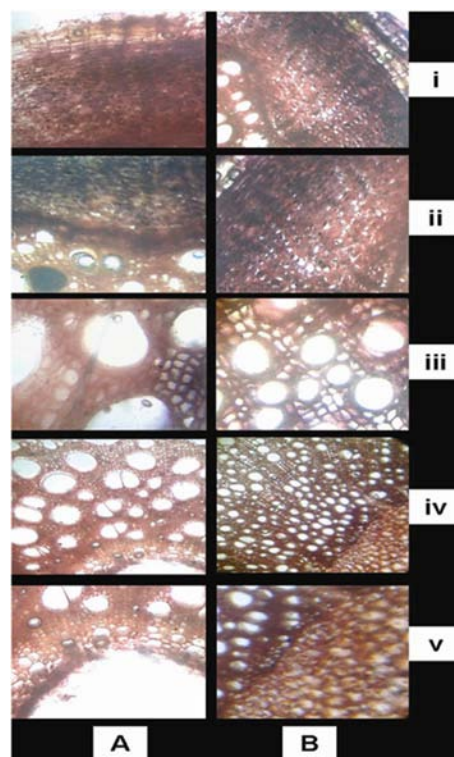
Fig. 3—General Schematic diagram of *Rubia cordifolia* and *Rubia tinctorum*

Fig. 4—T S of both stems (x450)

### Microscopy: Powder characteristics

*Rubia cordifolia* is a pinkish brown colored powder with no distinct odor or taste whereas *Rubia tinctorum* is a brownish colored powder with slightly sweet taste. Diagnostic features of both the powders are pitted xylem parenchyma, annular and pitted xylem vessels, microsphenoidal and prismatic calcium oxalate crystals, acicular calcium oxalate crystals both

Fig. 5—Enlarged portions of detailed T. S. of (A) *Rubia cordifolia* and (B) *Rubia tinctorum* (x450) i: Cork; ii: Cortex; iii: Collenchyma; iv: Collateral vascular bundles; v: Pith

isolated and in bundles, starch grains and cork in surface view (Fig. 6).

### Thin Layer Chromatography

TLC of the alcoholic extracts of both species using n-Butanol: Acetic acid: Water (4:1:5) shows in natural sunlight two spots at  $R_f$  0.9 and 0.95. In UV light (366 nm), TLC of extracts of both species show two fluorescent spots at  $R_f$  0.9 (grey) and 0.95 (pinkish red). After exposure to Iodine vapors, the plates of extracts of both species show yellowish spots at  $R_f$  0.25, 0.35, 0.5, 0.7, 0.9 and 0.95. Upon spraying methanolic sulphuric acid and heating at 110°C, spots were observed at  $R_f$  0.3 (dark grey), 0.4 (dark grey), 0.5 (dark grey), 0.7 (light grey), 0.9 (light grey) and 0.95 (bluish).

### Discussion

A detailed comparative pharmacognostic study of stem of *Rubia cordifolia* and *Rubia tinctorum* has been performed. Morphologically, the stems of both the species show many variations. *R. tinctorum* is thicker, dark reddish brown, rough and has no nodes, whereas, *R. cordifolia* is thin, light reddish brown,



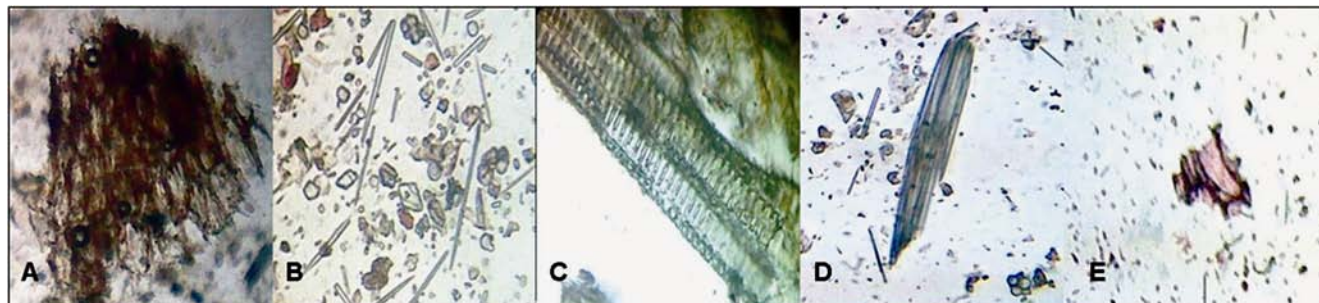


Fig. 6—Powder study (x450) of both species A: Cork cells in surface view; B: Microsphenoidal, prismatic and acicular calcium oxalate crystals along with starch grains; C: Annular xylem vessels; D: Bundle of acicular crystals; E: Pitted xylem parenchyma

smooth and may have nodes. Transverse section of both stems showed variations in the width of cork, cortex, stele and pith. Powder microscopy revealed the presence of annular and pitted xylem vessels, xylem fibers and acicular crystals in singular and bundles; however their sizes varied in both species. After performing preliminary phytochemical screening, some of the detected phytoconstituents like anthraquinones and tannins were estimated by their respective procedures, which also varied in both species. However, TLC profiles remained same for both species, thus making morphological and microscopic study most necessary for distinguishing the two species.

This study would be useful for researchers in distinguishing the two species and serve as a guide for further phytochemical and pharmacological explorations. Moreover, it serves as a gauge for herbal industries and traditional healers to use the correct species for *Manjistha* formulations which are widely consumed in the society.

### Conclusion

It is advisable to purchase *Manjistha* (*R. cordifolia*) in the form of entire crude drug and not in the form of powder directly owing to striking differences in morphology and transverse section of the stems of both species. The present work can serve as a very useful phytopharmacognostical tool in the identification, authentication and standardization of the raw material and prepared formulations of *R. cordifolia* and distinguishing it from *R. tinctorum* (which should be avoided as it contains a genotoxic compound), which will prove to be

crucial for quality control in herbal industries and for traditional medicine practitioners, and ultimately benefit the patients.

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