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COMPARATIVE PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES OF DIFFERENT SAMPLES OF MANJISHTHA (RUBIACORDIFOLIA LINN AND RUBIA TICTORUM LINN)

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ABSTRACT

Rubia cordifolia and Rubia tinctorum (Rubiaceae) known as, Manjishtha, Indian madder are most commonly used plants in traditional system of medicine.The plant contains substantial amounts of anthraquinones, triterpenoids especially in the roots which is responsible for nti-tumor, anti-inflammatory, urinary disorders, anti-stress antimicrobial, hepatoprotective, radio protective, anticancer, antimicrobial, antifungal, hypotensive, analgesic, antimalarial, antioxidant, antileukemic & mutagenic functions, immunomodulatory, and antioxidant activity. This study is aimed at assessing the scientific evaluation of four market samples of Manjishtha for the study of pharmacognostical and physiochemical analysis, which covered the macroscopic and microscopic features and Phytochemical parameters such as pH, total ash value, water-soluble extract values were assessed in the preliminary physicochemical screening. The ethanol extract of rhizomes was subjected to TLC for the separation of components.

KEYWORDS: pharmacognosy, phytochemistry, Rubia cordifolia, Rubia tinctorium, Indian and Irani Manjishtha.

INTRODUCTION

Manjishtha, Rubia cordifolia Linn is one of the most commonly used drug in traditional system of medicine for skin and other mainly inflammatory diseases like odema- *sotha*, rheumatism- *amavata*, ascites- *udar* and such others.[1] It is common throughout the hilly tracts of India from the North Western Himalayas, eastward ascending to 2500m and also reported from Greece, Africa and other Asiatic countries like China, Japan, Afghanistan, Vietnam and Malaysia.[2,3] The root of Manjishtha is sweet, bitter, acrid and used as anti-inflammatory[4], haemostatic[5], antidysentric, antipyretic, analgesic, anthelmintic, improves the voice, the complexion and cures the Kapha, the inflammation diseases of the uterus, the vagina, eye, ear and the blood. It is also used in the cure of leucoderma, ulcers, urinary discharges, jaundice, and piles[2] Rubia cordifolia is used in Ayurveda as an ingredient of popular formulations like *Chandanasava, Ashwagandhadyarishtha, Jatyadi Ghrita, Jatyadi Taila, PhalaGhrita, Pinda Taila*[6]

It was found that *Manjishtha* offered in 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.[7]

MATERIALS AND METHODOLOGY

Plant Material

Various samples of root of *Manjishtha* were collected from Pune, Mumbai, Amritsar and Mysore market in the month of Oct-Nov 2016.Identification and authentication of the sample was done with the help of different floras[8] and authenticated at the department of Botany, Savitribai Phule Pune University. The sample was analyzed by using different organoleptic, qualitative and quantitative analyses. A chromatography analysis was also carried out.

Results of Authentication were found to be Indian Manjishtha as *Rubia cordifolia Linn* and Irani variety as *Rubia tinctorum Linn*. Pharmacognostical study containing Organoleptic, Microscopic and physicochemical analysis has been performed.

Organoleptic evaluation:

All the market samples (roots) were studied as per standard procedures. In the Organoleptic evaluation various sensory parameters such as color, odor, taste and texture were investigated.[9]

Microscopic study

Roots were processed as per the standard procedures for histological examinations, and microscopic characters were drawn. Microscopic examination was conducted by mounting the material in medium.

Physico-chemical studies

The physico-chemical parameters such as loss on drying, total ash content, pH, and extractive values, (water-soluble and alcohol soluble) were determined. These parameters were analyzed in accordance with the Ayurvedic Pharmacopeia of India.[10]

Preliminary qualitative tests

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

Chromatographic study

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

OBSERVATION AND RESULTS

Authentification

4 different samples from various markets were authentified as follows-

Table 1: List of plants collected and authenticated.

Name	Collected from market	Botanical identity
Sample A	Pune	*Rubia cordifolia Linn*
Sample B	Amritsar	*Rubia tinctorun Linn*
Sample C	Mysore	*Rubia tinctorun Linn*
Sample D	Mumbai	*Rubia cordifolia Linn*

Morphological characteristics of crude drug

The morphological characteristics of all the market samples are shown in [Table 2].

Table 2: Organoleptic examination of all collected samples.

Observations	Sample A	Sample B	Sample C	Sample D
Morphological nature	Roots and stem mixed 50:50	Roots and stem mixed 75:25	Roots and stem mixed 50:50	Roots and stem mixed 70:30
Origin	Pune market	Amritsar market	Mysore market	Mumbai market
Size and Shape	Shape cylindrical, size variable usually the pieces are from 1.5" to 5" in length	Shape cylindrical and twisted length variable from 2" to 6" in length	Shape cylindrical and twisted length variable from 1" to 5" in length	Shape cylindrical, size variable usually the pieces are from 1.5" to 5" in length
Colour	Light brown roots	Dark brown roots	Light brown roots	Dark brown roots
Odour	Characteristic	Characteristic and pleasant	Characteristic and pleasant	Characteristic
Surface characters	Surface longitudinally furrowed	Surface longitudinally deeply grooved	Surface longitudinally deeply grooved	Surface longitudinally furrowed
Texture and Fracture	Brittle, External bark gets peeled easily	Brittle, Fibrous. Roots very hard. Difficult to peels off the outer bark.	Brittle	Brittle
Inner Surface	Yellowish Brown	Reddish brown	Reddish brown	Yellowish Brown
Taste	Bitter and astringent	Bitter, astringent and slightly sweet	Bitter and astringent	Bitter and astringent

Microscopy section of Root

Rubia cordifolia Linn-Manjishtha- Sample D

The transverse section of root is somewhat circular in outline. The cork is well developed. The cork cells are tangentially elongated and 20-40 cells wide. The cells are thick walled and occasionally contain tannin. Phallogen is not distinct. Secondary cortical cells are thin walled, red in colourans polygonal in shape. Secondary xylem forms a continuous cylinder of reddish, consists of vessels and tracheids. The vascular tissues are comparatively smaller and widely distributed. Vessels are numerous and uniformly distributed. Secondary phloem composed of thin walled cells like sieve elements and phloenparenchyama but lacks in phloem fibers, which forms a wide zone of reddish colour.Cambium is distinct and characterized by absence of medullary rays. The entire portion of root is reddish in color, indicates presence of anthroquinone.

Powder microscopy

Powder is a light reddish brown in color, shows numerous fragments of cork., lignified xylem vessels, trachieds and fibers with pitted and reticulate xylem parenchyma having red colored contents, acicular and sandy crystals as black granular masses.Presence of stone cells.

Microscopy section of Root: *Rubia tinctorum Linn-Manjishtha* – Sample B

The transverse section of root is circular in outline with deep indentations.The cork is well developed. The cork cells are tangentially arranged elongated and 50-60 cells wide. The cells are thick walled, large and contains red pigments. Phallogen is not distinct. Secondary cortical cells are thin walled, red in colorants polygonal in shape. Intracellular spaces are present in cortical parenchyma. Secondary xylem forms a continuous cylinder of dark reddish, consists of many vessels and tracheids. The vascular tissues are comparatively larger than Indian Manjishtha and compactly arranged in groups. Vessels are numerous and uniformly distributed.Secondary phloem composed of thin walled cells like sieve elements and phloemparenchyma but lacks in phloem fibers, which forms a wide zone of reddish color.Cambium is distinct and characterized by absence of medullary rays.The entire portion of root is dark reddish to brown in color.

Powder microscopy

Powder is a dark reddish brown in colour, shows numerous fragments of cork, Lignified xylem vessels, trachieds and fibers with pitted and reticulate xylem parenchyama having Red colored contents, acicular and sandy crystals as black granular masses. Presence of stone cells.

Phytochemical analysis

Physical analysis of root powder showed particle Size moderately Coarse 80% passes through 710 micrometer and 20% passes through 250 micrometer. The value of total ash shows the presence of residue after ignition, representing the plant residue, and external materials. Water and alcohol soluble extractive, combined anthroquinone values were determined.

Table No 3. Physicochemical analysis of all samples of Manjishtha

Parameters	Sample A	Sample B	Sample C	Sample D
Moisture content	11.65%	12.65%	13.65%	11.65%
Total ash value	10.5%	15.4%	15.5 %	11.3%
Water soluble ash	3.3%	4.1%	4.0%	3.5%
Acid insoluble ash	0.56%	0.65%	0.67%	0.57%

Water soluble extractive	37.0%	65.0%	67.0 %	37.02%
Alcohol soluble extractive	4.8%	38.00%	40.0 %	4.9%
Combined anthroquinone	0.62%	1.26%	1.29%	0.65%

Preliminary qualitative analysis

The qualitative analysis revealed the occurrence of flavonoids, alkaloids, tannins, anthraquinone and saponins glycosides that will be the land marks in authentication of this plant. The presence of anthraquinoneis mostly responsible for the anti-inflammatory activity proven in Ayurveda.

Table No 4. Phytochemical screening of Sample B and D.

Sr.No	Tests	Rubia cordifolia Linn Sample D	Rubia tinctorum Linn Sample B
I	Alkaloids	Negative	Negative
II	Carbohydrates	Positive	Positive
III	Saponins	Positive	Positive
IV	Flavonoids	Negative	Positive
V	Anthraquinone	Positive	Positive
VI	Tannins	Negative	Negative
VII	Volatile oil	Negative	Negative

Chromatographic study and UV visible spectrophotometric analysis

Thin-layer chromatography[11] was executed for the normal phase separation of components of alcohol extracts of Manjishtha, Sample B and Sample D. For TLC screening, solvent system was prepared by taking N-Butenol: Acetic acid: Water in a proportion of 4:1:5. Stationary phase for the TLC profile was silica gel G60F254. The spots obtained from the extract were examined under ultra violet light of wavelength 254 and 366 nm. The resolution factor was calculated by using the formula R_f = distance travelled by solute/distance travelled by solvent.

Table 4: TLC profile.

TLC profile	*Rubia cordifolia Sample D*	*Rubia tictorum Sample B*
Under visible light	**Three spots**	
	0.60 [yellow]	0.63 [yellow]
	0.84 [violet]	0.84 [violet]
	0.90[yellow]	0.89 [yellow]
UV 254 NM	**Three spots**	
	0.60 [brown]	0.63 [brown]
	0.84 [blue]	0.84 [blue]
	0.90 [blue]	0.89 [blue]

UV 366 NM		
	0.60 [Red]	
	0.84 [blue]	0.84 [blue]
	0.90[flu.light blue]	0.89[flu.light blue]
Methanolic sulphuric acid reagent	**Six spots**	
	0.29 [yellow]	0.32 [yellow]
	0.52 [blue]	0.54 [blue]
	0.59 [yellow]	0.62 [yellow]
	0.64 [green]	0.67 [green]
	0.80 [yellow]	0.84 [yellow]

FIGURES

Figure 1. Rubia cordifolia Linn

Figure 2. Rubia tinctorum Linn

Figure 3. Rubia cordifolia Linn Roots and powder.

Figure 4. Rubia tinctorum Linn Roots and powder.

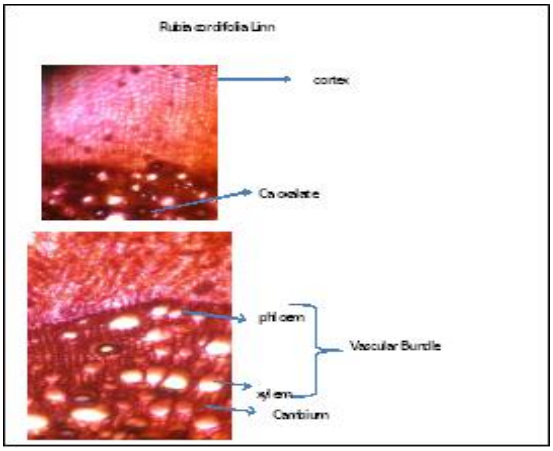

Figure 5. T.S. of Rubia cordifolia Linn.

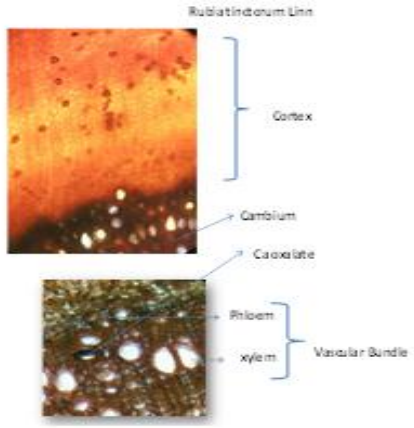

Figure 6. T.S. of Rubia tinctorum Linn.

DISCUSSION

The quality control parameters for the raw materials were established with the help of Organoleptic, microscopic, physicochemical parameters and thin layer chromatography. These values vary within fairly wide limits and are therefore important parameters for the purpose of evaluation of crude drugs. Direct contamination, such as by sand or earth, is immediately detected by the ash value. The total ash, acid-insoluble ash, and water soluble ash were found to be within limit as specified in Ayurved pharmacopeia and was observed slightly higher. This may be due to contamination or sometimes due to unwanted parts of the drug. The results obtained for alcohol and water soluble extractives were within the limit of pharmacopeia which gave an idea about the nature of compounds present.

Preliminary physiochemical screening was performed for establishing the profile of extract for its nature of chemical composition. The qualitative chemical tests carried out for the identification of the nature of phyto-constituents present. All the samples showed the

presence of Triterpenoids, glycosides and saponins. Thin layer chromatography is an important analytical tool in the separation, identification and estimation of different classes of natural products. The RF values of the separated components helps in ascertaining the number of similar type of compounds present in the samples.

CONCLUSION

After analysis of all market sample of *Manjishtha* by different parameters such as microscopic, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, TLC, may serve as diagnostic tools for identification study may act as a stepping stone for further research work.

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