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Phytochemical analysis of *Manjishtha* (*Rubia cordifolia* Linn.) and it's therapeutic relevance

Radha Gupta and Basant Gupta

Abstract
Aim: The study was conducted to evaluate Phytochemistry of *Manjishtha* root to compare the relevant therapeutic Ayurvedic application.
Materials and Methods: *Manjishtha* root was evaluate to determine total, water soluble, acid insoluble ash & extractive values. Organic matter has determined the presence or absence of carbohydrate, alkaloids, amino acids, protein, saponin, glycosides, phenolic compound, tannins from aqueous & alcoholic extract. TLC of ethanolic extract has dropped on Silica Gel & Toluene: Ethyl Acetate: Formic Acid.
Results: 3 tests in Aqueous and 2 tests in alcoholic extract were found positive of carbohydrate. Alkaloids were found positive in 2 tests for aqueous extract & 2 tests from alcoholic extract. In both extract the amino acids was found positive which have major role in the detoxification of blood. Foam test was found positive in aqueous extract which is done for saponin which is a cleansing agent & washout the poison from vegetables. Keller kilini test was found positive only in aqueous extract which is done for glycosides. Phenolic compound was positive for both extract. Phenol plays a role as antiseptic along with anticarcinogenic, antioxidant properties. All the 4 tests in both extract were found positive which is done for tannins which play a major role as chemical antidote by precipitating most of the poisonous compound.
Conclusion: Carbohydrate, alkaloids, amino acids, saponin, glycosides, phenolic compound and tannins were found as a major constituent in *Rubia cordifolia* Linn. root which will play a major role as a antitoxin, detoxification of blood, antiseptic, antimutagenic, anticarcinogenic and antioxidant agent.

Keywords: Manjishtha, *Rubia cordifolia* Linn., phytochemistry, ayurvedic applications

Introduction
Phytochemistry is the branch of natural product chemistry in which qualitative and quantitative analysis of herbal drugs take place. Phytochemistry is in the strict sense of the word, the study of phytochemicals. The systematic investigations of plant materials for its phytochemical behavior involves for different stages. The procurement of drug material and quality control, Examination, Purification and Characterization of the constituents for pharmaceutical interest and in process of quality control, Investigation of Bio-synthetic pathways of a particular compound, Quantitative evaluations. Standardization starts right from the collection of raw materials to the extreme clinical application. In case of *Ayurvedic* medicines, the therapeutic efficacy is a total effect of its chemical constituents. So, the quality and purity refers to the total profile of the drug rather than any of its character. Therefore, a multidimensional approach is essential for standardizing an *Ayurvedic* drug. This multidimensional approach should cover every minute aspect of *Ayurvedic* drug specifically the name, botanical source, and geographical source, organo-leptic, morphological, anatomical, physical, chemical and biological. For the proper identification & Authentification, the present research drug i.e. *Rubia cordifolia* Linn. was subjected to preliminary Pharmacognostical & Phytochemical analysis.
The phytochemical study of *Manjishtha* stem is available in The Ayurvedic pharmacopoeia of India (A.P.I.), but the phytochemical study of *Manjishtha* root is not available in A.P.I. till, hence it is need to standardize the *Manjishtha* root for utilization of proper medication and relevant therapeutic application.

Aims and Objectives
- To procurement of drug material and quality control.
- To Examination, Purification and Characterization of the constituents of *Manjishtha* root for pharmaceutical interest and in process of quality control.

- To Quantitative evaluations of *Manjishtha* root.
- To study the Pharmacognostical & Phytochemical study of *Manjishtha* root for relevant therapeutic application in medicine.

Materials and Methods

Materials

Collection of Plant Materials-

The *Manjishtha* used for the study was collected from Rasashastra Pharmacy, National Institute of Ayurveda, Jaipur.

Instruments and apparatus

Gooch crucible, filter paper, evaporating dish, silica dish, beaker, soxhlet apparatus, capillary tube, Muffle furnace, Applicator

Chemicals and reagents

Water, ethanol, acetone, methanol, hexane, filter paper, wagner's reagent, Molisch's reagent, Benedict's reagent, Fehling's A and B reagent, Dragendorff's reagent, potassium mercury Iodide, potassium iodide, potassium iodobismuthate, glacial acetic acid, ferric chloride, petroleum ether, potassium permanganate solution, Indigo carmine solution, bismuth subnitrate, picric acid, copper sulphate, benzene, tartaric acid, concentrated sulphuric acid, sodium hydroxide, cupric citrate, hydrochloric acid, acetic anhydride, ethanoic acid, chloroform, ninhydrin solution [1, 2]

Methods

1) Determination of total ash

Silica Crucibles were cleaned, dried well and then weighed to constant weight and labeling was made. Drug sample were then weighed accurately and placed in the Silica Crucibles respectively. These crucibles were placed in a muffle furnace at a temperature of 450°C ±5°C till were become totally free from Carbon. The time taken for this process was about 6 hrs. The crucibles containing the ash were allowed to be cooled in desiccators and subsequently weighed to constant weight [3]

Determination of water soluble ash

Water soluble ash value was determined as per Pharmacopoeia of India 1996. Boil the total ash for 5 minutes with 25 ml of water; collect the insoluble matter in a Gooch's Crucible or on an ash less filter paper, Wash with hot water and ignite for 15 minutes at a temperature not exceeding 4500 C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash. Calculate the percentage of water soluble ash with reference to the air dried drug [4].

Determination of acid insoluble Ash

Acid insoluble Ash value was determined as per Pharmacopoeia of India, 1996. Boil the total ash with 25 ml of 2M Hydrochloric acid for 5 minutes. Collect the insoluble matter in a Gooch crucible or on an ash less filter paper, wash with hot water, ignite, cool in a desiccator and weigh. Calculate the percentage of acid insoluble ash with reference to the air dried drug [5].

2) Determination of extractive values

The organic substances of the *Manjishtha* show their solubility in various, solvents in different quantities. So for this purpose of determination of extractive values solvents were selected according to their polarity.

1. Ethanol
2. Water
3. 5 gm sample was taken in a dry conical flask. 200 ml solvent was added to it and was shaken for some time. The sample was kept overnight. Next day, it was filtered and 20 ml filtrate was taken in a pre-weighed evaporating dish, solvent was evaporated by heating on a water bath, dried in an oven till constant weight, cooled and weighed. From the weight of the residue obtained, the solvent soluble extractive percentage was calculated the residual mass remained in filter paper is dried as such and is collected fully. This mass in again put into the conical flask and added with next solvent and fitted with reflux condenser, and extract is prepared in the same method the content of the extractable matter is calculated in the following manner [6].

3) Qualitative examination of organic matter

The extracts obtained from both the research drugs were subject to qualitative examination as per the Pharmacopoeia of India (IP).

(1) Test for Carbohydrate

- **Molich's Test:** 2 ml of the Aqueous Extract of Drug is taken in test tube and 2 ml of the Molisch's reagent is added and shaken carefully, then about 1 ml. of conc. H_2SO_4 is poured from side of the test tube and allowed to stand for one 1 minutes. A Red Brown ring at the junction of the two layers indicates the presence of Carbohydrate [7].
- **Benedict's test:** Treat the test solution with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate forms if reducing sugars are present [8].
- **Fehling's test:** Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) reagents are mixed and few drops of sample is added and Boiled, a brick red precipitate of cuprous oxide forms, if reducing sugars are present [9].

(2) Test for Alkaloids

The following Colour tests are used to detect the presence of an Alkaloid in the sample.

- **Mayer's reagent:** It is Potassium Mercury Iodide soln. & gives a White or Pale Yellow ppt., except with Alkaloids of the Purine groups and few others [10].
- **Dragon Droff's reagent:** It is soln. of Potassium Iodide and Bismuth sub nitrate. They form Orange colour ppt. with the reagent [11].
- **Wagner's test:** Alkaloids give a reddish brown precipitate with Wagner's reagent [Solution of iodine in potassium iodide]. [12]
- **Hager's test:** Alkaloids give yellow colour precipitate with Hager's reagent [saturated solution of Picric acid]. [13]

(3) Test for Proteins

- **Ninhydrin Test:** To an Aqu. Soln. of Protein, Alc. soln. of Ninhydrin is added and then heated. Formation of Red/Blue to Violet colour suggests presence of Proteins. [14]
- **Millons test:** Any compound containing containing a phenolic hydroxyl group gives Millons' test positive. The

Millon reagent is a solution of mercuric and mercurous ions in nitric and nitrous acids. Take 1 ml of protein solution in a test tube and add few drops of Millons reagent. White precipitate is produced, which turns red after heating for 5 minutes on water bath [15].

- **Xanthoprotic Reaction:** Protein usually forms yellow colour soln. when warmed with conc. HNO_3. This colour becomes orange, when the solution made Alkaline [16].

(4) Test for Tannin

- When Aqu. estract of the drug is treated with Vanillin HCL alcohol reagent (Vanillin 1gm + 10 ml Alcohol) Brick or Red colour is formed, showing the presence of tannin [17].

(5) Test for Glycoside

- **Killer – Killiani Test:** To an Extract of drug in Glacial Acetic Acid, a few drops of $FeCl_3$ and conc. H_2SO_4 are added. Formation of Reddish Brown colour at the junction of two layers and changing of the upper layer into Bluish Green indicates presence of Glycoside [18].

(6) Test for Saponin

- About 1 ml of Aqueous Extract is diluted by distilled water up to 10 ml and shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of froth indicates presence of Saponin [19].

(7) Test for Phenols

- 2 ml of Drug extract is taken in a test tube and added 2 ml of $FeCl_3$ solution. Blue – Violet/ Red or Deep Green colour of the solution. is suggestive to presence of Phenols [20].

(8) Flavonoids

- **Shinoda test:** To dry powder or extract, add 5 ml. 95% ethanol, few drops conc. HCL and 0.5 g magnesium turnings. Pink color observed [21].

Thin layer chromatography (T.L.C.)

Thin layer chromatography is a technique to separate the compounds from a mixture based on adsorption principle. It has the advantage of faster runs, better separations, and the choice between different adsorbents. It enables the qualitative, semi qualitative and qualitative evaluation of phytochemical constituents of herbal drugs. This allows the calculation of an Rf value and can be compared to standard compounds to aid in the identification of an unknown substance.

Chromatography plates

T.L.C. plate coated with 0.25 mm layer of silica gel GF 254 with fluorescent indicator, (Mercks) were used. The dimensions of each plate have 10 cm length and 2 cm width. [22]

Detection

- Long wave and short wave of U.V. radiation.
- Iodine vapour
- Dragandroffs

Observations and Results

Table 1: Showing ash values of the *Rubia cordifolia* Linn**.**

Sr.	Test	Value
1.	Total Ash	6.58 %
2.	Water Soluble Ash	21.68%
3.	Acid insoluble Ash	0.95 %

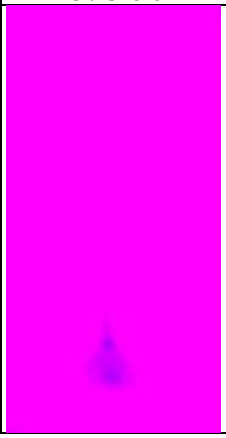
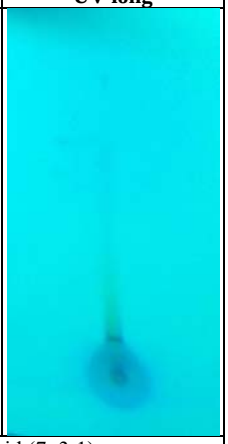
Table 2: Determination of Extractive values in extraction of *Rubia cordifolia* Linn.

Sr.	Solvent	Percentage of Extract $\{(W_2-W_1)/X\} \times 100$
1.	Aqueous Extract	24.64 %
2.	Alcoholic Extract	16.52 %
3.	Petroleum Ether Extract	5.69 %

Table 3: Showing observation of Qualitative analysis of Organic matter in *Rubia cordifolia* Linn.

Sr.	Tests	Aq. Ext	Al. Ext
1. Carbohydrate			
A.	Molish test	+ ve	-ve
B.	Benedict test	+ ve	+ ve
C.	Barfoad test	-ve	-ve
D.	Fehling test	+ ve	+ ve
2. Alkaloids			
	Dragandrof test	+ ve	+ ve
	Wagner's test	-ve	-ve
	Mayer's test	-ve	-ve
	Hager's test	+ ve	+ ve
3. Amino acids			
	Ninhydrine	+ ve	+ ve
4. Protein			
	Biuret test	+ ve	-ve
	Xenthoprotic test	-ve	+ ve
	Millon test	-ve	-ve
5. Saponin			
	Foam test	+ ve	-ve
6. Glycosides			
	Keller kilini test	+ ve	-ve
	Borntrager's test	-ve	-ve
7. Phenolic compound		+ ve	+ ve
8. Flavonoids		-ve	-ve
9. Tannins			
	$Fecl_3$	+ ve	+ ve
	Lead acetate	+ ve	+ ve
	Pot. Dichromate	+ ve	+ ve
	Gelatin Test	+ ve	+ ve

Table 4: Show the chromatography of the alcoholic extract of *Rubia cordifolia* Linn.

Visualization	Day light	UV Short	UV long
			
Mobile Solution	Toluene: Ethyl Acetate : Formic Acid (7: 3:1)		
Stationary phase	Silica Gel G		
Rf Value	0.64, 0.68, 0.71, 0.74		

Visualization	Iodine vapour	Dragandroffs
		
Mobile Solution	Toluene: Ethyl Acetate : Formic Acid (7: 3:1)	
Stationary phase	Silica Gel G	
Rf Value	0.64, 0.68, 0.71, 0.74	

Discussion

Phytochemical analysis of *Manjishtha* stem is available in The Ayurvedic pharmacopoeia of India (A.P.I.), but that of *Manjishtha* root is not available. The observed phytochemical results of *Manjishtha* root are very similar to that of stem, which is available in A.P.I. [23] The phytochemical study of root has proceed and its theraputic relevance has compared with therapeutic indication mention in Ayurveda.

Priya Nighantu has mentioned about *raktashodhak* (blood purifier) property of *Manjishtha* which is similar to therapeutic properties of its one of ingredient amino acid which having the role in the detoxification of blood [24, 25].

Foam test also positive in one sample it means that it contain saponin somewhat. Saponin is best cleansing agent hence the decoction of *Manjishtha* can be used to washout the poisons from dermal layer of vegetables [26]. *Ayurveda* has also mentioned the *Tikta* (Bitter) and *Kashaya rasas* (Astringent) of *Manjishtha* which will be helped during wash to absorb the poisonous compound deposited on vegetables [27, 28].

Manjishtha found the phenolic compound in phytochemical test. As phenol is best antiseptic and used to prevent the infectious pathology, the decoction of *Manjishtha* root may be use as an antiseptic solution to prevent the infection [29, 30]. Recently phenolic compound has found significant role in the prevention and treatment of cancer [31]. Many researches has proved the antimutagenic, anticarcinogenic, antioxidant properties of *Manjishtha* [32].

Ayurveda has already mentioned the *Raktashodhak* property of *Manjishtha* which is useful in the treatment of skin and cosmetic diseases including acne vulgaris [33, 34]. The recent research suggested the antioxidant activity of phenolic compound which is present in *Manjishtha* which will help to prevent the acne [29, 30]

All of the 4 test including $FeCl_3$, Lead acetate, Pot. Dichromate, Gelatin test applied to detect the presence the tannin in *Manjishtha* have positive, It means that the tannin is main ingredient of root. Solution of tannic acid acts as a chemical antidote by precipitating most of the poisons like

alkaloids and metallic poisons [35]. The *Aacharya Charak* has also included it, in *Vishaghna Mahakashaya* [36].

Out of six test of detection of protein in *Manjishtha* only two are positive. It means that it having low protein.

The one test for glycosides has found positive out of four.

Now a days the fashion of addition of colouring agents in the dietary products has been increased. Inspite of long term hazards and carcinogenic effect of that is artificial chemical agent [37, 38] *Manjishtha* has a natural colouring agent and the dyes produce from *Manjishtha* can be used as a dietary colour [39]. As it having phenolic compounds which are useful for cancer, inflammation and have antioxidative property it will gives additional benefits to human beings. *Ayurveda* has already mentioned the *Raktashodhak* property of *Manjishtha* which will be helpful to purify and detoxify human being.

The Thin Layer Chromatography has done by using the stationary phase Silica Gel G, mobile phase Toluene: Ethyl Acetate : Formic Acid (7: 3:1) and T.L.C. plate coated with 0.25 mm layer of silica gel GF 254 with fluorescent indicator. Rf value has measured which are 0.64, 0.68, 0.71, 0.74.

Conclusion

Ayurveda has a store of natural herbal medicine having specific - nonspecific roles to prevent and cure the disease. *Rubia cordifolia* Linn. was found positive for carbohydrate, alkaloids, amino acids, saponin, glycosides, phenolic compound and tannins as a medicinal phytochemical active principal which will be played a major role as a antitoxic, detoxification of blood, antiseptic, antimutagenic, anticarcinogenic and antioxidant agent. Thus the Manjishtha has proved its therapeutic indications mentioned in Ayurveda on the basis of medicinal chemistry.

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