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RESEARCH ARTICLE

Preliminary Photochemical Investigation of Ethanolic and Petroleum ether Extracts of *Rubia cordifolia* Leaves

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Abstract:

The present study is designed to investigate the phytochemicals of leaves of *R. cordifolia* L. in their ethanol and petroleum ether extracts to add the piece of information. The extraction yield of ethanolic extract is 16.34% while that of the petroleum ether extract is 0.56% only. Both extracts were pleasant smelling. Upon phytochemical screening the ethanolic extract reported to be rich in carbohydrates, alkaloids, tannins, phenols, and flavonoids but the petroleum ether extracts contains carbohydrates, tannins, and phenols only. With reference to the *Atraireya Aranyaka* Manjishta is well known to Indian culture of medicines from the time of *Vedic* period; in context to the information the present study contributes the further exploration of plants in development of new therapeutics upon extensive studies.

Key words: *R. cordifolia* L. phytochemical screening, ethanolic extract.

INTRODUCTION

Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments.[1] The discovery of medicinal plants has usually depended on the experience of the populace based on long and dangerous self experiment.

Rubia cordifolia L., is a plant which belongs to the family *Rubiaceae* and is also known with a name Manjistha in general which was originated from Sanskrit language.[2.3] *R. cordifolia* L., is a climber perennial, prickly or scabrous herb plant distributed in temperate and tropical forests of Africa and Asia especially in the hilly regions with an altitude ranging upto 3500 m.[2] The plant is used as traditional medicine for many ailments in India, China, Tibet, Nepal, and Sri Lanka. In south of India, the frequent distribution of this plant is seen adjacent to rivers and streams upper Ghats in evergreen forest in level of 3750 m altitude.[4-7] This plant is reported to be used in treatment of urinary tract disorders and skin problems.[2] Basically, the stem and roots are the main active parts of this plant are used in *Ayurvedic* system of medicine. This plant is used as blood purifier activity, anticancer, astringent, antidysentric, antiseptic, deobstruent, antirheumatic, hepatoprotective.[3,8,9] In Unani medicines *R. cordifolia* L. is prescribed as deobstruent, diuretic, resolvent, detergent, detoxifier and blood purifier.[10]

The root extract has been used against arthralgia, arthritis, cephalalgia, cough, diabetes, discolouration of the skin, dysmenorrhoea, emmenagogue, general debility, hemorrhoids, hepatopathy, intermittent fevers, jaundice, leucorrhoea, neuralgia, pectoral diseases, pharyngitis, ophthalmopathy, otopathy, splenopathy, strangury, slow healing of broken bones, tubercular conditions of the skin and mucous tissue, tuberculosis and urethrorrhoea.[7,11] Manjishthahas evaluated for its wound healing property. The antiviral and free radical scavenging activity leaves of this plant has also been studied.[9, 11-13] The stem part of *R. cordifolia* L. were told to cure scorpion sting and snake bites besides the medicinal significance, the plant is also used as natural food colorants and as a natural hair dye.[3, 14]

The phytochemical studies of this plant were conducted by several workers and roots and stems were investigated extensively in earlier efforts. As very less work has been done on the leaves of *R. cordifolia* L. regarding the phytochemical studies, thus the present study is designed to investigate the phytochemicals of leaves of *R. cordifolia* L. in their ethanol and petroleum ether extracts to add the piece of information as an objective.

Materials and Methods

Sampling & Processing:

Leaves of *Rubia cordifolia* L. Were collected from plants samples obtained from Sanjeevani Nursery, Bhopal (M.P) India. The collected plant parts were thoroughly washed, cleaned and subjected to drying at room temperature till their complete dehydration. Then the material was subjected to pulverization to make fine powder and stored in a container.

Phytochemical Extraction

The pulverised fine powder of *R. cordifolia* leaves were subjected to soxhlet extraction with 80% ethanol and petroleum ether separately at 80^{o}Cand 70 ^{o}C temperatures respectively till the exhaustion. Finally, the obtained menstrum is further concentrated in hot water bath at 50^{o}C till the complete evaporation of the solvents.

Test of Phytochemicals

Chemical tests were carried out for different extract to detect the presence of bioactive components in them by using standard methods described by Harbourne and Sofowora.[15,16] A small portion of the dry extracts were subjected to test the presence of carbohydrates, proteins, alkaloids, tannins, terpenoids, saponins, flavonoids and glycosides.[17,18]

a. Test for Carbohydrates:

Dissolved small quantities of extracts in distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates.

b. Test for Alkaloid:

3 ml each extract was stirred with 3 ml of 1% HCl on steam bath. After that the extracts were cooled to room temperature and Mayer and Wagner's reagent was added to mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid

c. Test for Tannins:

About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of 1% $FeCl_3$ Solution were added. Formation of blue, green or brownish green colour indicated the presence of tannins.

d. Test for Saponins:

A small amount of extract was shaken with 4ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

e. Test for Flavonoids:

To 3ml of test sample, 1 ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

f. Test for Terpenoids:

2 ml of the extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. Development of a greyish colour indicates the presence of terpenoids.

g. Tests for glycosides:

Sodium hydroxide reagent: Dissolved a small amount of extract in 1ml water and added sodium hydroxide solution. Development of yellow colour indicates the presence of glycosides.

Keller-Kiliani's test: dissolved the extract in water followed by glacial acetic acid. Than one drop of 5%$FeCl_3$ and conc. H_2SO_4 was added. Formation of reddish brown colour at junction of two liquid layers indicates the presence of Glycosides.

h. Test for Phenols:

The dried plant extracts about 100mg was dissolve separately in double distilled water; few crystals of ferric sulphate were added. Formation of dark violet colour indicates the presence of phenolic compound.

Results and Discussion

From the results of current study it is clear that the extraction yield of ethanolic extract from R. cordifolia leaves is higher than that of its petroleum ether extracts. The ethanolic extract was reported to be dark maroon, viscous, pleasant smelling matter with16.34% in yield. On the other hand petroleum ether extract is found to be sticky powder, pleasant smelling dark yellow substance with a little yield of 0.56% only (Table 1). This is the major difference in the extraction yield when compare to the use of solvents for extraction. In addition, upon phytochemical evaluation with the use of specific tests, the constituents of ethanolic extract include carbohydrates, alkaloids, tannins, phenols, and flavonoids. While petroleum ether represents the presence of carbohydrates, tannins, and phenols in it.

Chemical constituents of *R. cordifolia* L. includesanthraquinones, iridoids, hexapeptides, rubiprasins, quinones, and triterpenoids.[3,19] Chemical constituents of *R. cordifolia* L. include santhraquinones, iridoids, hexapeptides, rubiprasins, quinones, and triterpenoids.[19] Anthraquinone, anthraquinone glycoside, napthaquinone, napthaquinone glycoside, furomollngin, mollugin, alizarin, lucidine, pimeveroside, ruberythric acid, purpurin, xanthopurpurin, cyclohexapeptide, alkaloid and lignan have been reported from *rubaia* species.[9] A mixture of purpurin (trihydroxyanthraquinone) and munjisthin (xanthopurpurin-2-carboxylic acid) which are the coloring matters present in the roots of this plant also responsible for the antitumor properties of *R. cordifolia* L. Rubiadin is the major chemical constituent of this plant considered as a marker compound used in the quality control of this plant, and this rubiadin as also responsible for the hepatoprotective properties.[20,21]

Plant secondary metabolites from roots of *R. cordifolia* were investigated by Prabhjit Kaur *et al.*, (2008), Shivakumar *et al,* (2012), Pawar *et al.,* (2009), Tiwari *et al.,* (2012) and many others using ethanolic extracts for antiosteoporotic activity, diuretic activity, apoptosis etc. Some work has been done on stems of this plant. Very less work has been done on leaves of *R. cordifolia* for phytochemical instigation or other biological studies. Pointing to the studies made by Aditya *et al.,* (2013) who used methanolic extract from leaves of three plants including *R. cordifolia*, in anticancer properties. The present investigation determines the richness of ethanolic extract in phytochemicals especially alkaloids, tannins and phenols compared to the petroleum ether extracts prepared in the study. These studies could be further extended for the biological and pharmacological in development of new phytotherapeutics.

Conclusions

With reference to the *Atraireya Aranyaka* Manjishta is well known to Indian culture of medicines from the time of *Vedic* period (Jain, *et al*., 2015). Since there has been little work was reported in the phytochemical of leave of *R. cordifolia* L., so the present investigation on phytochemicals using two different solvents is an important step in reporting the further new information in phytochemical investigation of this plant which fulfills the objective. Present study reports the present of various phytochemical carbohydrates, alkaloids, tannins, phenols, and flavonoids in the leaves of this plant. This information could be explored further into development of new therapeutics upon extensive studies in future.

Table 1: Organoleptic properties of phytochemical extracts of *Rubia cordifolia*Leaves

S.N	Extract	Plant part used	Yield of Extraction	Color	Odor	Consistency
1.	Ethanolic	Leaf powder	16.34%	Dark Maroon	Pleasant	Viscous
2.	Petro-ether	Leaf powder	0.56%	Dark Yellow	Pleasant	Sticky Powder

Table 2: Phytochemical Analysis of *Rubia cordifolia* extracts from Leaves.

S.N	Constituents	Soxletion Extracts of Leaves of *R. cordifolia*	
		Pet. ether Extract	Ethanolic Extract
1	Carbohydrates	+3	+
2	Alkaloids	–	++
3	Glycosides	–	–
4	Tannins	+	+2
5	Phenols	+	+2
6	Terpenoids	–	–
7	Flavonoids	–	+
8	Saponins	–	–

[(+) means present, (+2) means Prominent, (+3) means highly prominent and (–) means absent]

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