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In-vitro anthelmintic activity and phytochemical screening of Rubia cordifolia L. root extracts

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Abstract

Rubia cordifolia L. commonly known as Manjistha have been traditionally use as an Ayurvedic medicines for the treatment of various kind of diseases like dysentery, colic pain, blood purification, chest pains and related inflammatory conditions in India as well as at the variousparts of the worlds. Beside its folk uses, the scientific studies revealing its application as effective anthelmintic agent is not studied scientifically yet. So, in search for the effective anthelmintic agent, *R. cordifolia* roots were investigated for *in-vitro* anthelmintic activity. In this study, the petroleum ether, chloroform, acetone, ethanol and aqueous extracts were tested against adult earthworms (*Pheretima posthuma)* along with phytochemical screening to determine the most possible constituents responsible for the activity. In this study we found that, the petroleum ether and chloroform extract had finest activity against adult earthworms as compared to other extracts. Said *in-vitro* anthelmintic activity was proposed due to its richness of variety of chemical constituents like alkaloids, steroids, flavonoids, terpenoids, tannins and anthraquinone glycosides.

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Introduction

Rubia cordifolia is a well known medicinal herb in the ayurveda commonly called as Indian madder (Common madder, Manjistha in Sanskrit) belongs to the family Rubiaceae. *R. cordifolia* is a variable, prickly creeper or climber up to 10 m long distributed throughout India. It is also found in other countries of Asia, Africa and Australia [1-5].

In the Pharmacognosy, beside various parts of the *R. cordifolia* plant, the roots hold a special attention for its application in the traditional as well as in the modern therapeutics. The root size can be measured up to 1m long with 10-12 mm thickness. The roots appeared red in colour dueto thin brownish cork which can peels off in flakes composing a red-brown inner bark marked by longitudinal furrows. It tastes sweet followed by acrid and bitter taste. Traditionally in India it has been used to improve the voice due to 'kapha' properties as described in the Ayurveda. It also found useful for the purification of blood, control of inflammation of vagina, eyes and ears along with treatment of jaundice and piles. It has long history as skin care and treatment in India and neighboring countries. In China, the roots have been used as tonic and astringent. While, in the Cape Province, the root decoction has been used for the treatment of colic, chest complaints and related inflammatory conditions. It also has application in the veteri-

nary sciences for the treatment of dysentery, liver flukes, intestinal worms and wounds.

Various research studies have been explored the variety of pharmacological actions of *R. cordifolia* roots like hepatoprotective, anticancer, anti-inflammatory, anti-hyperglycemic, anticonvulsant, radioprotective, wound healing and antioxidant activity might attributed by variety of phytoconstituents like quinines, iridoids, pentacyclic triterpenes, cyclic hexapepetide derivates and many more [6-15].

However, besides number of traditional and folk uses of *R. cordifolia* roots for the treatment of intestinal problems, till date no scientific study to evaluate its potency against common helminths have been carried out. So, the main objective of the study was to investigate the anthelmintic activity of *R. cordifolia* root extracts along with phytochemical screening.

Materials and methods

Chemicals

For this study, Nutrient agar (HiMedia, India), Petroleum ether, Chloroform and Acetone (CDH Fine Chemical, India), Ethanol (Changshu Hongsheng Fine Chemical, China) of AR grade were used. The albendazole was obtained as gift sample from Relief Lab Pvt. Ltd, Kalmeshwar. Water used for the extraction was double distilled prepared in the laboratory was used.

Plant Materials

The plant roots were collected from Katol, Maharashtra, India region (21.27351° N, 78.58588° E). The taxonomical authentication of said plant material was confirmed from the Department of Botany, Nabira Mahavidyalaya, Katol, Maharashtra. Further, the collected plant material was washed thoroughly with water to remove any adhering dust and undesired materials followed by drying in shade and grinding to fine powder through 100 mm sieve.

Extraction and phytochemical screening

The powdered plant material was successively extracted with solvents- petroleum ether, chloroform, acetone and ethanol using soxhlet apparatus for 7 days along with aqueous extract prepared through decoction. The liquid extracts thus obtained were filtered through Whatman filter paper no. 1 and the organic solvents were recovered by using rotary vacuum evaporator (Acculab, India). The extracts were further concentrated and dried using water bath and subjected to phytochemical screening for the presence of carbohydrates, proteins, alkaloids, saponins, anthraquinone glycosides, steroids, flavonoids, terpenoids, tannins and amino acids. For the phytochemical screening, various standards procedures were followed as given below [16, 17].

Tests for carbohydrates

Molisch's test (General test) To 2 ml of aqueous extract, add few drops of alpha-napthol solution in alcohol, shake and add conc. sulphuric acid from sides of the test tube. Violet ring is formed at the junction of two liquids.

Fehling's test (For reducing sugars) Mix 1 ml of Fehling's A and 1 ml of Fehling's B solution, boil for one minute. Add equal volume of test solution. Heat in boiling water bath for 5-10 minutes. Yellow followed by brick red precipitate is observed.

Barfoed's test (For monosaccharides) Mix equal volume of Barfoed's reagent and test solution in test tube. Heat for 5 minutes. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

Bial's Orcinol test (For pentose sugars) To boiling Bial's reagent add few drops of test solution. Green to purple colouration appears.

Selwinoff's test (For hexose sugars) Heat 3 ml Selwinoff's regent and 1 ml test solution on water bath for 1-2 minutes. Red colour is resulted.

Tests for proteins

Biuret test (General test) to 3 ml test solution add 4% NaOH and few drops of 1% $CuSO_4$ solution. Violet to pink colour forms.

Million's test Mix 3 ml test solution with 5 ml Million's reagent. White precipitate obtained. Warm precipitate, turn brick red or the precipitate dissolves giving reddish coloured solution.

Test for sulphur containing proteins Mix 5 ml test solution with 2 ml of 40% sodium hydroxide solution and 2-3 drops of 10% lead acetate solution. Boil. Solution turns black to brownish due to lead sulfide formation.

Tests for alkaloids

For the alkaloidal test, 2 ml of dilute hydrochloric acid added to 1 g of dry extracts, shaken well, filtered and use for following tests.

Mayer's Test To 3 ml of the filtrate add 1 ml of Mayer's reagent. The creamy precipitate indicates the presence of alkaloids.

Wagner's Test To 3 ml of the filtrate add 1 ml of Wagner's reagent. The reddish brown precipitate indicates the presence of alkaloids.

Hager's Test To 3 ml of the filtrate add 1 ml of Hager's reagent. The yellow precipitate indicates the presence of alkaloids.

Dragendroff's Test To 3 ml of the filtrate add 1 ml of Dragendroff's reagent. The appearance oforange brown precipitate indicates the presence of alkaloids.

Tests for saponin glycosides

Foam test Shake little quantity of extract with water. The formation of persistent foam for 10 minutes confirms the presence of saponins.

Haemolysis test Mix small amount of extract with blood on the slide. The haemolytic zoneoccurs due to the saponins glycosides.

Tests for anthraquinone glycosides

Borntrager's test To 3 ml of extract, add dilute sulphuric acid solution, boil and filter. To the cold filtrate, add equal volume of benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammonical layer turns pink or red.

Tests for steroids

Salkowski's test To the 2 ml test solution, add 2 ml chloroform and 2 ml conc. sulphuric acid. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Legal's test (For cardenolides) to the 1 ml of test solution, add 1 ml pyridine and 1 ml sodium nitroprusside solution. Pink to red colour appears.

Tests for flavonoids

Shinoda tests Dissolve extract in 5 ml of 95% v/v ethanol and add few drops of conc. hydrochloric acid and 0.5 g of magnesium turnings. The pink, crimson or magenta colour represents flavonoids.

Tests for terpenoids

Salkowaski's test To the extract, add 2 ml of chloroform and 2 ml of conc. sulphuric acid from the side of test tube. Shake it for few minutes. Red colour forms.

Liebermann-Burchard's test Dissolve extract in chloroform, add few ml of acetic anhydrideand heat it. Cool it and add few drops of conc. sulphuric acid from the side of the test-tube blue colour forms.

Tests for tannins

Ferric chloride test with the 5% ferric chloride solution extract gives dark green to deep blue colour.

Lead acetate test Add 10% w/v solution of basic lead acetate in distilled water to extract. Precipitate is obtained.

Potassium dichromate test with the extract, potassium dichromate solution produce dark precipitate.

Tests for amino acids

Ninhydrin test Heat 3 ml test solution and 3 drops 5% ninhydrin solution in boiling water bath for 10 minutes. Purple or bluish colour appears.

Anthelmintic Assay

For this assay, the adult earthworms (*Pheretima posthuma)* were used due to similarity of its anatomical and physiological resemblance to that of the intestinal roundworms of humans. The collected earthworms were washed with water to remove any adhering soil and earthy matters. The petroleum ether, chloroform, acetone, ethanol and aqueous extracts of *R. cordifolia* roots were investigated for said anthelmintic assay. In brief, the earthworms to be tested were divided into three groups. In which first group of earthworms was treated with 20 ml solution of prepared extracts (petroleum ether, chloroform, acetone, ethanol and aqueous extracts) of different concentrations of 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml in petri plates (5 earthworms in each petri plate). Second group was treated with albendazole (reference standard drug) of 20 mg/ml concentration. While, saline solution was use as control for third group.

The mean time (minutes) of paralysis and death or worms were noted down. Time for paralysis was noted when no movement of any kind could be seen unless the worms were violently shaken, this is when paralysis occurred. While, after determining that the worms did not move when violently shaken, the time of the worms death was recorded

Results and discussions

The plant materials used in the current study, to determine its effectiveness as an anthelmintic agent, was confirmed as *Rubia cordifolia* L. belonging to family Rubiaceae. A herbarium specimen (No: 01/PHCOG-Rub-02/23) was deposited to Department of Botany, Nabira Mahavidyalaya, Katol along with Pharmacognosy Department of Smt. Kusumtai Wankhede Institute of Pharmacy, Katol, India for future reference.

The phytochemical screening of *R. cordifolia* roots reveals the presence of carbohydrates in ethanol as well as in aqueous extracts; proteins in aqueous extract; alkaloids in petroleum ether, chloroform and acetone extracts; saponins glycosides in aqueous extracts; anthraquinone glycosides in chloroform extract; steroids in petroleum ether and chloroform extracts; flavonoids, terpenoids and tannins were reported in all extracts. The details of phytochemical screening are given in the table (Table1).

Table 1: Phytochemical screening

Phytoconstituents	Tests	Petroleum ether extract	Chloroform extract	Acetone extract	Ethanol extract	Aqueous extract
Carbohydrates	Molisch's	-	-	-	+	+
	Fehling's	-	-	-	+	+
	Barfoed's	-	-	-	+	+
	Bial's Orcinol	-	-	-	+	+
	Selwinoff's	-	-	-	+	+
Proteins	Biuret	-	-	-	-	+
	Million's	-	-	-	-	+
	Sulphur containing	-	-	-	-	+
Alkaloids	Mayer's	+	+	+	-	-
	Wagner's	+	+	+	-	-
	Hager's	+	+	+	-	-
	Dragendroff's	+	+	+	-	-
Saponin glycosides	Foam	-	-	-	-	+
	Haemolysis	-	-	-	-	+
Anthraquinone	Borntrager's	-	+	-	-	-
glycosides						
Steroids	Salkowski's	+	+	-	-	-
	Legal's	+	+	-	-	-
Flavonoids	Shinoda	+	+	+	+	+
Terpenoids	Salkowaski's	+	+	+	+	+
	Liebermann-Burchard's	+	+	+	+	+
Tannins	Ferric chloride	+	+	+	+	+
	Lead acetate	+	+	+	+	+
	Potassium dichromate	+	+	+	+	+
Amino acids	Ninhydrin	-	-	-	-	-

+ represents "present" and – represents "absent"

The anthelmintic assay carried out on the *P. posthuma* showed that (Table 2), the petroleum ether extract of roots had paralysis time within 7 to 22 minutes and death time within 19 to 34 minutes. Chloroform extract had paralysis time within 7 to 29 minutes and death time within 23to 38 minutes. Acetone extract had paralysis time within 32 to 43 min-

utes and death time within 41 to 110 minutes. Ethanol extract had paralysis time within 36 to 41 minutes and death time within 42 to 102 minutes.

Table 2: Anthelmintic assay

Extracts/Drugs	Concentrations	Paralysis (Mean±SD) (min)	Death (Mean±SD) (min)
Albendazole (Standard)	25 mg/ml	8.2±0.2	18.2±0.1
	50 mg/ml	7.8±0.3	14.2±0.1
	75 mg/ml	6.0±0.2	13.3±0.1
	100 mg/ml	4.3±0.1	11.1±0.2
Petroleum ether extract	25 mg/ml	22.2±0.3	34.3±0.2
	50 mg/ml	12.0±0.3	27.6.2±0.1
	75 mg/ml	7.9±0.3	22.2±0.1
	100 mg/ml	7.0±0.1	18.9±0.3
Chloroform extract	25 mg/ml	28.7±0.2	37.7±0.3
	50 mg/ml	18.9±0.1	33.6.2±0.3
	75 mg/ml	14.2±0.1	28.2±0.1
	100 mg/ml	8.6±0.1	22.9±0.1
Acetone extract	25 mg/ml	43.1±0.3	109.7±0.2
	50 mg/ml	38.2±0.1	52.0±0.2
	75 mg/ml	35.3±0.3	43.2±0.3
	100 mg/ml	32.1±0.1	40.7±0.3
Ethanol extract	25 mg/ml	41.1±0.2	101.7±0.1
	50 mg/ml	39.3±0.2	48.8±0.2
	75 mg/ml	38.1±0.2	43.72±0.3
	100 mg/ml	36.3±0.1	41.5±0.1
Aqueous extract	25 mg/ml	56.3±0.3	146.9±0.3
	50 mg/ml	55.1±0.3	132±0.1
	75 mg/ml	49.3±0.1	120.4±0.4
	100 mg/ml	40.2±0.3	104.0±0.4

While aqueous extract had paralysis time within 40 to 56 minutes and death time within 104 to 147 minutes as compared to albendazole (standard) with paralysis time within 4 to 8 minutes and death time within 11 to 18 minutes at variable concentrations of 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml respectively.

Conclusions

Concluding the study, *in-vitro* anthelmintic activity of root extracts of *Rubia cordifolia* L. was carried out using petroleum ether, chloroform, acetone, ethanol and water as solvents. The results depicted that, the petroleum ether and chloroform extracts showed good activity against *P. posthuma* might be due to presence variety of phytochemicals like alkaloids, steroids, flavonoids, terpenoids, tannins and anthraquinone glycosides as screened through various phytochemical tests. The potency of acetone and ethanol extract was found to somewhat similar but lesser than petroleum ether and chloroform extract while aqueous extract could be assumed as ineffective in this study.

Conflict of interest

The authors declare that there is no conflict of interest.

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