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ORIGINAL ARTICLE

Exploring The Therapeutic Potential of *Rubia cordifolia*: Extraction, Phytochemical Evaluation and Microbiological Perspectives

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ABSTRACT
Exploring plant extracts for their therapeutic effects is becoming more popular as people become more aware of and choose natural medicines. Indian madder, also known as Rubia cordifolia, has long been used in ayurvedic medicine to treat a variety of skin conditions. In this research work the extract of Rubia cordifolia is evaluated to know the active constituents present in crude drug. During the phytochemical screening of alcoholic extract of Rubia cordifolia, the tests for alkaloids, Tannins, Phenolic compounds and Flavonoids was found negative. In case of glycosides, the tests for anthraquinone glycosides was found positive. Thus from this, we can concluded that the tested alcoholic extract of Rubia cordifolia contains anthraquinone glycosides. The same extract is also evaluated for its antimicrobial activity and it found to be active against pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Candida albicans. Thus this study reflects the potential antimicrobial activity of Rubia cordifolia present due to anthraquinoine glycosides and it opens the opportunity for multiple formulation to use Rubia cordifolia extract as an active constitute to make effective antimicrobial effect.
Keywords: Traditional medicines, *Rubia cordifolia*, Extraction, phytochemical screening, antimicrobial activity.

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INTRODUCTION

The antibacterial and antifungal infection of the skin is common problem in whole world. These infections should treat by using systemic or topical antibiotics. The systemic treatment is more critical as it creates many severe adverse effects [1]. Thus, topical antibiotic treatment is one of the better options to treat the topical infections. However, the consistent use of antibiotics produces the resistance within the bacteria. In addition, this multi-drug resistant bacteria and fungus is the main problem in treating infectious diseases. The amount of multi drug resistant bacteria and fungus is increasing rapidly. Therefore, it is urgently necessary to create new, powerful antimicrobial drugs that can stop these bacteria and fungi that are resistant to a variety of medications [2].

As more infectious diseases develop resistance to synthetic medications, finding new antibiotics is a global problem facing the pharmaceutical and research sectors. The improper use of orthodox medicines has led to problems with resistance, pollution, and environmental degradation. As a result, there is a growing interest in nature as a source of safer and more effective alternatives for treating human

infections [3, 4]. The drug's capacity to enter the skin sufficiently to produce the desired effects is what determines whether topical delivery will be successful.

In India and other nations, Ayurveda is a widely used traditional medical system. Plants are used in the Ayurvedic medical system to treat illnesses and ailments. Natural products continue to be one of the best options for the discovery of therapeutical, despite the availability of various approaches. They are employed directly as medicinal agents, as well as a model for pharmacologically active compounds or as a raw material for drug synthesis [5, 6].

Trees, shrubs, and frequently herbs are included in the family Rubiaceae, which has roughly 450 genera and 6500 species. Alpha-sitosterol, daucosterol, gallic acid, tannins, rubimallin, alizarin, and hydroxyanthraquinones are the main ingredients of *Rubia cordifolia.* Extracted from *Rubia cordifolia*, rubiadin is a dihydroxyanthraquinone with strong antioxidant properties that, in a dose-dependent manner, inhibits lipid peroxidation. The plant's traditional medicinal uses include treating skin conditions and purifying the blood. Interesting in vivo biological activities, including antifungal, antimicrobial, analgesic, hypotensive, antioxidant, antimalarial, antileukemic, and mutagenic properties, are displayed by the Rubiaceae family's anthraquinones [7]. This plant is also utilised to make natural hair dyes and food colouring. Because natural dyes and colouring materials are used in food, medicine, and other consumables for human consumption, there is a growing interest in their isolation [8, 9]. *Rubia cordifolia* extract improves memory retention activity and has a notable preventive effect on neurodegeneration. *Rubia cordifolia* ethanolic extracts have anti-inflammatory properties [10, 11].

Rubia cordifolia has strong and excellent antibacterial, antifungal, anti-inflammatory, and antioxidant activity [12]. The world over, *Rubia cordifolia* is well-known for its therapeutic applications in treating skin conditions like dermatitis, eczema, ulcers, etc. It has long been used in India to treat a variety of skin infections. It is also used as an antiseptic for wounds and to relieve skin rashes. Folk medicine uses it to treat eczema, ulcers, and swellings [13]. Even at low concentrations, *Rubia cordifolia* demonstrates strong antibacterial and antifungal activity, making it a potent antimicrobial agent. Due to these characteristics, *Rubia cordifolia* is a useful medication for a variety of skin infections [14, 15].

Figure 1: Roots of Rubia cordifolia

MATERIAL AND METHODS

Preparation of alcoholic extract of *rubia cordifolia*

The cold maceration method of alcoholic extraction was used to extract the actives from *Rubia cordifolia*. For five days, 250 grams of *Rubia cordifolia* powder were stirred continuously at predetermined intervals in 500 milliliters of 95% ethanol. After the alcohol was filtered, excess ethanol was eliminated using a simple distillation process to produce concentrated alcoholic extract [16].

Phytochemical screening of alcoholic extract of *rubia cordifolia*

Table 1: Phytochemical screening of extract of *Rubia cordifolia* [17, 18].

Test	Observation
Tests for alkaloids	
a) *Dragendorff's test*	Precipitate having Orange brown color. Precipitate is produced.
b) *Mayer's test*	Precipitate having yellow color.
c) *Hager's test*	Precipitate having reddish brown color.

d) *Wagner's test* e) *Murexide test for Purine alkaloids*	Purple color is produced.
Tests for tannins and phenolic compounds a) *5% Fecl3 Solution* b) *Lead acetate solution* c) *Gelatin Solution* d) *Bromine Water* e) *Acetic acid solution* f) *Potassium Dichromate* g) *Dilute iodine solution* h) *Dilute HNO_3* i) *Dilute NH_4OH and potassium ferricyanide solution* j) *Dilute potassium permanganate solution*	 Deep blue color. White color precipitate. White color precipitate. Discoloration of bromine water. Solution having red color is produced. Precipitate having red color. Formation of transient red color Reddish to yellow color Solution having red color Discoloration of solution
Test for flavonoids a) *Shinoda test* b) *To small quantity of residue, add lead acetate solution* c) *Addition of sodium hydroxide to the residue*	 Pink color produced. Precipitate having yellow color. Yellow coloration, which decolorizes after addition of acid.
Tests for glycosides **Tests for Cardiac Glycosides** a) *Baljet's test* b) *Legal's test* c) *Keller- killiani test* d) *Liebermann's test*	 Yellow to orange coloralong with sodium picrate. Appears pink to red color Reddish brown colour appears at the junction of two liquid layers and upper layer appears bluish green. Appearance of blue color
Tests for Anthraquinone glycosides a) *Borntrager's test for anthraquinone glycosides*: 3 ml extract + dil. H_2SO_4+ boil and filter+ cool+ add equal volume of benzene or chloroform + shake well + separate organic solvents + add ammonia b) *Modified borntrager's test for C- glycosides:* 5 ml extract + add 5 ml. 5 % $FeCl_3$ + add 5 ml. dil HCl + heat for 5 min. in boiling water bath + cool and add benzene + shake well + separate organic solvents + add dil. Ammonia	 Ammonical layers turns pink/ red Pinkish red color is produced.
Tests for Saponin Glycosides *Foam test:* shake the drug extract vigorously with water	 Persistent foam is produced.

Antimicrobial activity of alcoholic extract of *rubia cordifolia*

Using the standard disc diffusion method, the antibacterial and antifungal properties of *Rubia cordifolia*'s alcoholic extract were tested against *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans*. To cultivate bacteria and a fungus, nutrient broth/agar was employed. The desired amount of culture was aseptically transferred in nutrient broth and kept at 37 °C for three hours to form an inoculum in order to recover the lyophilized culture. After aseptically pouring the media into Petri plates, it was allowed to solidify for half an hour. The fresh inoculums of various cultures were spread onto nutrient agar plates that had solidified after 30 minutes. Placed in the correct locations on petri plates were sterile paper discs made of Whatman filter paper, 5 mm in diameter, which had been dipped in 10000 µg/ml *Rubia cordifolia* extract. After that, the agar plates were incubated for 24 hours at 37°C. The zone of inhibition was examined following a 24 hour incubation period [19, 20].

RESULT AND DISCUSSION

Preparation of alcoholic extract of *rubia cordifolia*

The alcoholic extract of *Rubia cordifolia* was prepared as per the protocol discussed in Materials and Methods. The alcoholic extract of *Rubia cordifolia* looks like as shown in Figure 2.

Figure 2: Alcoholic extract of *Rubia cordifolia*

The percentage yield of extract of *Rubia cordifolia* was found to be 5 %.

Phytochemical Screening of Alcoholic Extract of *Rubia cordifolia*

Table 2: Observations of phytochemical screening of extract of *Rubia cordifolia*

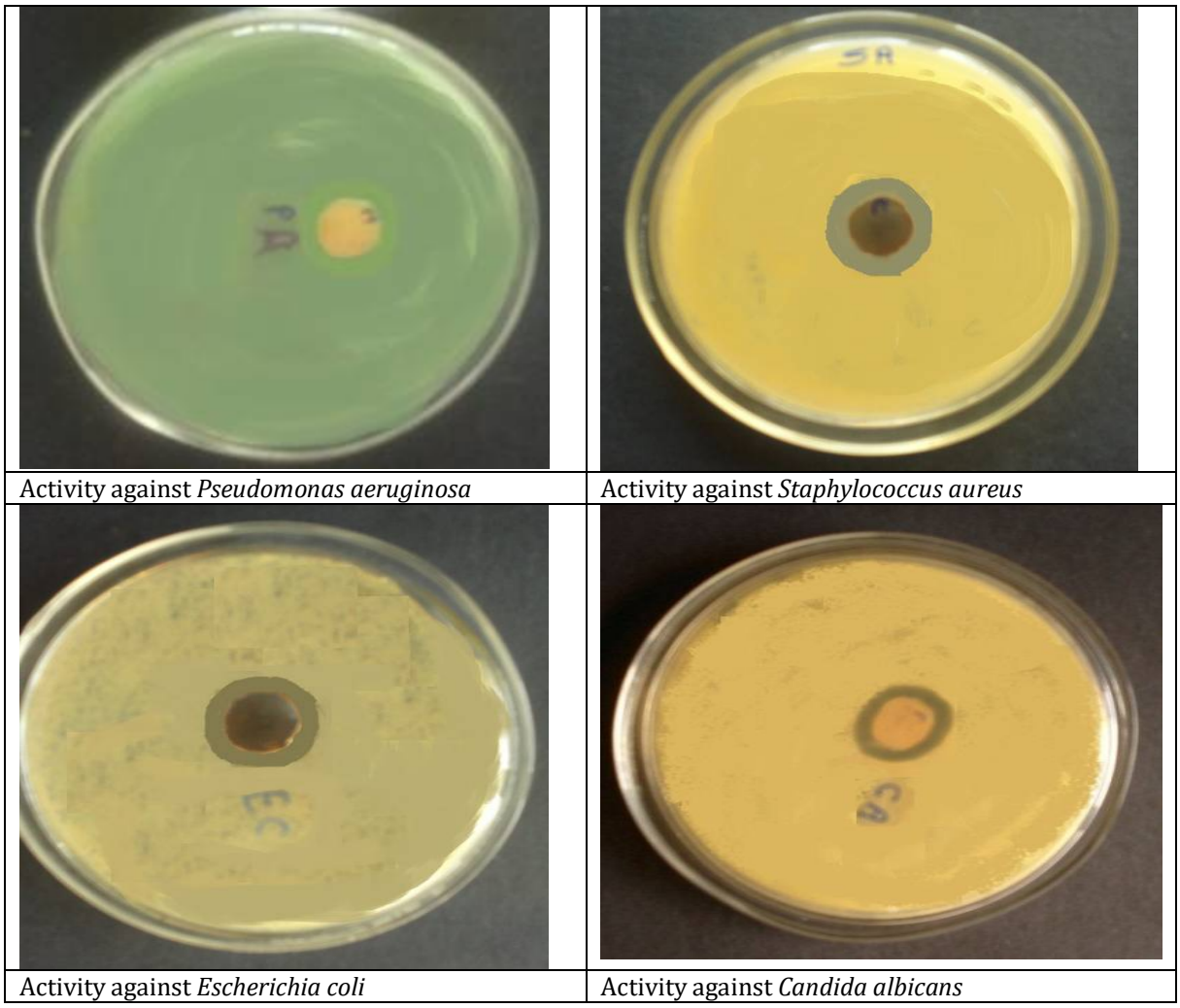
Phytochemical tests	**Observations**
Tests for alkaloids	
f) *Dragendorff's test*	_
g) *Mayer's test*	_
h) *Hager's test*	_
i) *Wagner's test*	_
j) *Murexide test for Purine alkaloids*	_
Tests for tannins and phenolic compounds	
k) *5% $Fecl_3$ Solution*	_
l) *Lead acetate solution*	_
m) *Gelatin Solution*	_
n) *Bromine Water*	_
o) *Acetic acid solution*	_
p) *Potassium Dichromate*	_
q) *Dilute iodine solution.*	_
r) *Dilute HNO_3*	_
s) *Dilute NH_4OH and potassium ferricyanide solution*	
t) *Dilute potassium permanganate solution*	_
	_
Test for flavonoids	
a) *Shinoda test*	_
b) *To small quantity of residue, add lead acetate solution*	
c) *Addition of sodium hydroxide to the residue*	_
	_
Tests for glycosides:	
Tests for Cardiac Glycosides	
e) *Baljet's test*	_
f) *Legal's test*	_
g) *Keller- killiani test*	_
h) *Liebermann's test*	_
Tests for Anthraquinone glycosides	
a. *Borntrager's test for anthraquinone glycosides*	+
b. Modified Borntrager's test for C- glycosides.	+
Tests for Saponin Glycosides	
a. *Foam test*	_

[(-) Absent, (+) Present]

During the phytochemical screening of alcoholic extract of *Rubia cordifolia*, the tests for alkaloids, Tannins, Phenolic compounds and Flavonoids was found to be negative. In case of glycosides, the tests for anthraquinone glycosides was found to be positive. Thus from this, we can concluded that the tested alcoholic extract of *Rubia cordifolia* contains anthraquinone glycosides.

Antimicrobial activity of alcoholic extract of *rubia cordifolia*

The antimicrobial activity of extract of *Rubia cordifolia* was studied against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans.* The results of zone of inhibition was as shown in Figure 3.



Activity against *Pseudomonas aeruginosa* | Activity against *Staphylococcus aureus*

Activity against *Escherichia coli* | Activity against *Candida albicans*

Figure 3: Antimicrobial activity of *Rubia cordifolia* extract

Table 3: Zone of inhibition for antimicrobial test of *Rubia cordifolia* extract

Bulk drugs	**Concentration**	**Zone of inhibition (mm)**			
		P. aeruginosa	***S. aureus***	***E. coli***	***Candida albicans***
Rubia cordifolia extract	10 mg/ml	17	17	16	15

From Table 3, *Rubia cordifolia* shows zone of inhibition against *pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Candida albicans. Rubia cordifolia* was found to be a good antibacterial and antifungal agent.

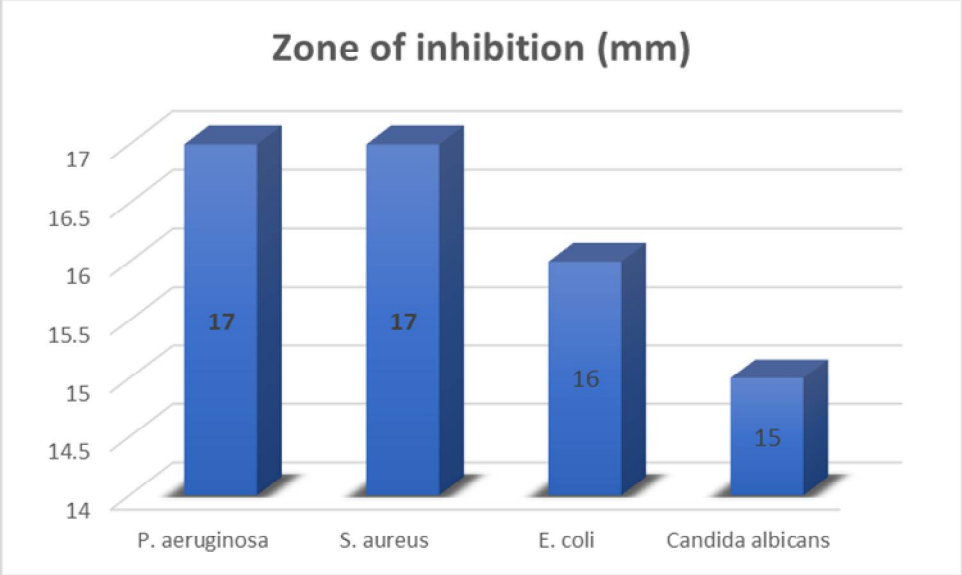


Figure 4: Zone of inhibition for antimicrobial test

CONCLUSION

Recently, there has been a rise in the use of conventional medicine to treat a wide range of conditions and the prescription of combination therapy for numerous illnesses. Due to the increased likelihood of drug interactions and side effects, it is necessary to assess herbal therapy on an individual basis. An ethno botanical plant called *Rubia cordifolia* is rich in components that have amazing abilities to both treat and prevent various pathologies. Alcoholic extract of *Rubia cordifolia* was used for studying phytochemical evaluation. The preliminary phytochemical tests of ethanolic extract showed presence of anthraquinone glycosides. During antimicrobial activity, *Rubia cordifolia* shows zone of inhibition against *pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Candida albicans. Thus Rubia cordifolia* was found to be a good antibacterial and antifungal agent.

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