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Research Article

ETHANO-BOTANICAL STUDIES ON RUBIA CORDIFOLIA LINN

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ABSTRACT

Rubia cordifolia is commonly known as Manjistha. Roots and stems are active part of plant. Plant has many pharmacological actions like blood purifier activity, anticancer, astringent, antidysentric, antiseptic, deobstruent properties and antirheumatic, hepatoprotective. Hepatoprotective action is mainly shown by Rubiadin. Plant contains various chemical constituents like Anthraquinones, Iridoids, Hexapeptides, Rubiprasins, Quinones and Triterpenoids. In the presence study phytochemicals like Phenols, Flavonoids, Saponins, Tannins, Glycosides and Steroids are detected. Further Quantitative analysis revealed a dose-dependent increase in concentration of phenols and flavonoids with increase in concentration of plant extract. It was found that antioxidant and anti-irritant properties exhibited by the solvent extract of the plant under study.

INTRODUCTION

Traditionally ***Rubia cordifolia*** is said to be a very important plant which has the beneficial effects in treatment of number of ailments including alzheimer, diabetes, cancer, acne, inflammation, allergy, enterocolitis, bacterial and viral infection. Other reported activities are immunomodulator, analgesic, diuretic, gastroprotective, hepatoprotective and nephroprotective. ***Rubia cordifolia*** show potent antioxidant activity against lead nitrate and radiation induced toxicity[1,2]. ***Rubia cordifolia*** has been evaluated for its wound healing activity[3]. The leaves of this plant also studied for its antiviral and in-vitro free radical scavenging activity[4]. Apart from its medicinal value, Manjistha has also been used as natural food colorants and natural dyes. The coloring pigments present in the roots are purpurin and munjistin in major amounts. Madder root extract has investigated for its dying characteristics and yielded beautiful orange red to scarlet shades when applied onto the woolen yarn[5].

Rubia cordifolia Linn belonging to family Rubiaceae is a well known ayurvedic herb popularly known as Indian Madder (English), manjeshta (Marathi), majit or manjit (Hindi), manjishtha, aruna, chitra, raktaangi, manjusha (Sanskrit) manjeethiraani (Unani), manjitti (Siddha). Synonyms are Rubia manjista Roxb. R. secunda Moon, R. mungisth.[6]

The WHO Traditional Medicine Strategy 2014 - 2023 estimated that the use of traditional medicines in developing nations will increase by 20% till 2020.The major compounds of this plant are anthraquinones alizarin and purpurin and their derivatives, ruberythric acid (alizarin-primeyeroside), pseudopurpurin and lucidinprimeveroside, rubiadin (1,3-Dihydroxy-2-methylanthracene9,10-dione), munjistin, quinizarin, lucidin and 1,8-ihydroxyanthraquinone [7].Apart from the above mentioned medicinal properties of this plant, its cytotoxic activity on cancer cell lines have also been studied previously. Because medicinal plants are generally replaced by an adulterant plant in the market due to their easy availability and low cost, the key question is how to identify a genuine plant? This is crucial, since adulterant plants possess low therapeutic potential. In the present study, we evaluated the cytotoxic property of the authentic plant on breast cancer cell line (MCF-7) and simultaneously compared the same with the extract of the market sample.

In this paper effort is made to study some of the ethano-botanical properties of *Rubia cordifolia* with important analysis.

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MATERIAL AND METHODS

Plant material (Bark) was collected from an ayurvedic shop from Mulund, Mumbai. Plant material of *R.cardifolia* was extracted using soxhlet extractor using ethanol as solvent for extraction. After extraction process was completed, the solvent was removed or recovered by means of rotary evaporator.

Phytochemical Analysis

Flavonoid analysis was done by standard Alkaline reagent test using quercetin (5-100µgm/ml) was used as a standard. Absorbance was measured at 510 nm spectrophotometrically [8] whereas phenolic compound analysis was carried out by ferric chloride test using gallic acid as standard and absorbance were measured at 765 nm spectrophotometrically.

Qualitative analysis of Tannins (Braemer"s test) in which Extract was added (500 µl) in test tube and in control tube. The sample was sonicated using a sonicator for 5 minutes so that all samples should get mixed properly. Then 10% alcoholic $FeCl_3$ was added in test tube and color change was observed saponins (foam test) and glycosides[9].

Qualitative analysis of Saponins (Foam test): The stock solution was prepared in D/W and it was continuously mixed for 15 min. After mixing it properly, presence of froth was observed[9].

Qualitative analysis of Glycoside: The sample extract was prepared in D/W. The stock sample was mixed with chloroform and conc. H_2SO_4 after shaking for few seconds color change was observed[9].

Antioxidant Assay

Mandal method was used for antioxidant determination. Methanolic extract of plant material was used with varying concentration ranging from (1-100 ug/ml) for T. arjuna. Stock solution was added according to varying concentration in TEST & BLANK tube. After adding stock and diluents, DPPH reagent was added in dark in TEST and incubated for half an hour. Absorbance was measured at 517 nm spectrophotometrically. Ascorbic acid has been used as a standard[8].

Anti-inflammatory assay

Materials used for Anti-inflammatory assay was whole human blood obtained from Clinico Laboratory, Mulund (E). The blood sample was diluted by adding into approximately 50 ml of phosphate buffer saline. From the diluted sample, approximately 12-15ml of blood was drawn in centrifuge tube and it was subjected to centrifugation. After centrifugation, the supernatant was discarded and the pellet was again mixed with some amount of blood to get more concentrated RBC. Then absorbance was measured at 560 nm to check 8 x 10^9cells/ml. This RBC sample was then used for the experimental purpose.

The method originally devised by N. Sampath Kumar [10] was used with slight modification. Test sample for both the plants i.e. ***Rubia cordifolia*** was prepared in (0.25%) hypotonic saline to produce a range of concentration (25-100µg/ml for *Rubia cardifolia*). Aliquot was taken from the stock solution and various concentrations were prepared. After adding test sample, total volume was made up to 1ml by adding hypotonic saline. From this 25 µl of mixture was discarded. Rapidly 25µl of RBC suspension was added to each tube containing 8 X10^9cells/ml and was shaken gently. Incubated at 45^0C for 30 minutes in water bath. After incubation, the reaction was terminated by a rapid high-speed centrifugation at 3500 rpm for 1 minute. After centrifugation, supernatant was transferred to another eppendorf tube and absorbance was measured at 560 nm. From the measured absorbance % protection for membrane, stabilization was determined.

RESULTS AND DISCUSSION

Phytochemical analysis

Table 1 Results for Phytochemicals are mentioned in following tables.

Phytochemical constituents	Method Followed	Result
Phenols	Ferric Chloride Test	+
Flavonoids	Alkaline Reagent Test	+
Saponins	Foam Test	+
Tannins	Braemer's Test	+
Glycosides		+
Steroids	Libermann Burchrad's Test	+
Alkaloids	Wagner's Test	-
Terpenoids		-

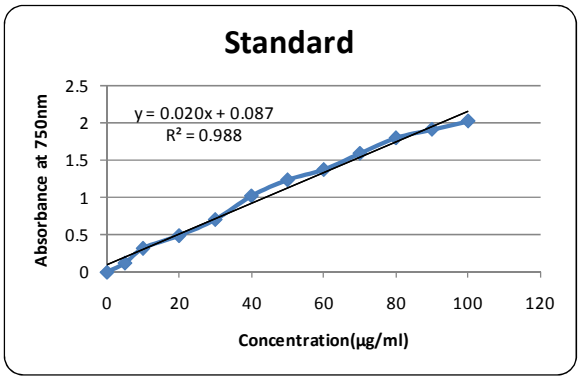
In *Rubia cordifolia,* presence of phytochemicals like Phenols, Flavonoids, Saponins, Tannins, Glycosides and Steroids was observed whereas alkaloids and terpenoids were not detected. Further Quantitative analysis revealed a dose-dependent increase in concentration of phenols and flavonoids with increase in concentration of plant extract. The similar observation was recorded by Deoda[11], the qualitative chemical tests carried out for the identification of the nature of phyto-constituents present in which methanol and chloroform extracts showed the presence of Triterpenoids, glycosides and saponins whereas in present study terpenoids were absent. The screening of biologically active compounds from various solvent extracts of root, stem and leaf in R. cordifolia in study by Seetaram *et al*.[12] revealed the presence of anthraquinones, glycosides saponins, steroids, phenols and flavonoids.

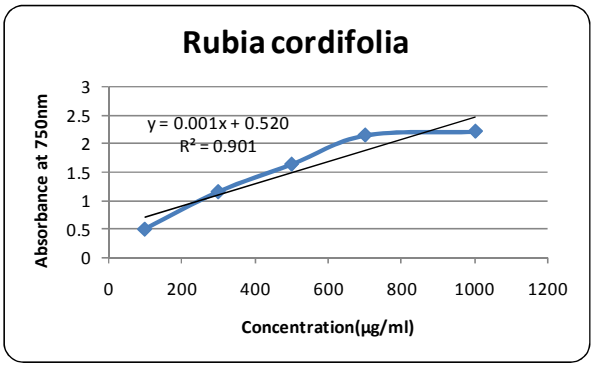
Table 2 Total phenols contents in *R.cardifolia*

Conentartion (ug/ml)	Absorbance
100	0.5046
300	1.1564
500	1.6358
700	2.1425
1000	2.2093

Graph showing total phenolic content gradation from low concentration to high (left to right) in the Standard used (Gallic Acid) and in *R.cardifolia*

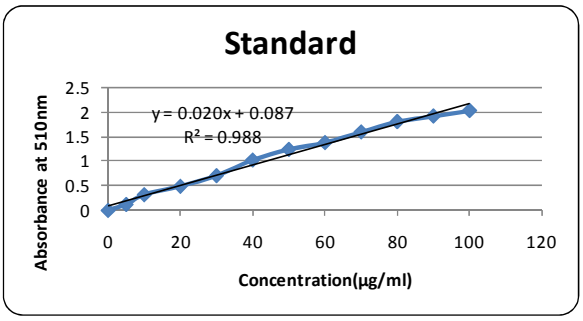
In investigation of Mandal *et.al*.,[8] total phenolic contents compounds of methanol, ethyl acetate and chloroform extracts were expressed as mg of pyrocatechol equivalent per gram of dry weight. The level of total phenolic compounds was found 70.38 mg per gram of extracts respectively. The reducing power of extracts was determined. The gradual increase in absorbance with concentration is indicative of enhancement of reducing power with concentration.

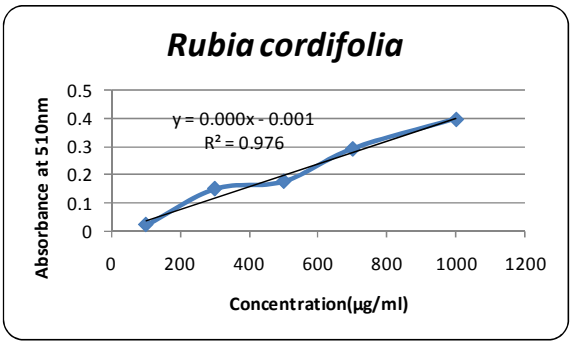




Desai *et.al*.[13] showed the dried roots of field grown plant showed highest phenolic content in methanol extract (41.021±0.813 mg GAE g $^{-1}$), and the lowest content was observed in aqueous extract of fresh roots (8.929±0.948 mg GAE g $^{-1}$). In dried hairy roots, highest phenolic contents was recorded in the methanol extract of hairy roots grown on B5 medium (139.719±0.856 mg GAE g $^{-1}$), where as the lowest content was observed in aqueous extract of hairy roots grown on MS medium (56.210±0.687 mg GAE g $^{-1}$). In the fresh hairy roots, the highest phenolic content was observed in ethanol extract of hairy roots grown on MS medium (75.719±1.893 mg GAE g $^{-1}$) and lowest content in aqueous extract grown on ½ MS medium (15.719±0.694 mg GAE g $^{-1}$)

Total flavonoid





Graph showing total Flavanoid content gradation from low concentration to high (left to right) in the Standard used (Quercetin)

In quantification of phytochemicals in hairy root cultures of ***Rubia cordifolia*** Linn, the highest flavonoid content was observed in methanol extract of dried roots (30.045±1.336 mg RE g $^{-1}$), and lowest content was observed in methanol extract of fresh roots (10.186±0.674 mg RE g $^{-1}$).[13] In dried hairy roots, highest flavonoid contents were recorded in the methanol extract of hairy roots grown on B5 medium (115.615±1.208 mg RE g $^{-1}$),where as the lowest content was recorded in ethanol extract of hairy roots grown on ½ MS medium (44.541±1.178 mg RE g $^{-1}$) .The flavonoid content was highest in methanol extract of fresh hairy roots grown on B5 medium (42.237±0.940 mg RE g $^{-1}$) which was lowest in hairy roots grown on MS medium (15.167±0.922 mg RE g $^{-1}$)

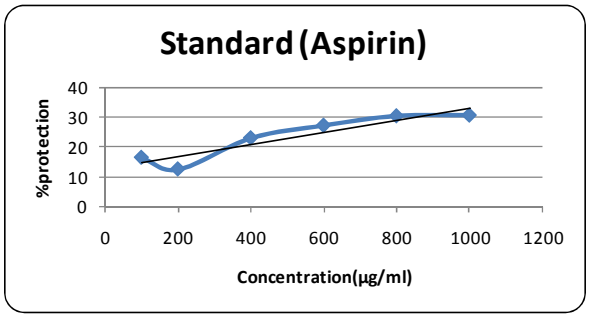
Estimation of Anti-oxidant activity by DPPH Assay

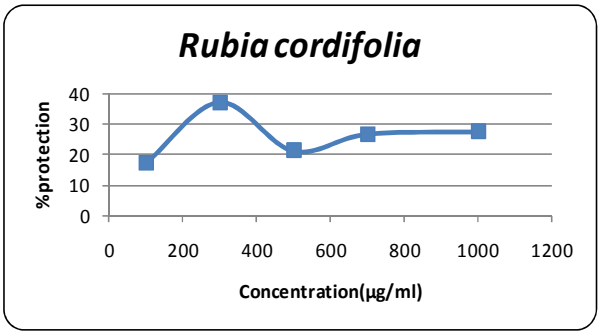
Antioxidant is defined as a substance which significantly delays or inhibits oxidation process. The antioxidant activity is measured indirectly by determining the inhibition rate of oxidation processes in the presence of an antioxidant. DPPH, an organic stable radical in its crystalline form and in solution, is widely used to determine the antiradical activity of a given compound or extract. The antioxidant activity of a given compound or extract is also often associated with its radical-scavenging activity.

Rubia cordifolia gave highest % radical scavenging at 700µg/ml tested by DPPH and FRAP methods. By DPPH method, the % radical scavenging at 700µg/ml was found to be 32.17±1.41 and By Reducing power assay, the reading was at 0.4961±0.01.

In study by Momin *et.al*. [14] of ethanolic extract of *T. arjuna* showed maximum activity of 89.4% at 100 µg/ml whereas ascorbic acid exhibited 96.26% inhibition at 50 µg/ml. Standard DPPH antioxidant activity and *R.cardifolia* activity in % radical scavenging

Anti-inflammatory Test





By anti-inflammatory testing, it was found that at 700µg/ml, *R.cordifolia* provided 25.93% protection, which is expected a decent value. Anti-inflammatory assay was carried out by following the standard protocol. Plant material of T.arjuna revealed very less protection at lower concentration i.e. 8% protection at 25 µg/ml. The result obtained indicates that the plant material used has very less antiinflammatory activity at the concentration used was an observation by[14] Momin *et.al*. Pharmacological evidences showed that RRREE was able to ameliorate the lead nitrate-induced oxidative damage by improving the activities of superoxidedismutase (SOD) and catalase (CAT), increasing the content of GSH, and suppressing LPO[15]. In a screening investigation of some phytochemicals, physcion and 1-hydroxy-2methylanthraquinone showed amelioration on the damage to mouse peritoneal macrophages induced by lipo-polysaccharide (LPS) and interferon-γ (IFN-γ), which was mediated through inhibition of iNOS protein expression and reduction of NO content [16].

Irritant potential Test In-vitro Hemolysis: Hemolysis is losing or rupturing of the Erythrocytes (red blood cells) and the release of their contents (cytoplasm) into surrounding fluid (*e.g.*, blood plasma). When hemolysis occurs outside the body, it is termed as in-vitro hemolysis.

Testing for irritancy potential, it was also found that, at 700µg/ml concentration, % hemolysis was around 42.09%. By the L50/Di value, it was found that *Rubia cordifolia* is a slightly-irritant plant by referring the standard table in INVITOX'37 and '99.

In study by [10]N.Sampat Kumar the EtOAc soluble fraction of *Bombax ceiba* at concentration range of 10 Lg - 150 Lg did not significantly protect the human and sheep erythrocyte membrane against haemolysis induced by hypotonic solution. At a concentration of 150 Lg, the extract produced 41.3 % and 36.11% inhibition of RBC membrane haemolysis in human and sheep bloods respectively.

CONCLUSION

The present study was carried out for "Ethano-botanical studies on *Rubia cordifolia* L. The present study reveals that the extract of *Rubia cordifolia* was good source of phytochemicals. The study also indicated that the extract of plant material possibly prevents the effect of oxidative stress exhibiting % radical scavenging activity. This may be due to present of antioxidants such as flavonoids and other phenolic compounds and can be used as natural antioxidant and as a possible food supplement in pharmaceutical industries. Invitro study indicates the importance of plant extracts as a source of preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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