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Original Research Article

Phytochemical analysis and In vitro antioxidant activity of rubia cordifolia

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Abstract

Plants as medicines have been used for thousands of years. Herbal extracts and formulations have long been regarded as a source of new and useful pharmaceuticals. The chemical composition of plant based medicines has become a new interest these days. Several bioactive constituents of plants have been isolated and studied for various pharmacological studies. Rubia species is one of the earliest plant resources that possessed commercial and important medicinal values. They were used as natural dyes in old days and used as drugs. We aimed to estimate the *In Vitro* antioxidant activity, total phenolic and total flavonoid contents of *Rubia cordifolia*. 2, 2- diphenyl-1-picryl-hydrazyl (DPPH•), superoxide scavenging and reducing power assay were used to assess the antioxidant activity of the plant. The results of phytochemical investigation revealed the presence of most of the phytoconstituents, reasonable amount of flavonoids and phenolic contents. Ethanolic extract of *Rubia cordifolia* leaves showed significant scavenging activity against DPPH and ample reducing power and superoxide scavenging activity.

Key Words: *Rubia cordifolia*, phytochemical, *in vitro* antioxidant.

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INTRODUCTION

The plants have been used as natural medicines. The use of plants as medicines has been in existence since prehistoric times. The different ways by which plants have been found useful as medicines such as crude extract of plants has been used directly due to the presence of natural chemical components such as berberine, morphine, psilocin, vincristine etc.[1] and natural compounds for the synthesis of drugs such as tubocurarine, colchicine, nicotine, quinine etc. for therapeutic purpose by general people. the modern medicines such as digitalis, vinblastine, aspirin, quinine and paracetamol had their origin from the natural compounds of medicinal plants viz., foxglove (digitalis purpurea), madagascar periwinkle (vinca rosea), willow bark (salix spp.), quinine bark (cinchona officinalis), respectively[2]. ayurvedic pharmacopoeia has recorded more than 300 medicinal plants that are commonly used for medicinal purpose. The knowledge of chemical constituents present in plants helps the scientists to understand the mechanism of drug action[3]. It has been observed that the use of crude drugs obtained from different geographical regions showed large dissimilarity and variations in clinical results[4]. *Rubia cordifolia* is a perennial, spiny climber with a stem, growing up to 12 m long. Leaves are ovate lanceolate, 5-7 nerved, 2-10 cm long and 2-5 cm broad, occur in whorls of 4-6. Flowers with fragrance, minute, whitish or greenish yellow in colour. Fruit is minute, glabrous, 1-2 seeded, dark purplish or blackish at maturity. The plant carries fruits and flowers in the months of august to October[5]. *Rubia cordifolia* is having red rhizomatous base and roots. It is an important raw material for the traditional herbal compositions such as aswagandharistam, gulguluthikthkarishtam, jaatyaadi ghrita, madhookasavam, majishthaadi taila, useerasavam etc. It is a member of Rubiaceae family, distributed in hilly areas of India up to 3750m. The plant is commonly

known as 'Indian Madder' and sold under the trade name 'manjistha'. The plant has different vernacular names such as chitravalli in Kannada, manjit in Hindi, ceevalli in Tamil, manchatti and manjatti in Malayalam and manderti in Telengu[6]. The plant is used to cure tuberculosis and intestinal ulcer. In the modern pharmacopeia, *Rubia cordifolia* is described to be protective against a various panel of cancer cell lines, such as P388, L1210, L5178Y, B16 melanoma, sarcoma180 and Lewis lung carcinoma[7]. The leaves of *Rubia cordifolia* also possess antiviral and in-vitro free radical scavenging activity[8]. *Rubia cordifolia* show powerful antioxidant activity against lead nitrate and radiation induced toxicity[9-10]. Free radicals are formed in our body due to the biological oxidation. Oxidation is a natural method in an organism for the manufacture of energy to fuel biological cycles. On the other hand, free radicals cause damage to the body and create oxidative stress[11-12]. The normal metabolism produce oxidation by-products which cause severe damage to DNA, protein and lipids, contribute to ageing and also to degenerative diseases including cancer, coronary artery disease, hypertension, diabetes etc[13]. The secondary plant metabolities like phenolics and flavonoids present in food substances of plants are natural antioxidants[14-15] can entrap few free radicals directly or through a sequence of reactions with antioxidant enzymes[16] and also show various biological effects, including antimutagenicity, antiageing and protective effects on oxidative stress[17-18-19]. Keeping in view the significance of medicinal plants and secondary metabolites, this research was carried out to evaluate the *Rubia cordifolia*, its phytochemical investigation along with its *in vitro* antioxidant activity.

MATERIALS AND METHODS

The leaves of *Rubia cordifolia* were collected from the village Barsoo, Tehsil Lar, District ganderbal (Latitude 34.241288 and Longitude 74.717246) during the month of July 2014. The plant was authenticated by Dr. Akhtar H. Malik (Curator, Centre for Biodiversity and Taxonomy, Department of Botany University of Kashmir). A voucher herbarium specimen was submitted to the Department of Taxonomy University of Kashmir bearing specimen number 2030-KASH Herbarium for further references.

Preparation of Rubia cadifolia: The leaves of *Rubia cordifolia* were dried in shade for a week at room temperature. The dried leaves were grinded to moderately coarse powder by mechanical grinding. The powder thus obtained was extracted in 90% ethanol using Soxhlet apparatus. The extract was dried under vacuum and the semi-solid material thus obtained was stored in bottles which were kept at -4 ^{o}C for further use.

Dried extract of *Rubia cordifolia* was weighed and percentage yield of each extract was determined by using the formula as:

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material used}} \times 100$$

The leaf extract were observed for colour, texture and odour, and packed in labeled air tight containers till further usage.

Stock solution of *Rubia cordifolia*: The stock solution of *Rubia cordifolia* extract was prepared in double distilled water 80 mg/ml freshly just before use.

Solubility of Extracts: The solubility of ethanolic leaf extract of *Rubia cardifolia* was observed in different solvents. **Phytochemical investigation of crude extract of *Rubia cordifolia:*** The standard procedures [20] were followed for the phytochemical analysis of the leaf extract. The extract were subjected to preliminary phytochemical investigstion to identify various phytoconstituents such as; alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins and tannins.

Results of Phytochemical studies: The results of phytochemical investigation were as follows:

Loss of weight on drying: After proper collection and identification, the plant materials i.e. the leaves were washed out and shade dried at room temperature for 7 days. The dried plant materials were grinded in a grinder. The loss of weight on drying of *Rubia cordifolia* was 78.94% (Table 1).

Percentage yield of plant extracts: The extract obtained was stored in air tight glass bottles at room temperature. The percentage yield of the extract was calculated as 7.16% (Table 2). The Organoleptic evaluation of the extract was performed (Table 3).

Solubility: The solubility of the extract was checked in different solvents for further studies like DPPH assay, Superoxide scavenging, reducing power, total phenolic content and total flavonoid content. (Table 4).

Observations of Phytochemical Screening: The qualitative phytochemical analysis was performed using standard procedures. The phytochemical analysis of ethanolic extract of *Rubia cordifolia* showed the presence of flavonoids, carbohydrates, tannins and phenolic compounds, saponins while as pet. ether extract of the same plant showed the presence of Carbohydrates, saponins, tannins and phenolic compounds (Table 5).

Antioxidant activity of Rubia cordifolia: The DPPH assay was used to estimate the antioxidant activity[21-22]. The DPPH radical reacts with suitable reducing agents losing colour, with the number of electrons consumed, which is measured spectrophotometrically at 517 nm. The standard curve has been plotted by using various

concentrations of ascorbic acid and its percentage inhibition by DDPH was calculated **(Fig.1 and 1A).** The result of ethanolic extract of *Rubia cordifolia* obtained was 56.6370µg/ml (Table 6). The effect was compared to that of the standard ascorbic acid with IC_{50} value 18.53µg/ml. The results, thus obtained suggest that ethanolic extract of *Rubia cordifolia* can serve as free radical inhibitors and exhibit significant DPPH radical inhibition. On the other hand the superoxide scavenging activity of extract was 42.1303µg/ml. which also revealed the antioxidant activity of extract (Table 7) and **(fig.2)**

Reducing Power Assay: Reducing power assay is another method used to determine antioxidant activity of *Rubia cordifolia* leaf extract[22]. The reducing power assay is higher as the absorbance of the reaction mixture is higher (Table 8) represents the absorbance of ethanolic leaf extract of *Rubia cordifolia* at different concentrations.

Presence of Total Phenolic Contents (TPC): Total phenolic content is estimated by using the method[23]. Different concentrations of gallic acid were made (Table 9) and absorbance taken at 765 nm (due to developed lue colour) using methanol as blank so as to draw a standard curve **(Fig. 3).** The total phenolic content in the ethanolic extract of *Rubia cordifolia* was 9.726 ± 0.572 µg/100 µg gallic acid equivalent (Table 10).

Presence of Total Flavonoid Content: Total flavanoid content estimation is done by using the procedure[24]. Different concentration of Quercetin were made and absorbance taken at 510 nm with water as blank (Table 11). Standard cure was plotted thereafter **(Fig. 4).** The total flavonoid content of ethanolic extract of *Rubia cordifolia* was 21.999 ± 0.166 µg/100 µg Quercetin equivalent (Table 12).

Table 1: Weight of leaves of *Rubia cordifolia* after drying and percentage loss

Sr. No.	Description	Weight (gm)	% Loss
1	Weight of fresh leaves of *Rubia cordifolia*	114	
2	Weight of dry leaves of *Rubia cordifolia*	24	78.94
3	Loss in weight on drying	90	

Table 2: Percentage yield of the extract of *Rubia cordifolia* leaves

Sr. No.	Plant Name	Solvent	Powdered Material (gm)	Volume of Solvent (ml)	Weight of Extract (gm)	Percentage Yield
1	*Rubia cordifolia*	Ethanol	120	2000	8.599	7.16

Table 3: Organoleptic evaluation of extracts of leaves of *Rubia cordifolia*.

Sr. No	Plant	Extract	Colour	Taste	Appearance	Smell
1	*Rubia cordifolia*	Ethanolic	Greenish black	Sour and Salty	Sticky and Oily	Characteristic smell

Table: 4: Solubility *Rubia cordifolia* leaf extracts in different solvents.

Sr. No.	Solvent	*Rubia cordifolia*
1	Water	insoluble
2	Methanol	Soluble
3	Pet. Benzine	Partially Soluble
4	Chloroform	Soluble
5	Acetone	Soluble
6	Ethyl acetate	Soluble
7	DMSO	Soluble

Table 5: Showing the presence of different phytochemicals in *Rubia cordifolia.*

Phytochemicals	Tests	Petroleum Extract	Ethanolic extract
Alkaloids	Mayer's Test	-	-
	Wagner's Test	-	-
	Hager's Test	-	-
	Dragendroff's Test	-	-
Terpenoids	Salkowski Test	-	-
	Libermann Burchards Test	-	-
Flavonoids	Lead Acetate Test	+	+
	Alkaline Reagent Test	-	+
Carbohydrates	Molish's Test	+	+
	Fehling's Test:	-	+
	Benedict's Test:	+	+
	Barfoed's Test	-	-
Glycosides	Killer Killians Test	-	-
	Borntrager's Test	-	+
	Legal's Test		-
Tannins and Phenolic compounds	$FeCl_3$ Test	+	+
	Dilute Iodine Solution Test	+	+
	Lead Acetate Test	-	+
Saponins	Gelatin Test	-	-
	Froth Test	+	+
Amino acids Proteins	Biuret's Test	-	-
	Millon's Test	-	-
	Ninhydrin Test	-	-

+ = Presence, - = Absence

Table 6: Showing % inhibition of DPPH by ethanolic extract of *Rubia cordifolia.*

S. No.	Conc. (µg/ml)	Absorbance (Control), A_c	Absorbance (Test), A_t	% Inhibition	IC_{50} (µg/ml)
1	20		0.675	23.6425	
2	40		0.556	37.1040	
3	60		0.376	57.4660	
4	80	0.884	0.289	67.3070	56.6370
5	100		0.211	76.1312	

Table 7: Showing % inhibition of superoxide scavenging by ethanolic extract of *Rubia cordifolia.*

Sr. No.	Conc. (µg/ml)	Absorbance (Control), A_c	Absorbance (Test), A_t	% Inhibition	IC_{50} (µg/ml)
1	20		0.547	35.94848	
2	40		0.394	53.86417	
3	60		0.339	60.30445	
4	80	0.854	0.291	65.92506	42.13
5	100		0.257	69.90632	

Table 8: Showing the Reducing Power of ethanolic extract of *Rubia cardifolia.*

Sr. No	Concentration µg/ml	Absorbance
1	20	0.551
2	40	0.599
3	60	0.630
4	80	0.671
5	100	0.740

Table 9: Showing different absorbance of Gallic Acid at different concentrations

Sr. No.	Concentration (µg/ml)	Absorbance (nm)
1	10	0.225
2	20	0.265
3	30	0.341
4	40	0.402
5	50	0.478
6	60	0.577
7	70	0.701
8	80	0.782
9	90	0.913
10	100	0.985

Table 10: Showing Total Phenolic Content in ethanolic extract of *Rubia cordifolia* leaves.

Sr. No.	Absorbance	Concentration (µg/ml)	Total Phenolic content in µg/100 µg Gallic acid equivalent
1	0.997	1000	9.363
2	0.995	1000	10.386
3	0.991	1000	9.431
Mean			**9.726**
S. D.			**0.572**

Table 11: Showing different absorbance of Quercetin at different concentrations

Sr. No.	Concentration (µg/ml)	Absorbance
1	20	0.051
2	40	0.060
3	60	0.080
4	80	0.082
5	100	0.103

Table 12: Showing Total Flavonoid content in ethanolic extract of *Rubia cordifolia* leaves.

Sr. No.	Absorbance	Concentration (µg/ml)	Total Flavonoid content in µg/100 µg Quercetin equivalent
1	0.303	1000	22.166
2	0.299	1000	21.833
3	0.301	1000	22.000
MEAN±SD	**21.999 ± 0.166**		

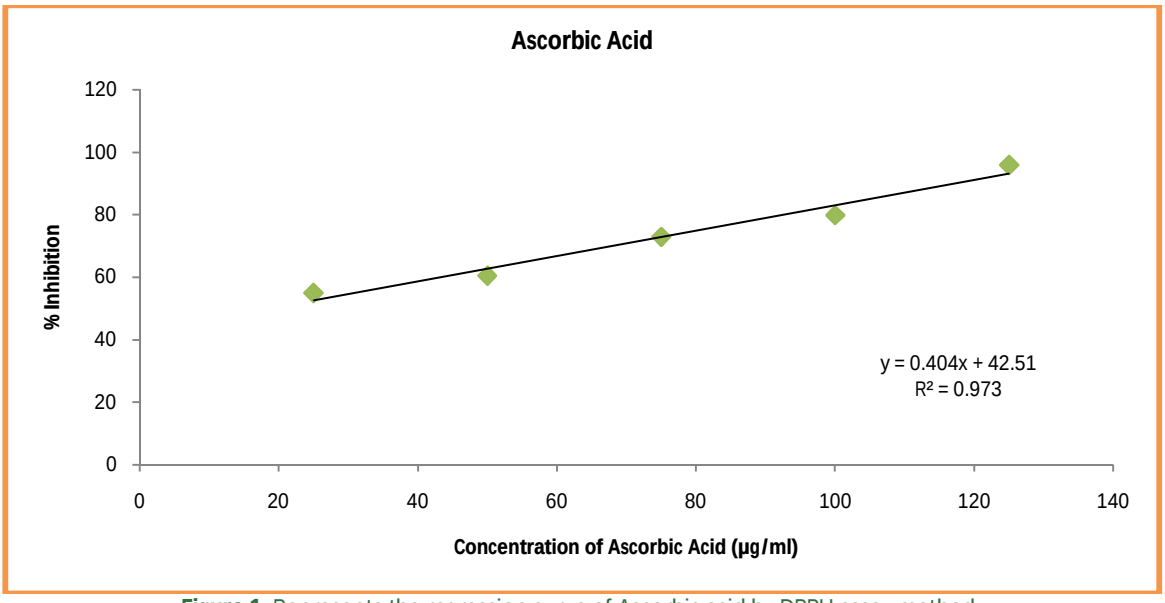

Figure 1: Represents the regression curve of Ascorbic acid by DPPH assay method.

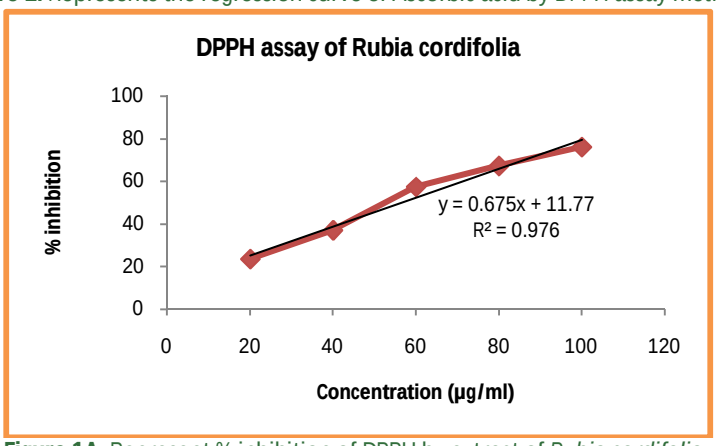

Figure 1A: Represent % inhibition of DPPH by extract of *Rubia cordifolia.*

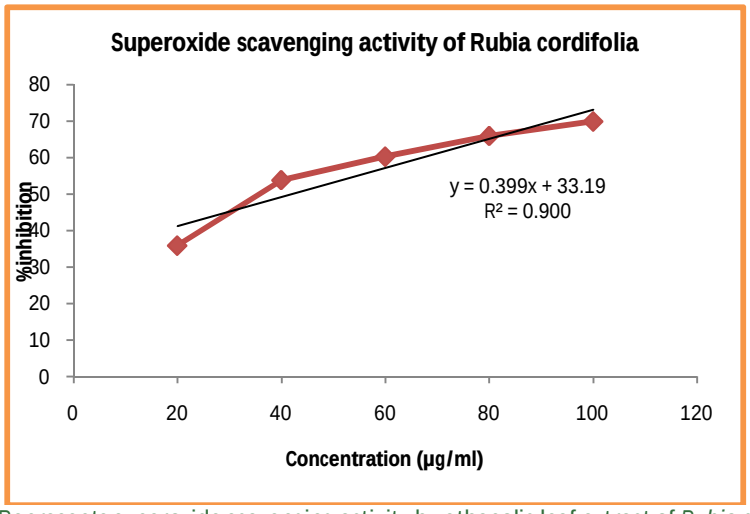

Figure 2: Represents superoxide scavenging activity by ethanolic leaf extract of *Rubia cordifolia*.

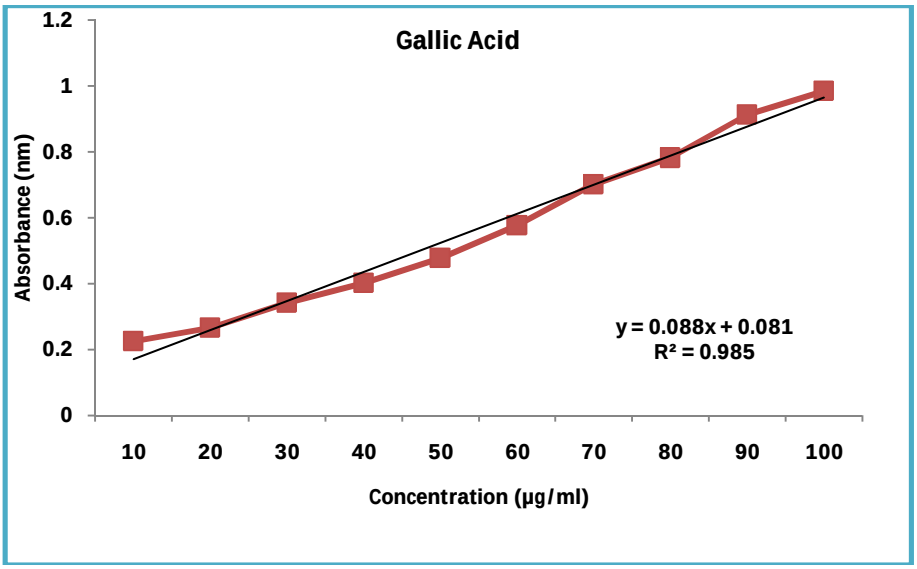

Figure 3: Represents the standard curve of Gallic Acid.

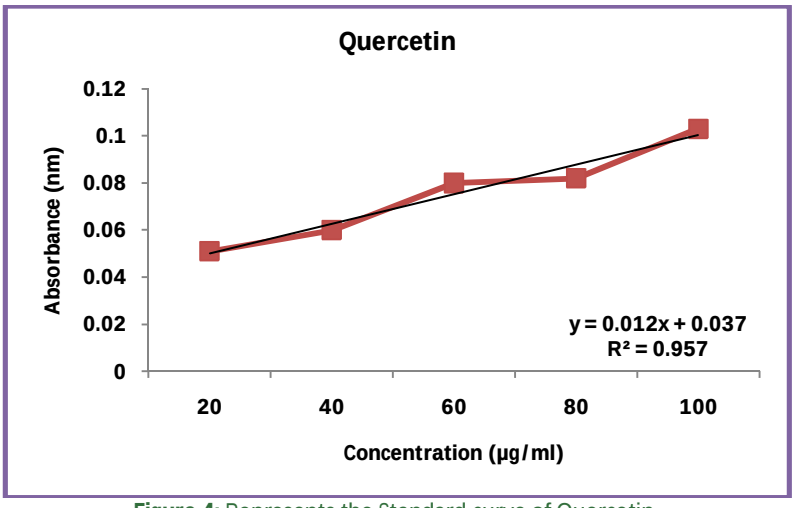

Figure 4: Represents the Standard curve of Quercetin.

DISCUSSION

The primary antioxidant activity has been determined with the help of stable radical DPPH. The DPPH anti-oxidant assay is based on the capability of DPPH a stable free radical, to change the colour in the existence of antioxidants[25]. The medicinal plants possess various constituents with disease remedial power. The harm caused due to the increased number of free radicals results in aging by numerous disease causative agents such as carcinogenesis[26], Alzheimer's disease and Parkinson's disease[27]. The metabolism is endorsed to the formation of highly reactive oxygen species, which start lipid peroxidation and the resulting destruction and damage to the cell membrane[28]. The extracts of *Rubia cordifolia* were effective in inhibition of free radicals and restoration of damage[29]. The leaf extract is administrated internally to reduce uterine pain by tribal people of Maharashtra[30]. Whereas, Baiga tribals in Madhya Pradesh consider leaf extract of *Rubia cordifolia* as blood purifier.[31]. The stem and leaf extract is used to treat mouth infection in children and in the treatment of pneumonia[32]. Leaf extract is used in the treatment of scabies and ringworm[33]. Phytochemicals by their biologically active polyphenols, such as flavonoids and phenolic acids, possess powerful antioxidant activities. These compounds act as oxidation terminators by scavenging free radicals[34]. In our findings, the in vitro antioxidant capacity of ethanolic extract of *Rubia cordifolia* observed through different methods (DPPH, reducing power assay) confirmed that phenolic compounds present in the extract are strong scavengers of free radicals.

CONCLUSION

The present findings reveal that the ethanolic extract of *Rubia cordifolia* possesses profound in vitro antioxidant and hepatoprotective activity due to the valuable amount of phytoconstituents. These findings indicate that the use of *Rubia cordifolia* might be considered as an accessory therapeutic strategy to combat hepatic disorders and oxidative stress-related diseases.

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