Research Article

In vitro Screening of Antioxidant Potential in *Rubia cordifolia* L.

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ABSTRACT

The present investigation has been carried out to evaluate the *in vitro* antioxidant potential of methanol and aqueous extracts of root, stem and leaf of *Rubia cordifolia* L. a medicinally important plant. The free radical scavenging activities of different parts of this plant were examined using DPPH (1, 1-diphenyl-2-picrilhydrazyl) method. The results showed that all the extracts possessed free radical scavenging potential with the highest amount possessed by aqueous and methanol extract of root with an IC_{50} value of 41.00 (µg/ml) and 61.95 (µg/ml) respectively. It can be concluded that various parts of this valuable medicinal plant may be utilized as natural medicine to eliminate the free radicals.

Keywords: Antioxidant potential, *Rubia cardifolia* L., DPPH.

INTRODUCTION

The medicinal plants and their derivatives have long been recognized as an tremendous source of therapeutically effective medicines as they contain bioactive compounds which are potential source of drugs and more reliable than synthetic products[1]. The bioactive compounds are by products of primary metabolisms of species. Therefore plants are regarded as a valuable repository of usual chemical compounds, since these bioactive compounds are usually chemical fingerprints of individual species[2]. Approximately 80% of people depend on plant based traditional medicines for their primary health care needs of developing countries[3]. A wide range of medicinal plant parts possessing a variety of pharmacological activities, such as root, stem, bark, leaves, flowers and fruits extracts which are used as powerful raw drugs. The plant derived drugs reflects its recognition of the validity of many traditional claims regarding the value of natural products in health care[4].

Rubia cordifolia L. (Rubiaceae) is a well-known rare medicinal plant commonly known as Indian madder. It is a hard climbing perennial herb grows in moist hill regions of India and tropical forests of Japan, Indonesia and Sri Lanka[5]. The nature of medicinal property of this plant is an essential raw drug for the traditional herbal formulations such as Aswagandharistam, *Gulguluthikthkarishtam, Jaatyaadighrita, Madhookasavam, Majishthaaditaila, Useerasavam* etc[6]. The stem of this plant is applied for treating cobra-bite and scorpion-sting[7], roots possess astringent and diuretic properties, being widely used in traditional medicinal formulation to treat amenorrhoea, dropsy, intestinal tony, chronic diarrhoea, renal calculi, jaundice and paralysis [5]. There is a need to study the pharmacologically important features in order to find out the importance of this valuable medicinal plant in traditional medicine. Hence this present investigation has been undertaken for screening *in vitro* antioxidant activity in root, stem and leaf extracts of *Rubia cordfolia* L.

MATERIALS AND METHODS

Collection of Plant material

The plants were collected from their natural habitat, Tirumala hills, Chittoor District, Andhra Pradesh, India. The plant was authenticated in Botany Department, Kakatiya University, Warangal, Telangana state, India.

Preparation of plant extract

The collected plant material was carefully washed under running tap water followed by sterilized distilled water and shade dried at room temperature for 8-10 days. These dried plant material was then homogenized to a fine coarse powder using an electronic blender and then stored in air tight containers until use. Methanol and water (aqueous) were used for extraction. 10 g of homogenized powders of root, stem and leaf were soaked in conical flasks containg solvents of 100 ml i.e. methanol and water separately. These conical flasks were then allowed to stand for 30 min on a water bath with occasional shaking, and were then shifted to rotary shaker at 200rpm for 24h[8, 9, 10]. Finally each solvent extracts (methanol and water) of different parts of root, stem and leaf were filtered separately through a sterilized Whatman No.1 filter paper and concentrated to dryness under vacuum at 40^0C using rotaevaporator. Thus the obtained dried extracts which were stored at 4^0C in labeled sterile bottles until further use[11, 12].

Anti oxidant potential by DPPH Method.

DPPH radical scavenging is one of the most extensively used method for *in vitro* antioxidant assay for plant extracts developed[13, 14] and modified[15]. The Antioxidant activity was assayed by 1, 1-diphenyl-2-picrilhydrazyl (DPPH) at the concentration of 0.2Mm in methanol (7.9mg/dl) using spectrophotometer[16]. The reduction in

absorbance of DPPH solution was monitored at 517 nm after addition of various concentrations of sample extracts to DPPH reagent and these solutions were maintained at room temperature for 20 min before taking the reading. The IC50 values for test samples were calculated from the calibration curves of concentration of extract (µg/ml) versus % of reduction in absorbance (% of inhibition) after subjecting to linear regression between 10–100 %. This activity was expressed as effective concentration at 50% (IC_{50}) that is the concentration of the test solution required to give a 50% reduction in absorbance of the test solution as compared to that of blank solution. In this assay ascorbic acid was used as a positive control. The inhibitory effect of DPPH was calculated according to the following formula:

Inhibition (%) = {(Absorbance $_{control}$- Absorbance $_{sample}$)/ Absorbance $_{control}$} x 100

Procedure

In clean and dry test tubes 0.1 ml of various concentration of test samples were taken (10µg, 20 µg, 40 µg, 60 µg, 80 µg, and 100 µg). To each test tube, 1ml of solvent (methanol/distilled water) and 2ml of DPPH reagent solutions were added with micropipette and mixed thoroughly, then immediately the intensity of color (O.D) in each tube was read at 517 nm using spectrophotometer. The O.D was read against blank prepared in identical way but without the addition of test sample. Standard graph was prepared using various concentrations of ascorbic acid solution against % of inhibition.

Statistical Analysis

All the experiments were repeated thrice and the results obtained. All statistical comparisons were made by unpaired 't' test and p-value < 0.05 were considered statistically significant. Simple correlation analysis was carried out and significance was tested by using standard methods [17].

RESULTS

The methanol and aqueous extracts of root stem and leaf of *R.cordifolia* were tested against DPPH radical scavenging activity. The IC_{50} values of free radical scavenging activity of methanol, aqueous extract and standard (ascorbic acid) were shown in **Table**.1, **Fig**.1, 2 & 3. The free radical scavenging potential of solvent extracts of various plant parts were in order of root › leaf › stem for aqueous and in methanol extract were root › leaf › stem.

DISCUSSION

The medicinal plants possess different biologically active constituents with disease curing capacity. The increased free radical mediated damage plays a crucial role in aging process by several disease causative agents such as carcinogenesis[18], Alzheimer's disease and Parkinson's disease[19]. The slow progression of these diseases the strategy employed to antagonize the formation of free radicals and their action[20]. The therapeutic point of view findings of *R.cordifolia* extractions were effective in inhibition of free radicals[21].

Table 1: IC_{50} values of Methanol and aqueous extracts of *R. cordifolia*.

Compound/Concentration (µg/ml)	IC_{50} Values of Solvent Extracts (µg/ml)	
	Methanol	Water
Root	61.95	41.00
Stem	84.59	82.84
Leaf	70.56	79.61
Control	8.28	8.28

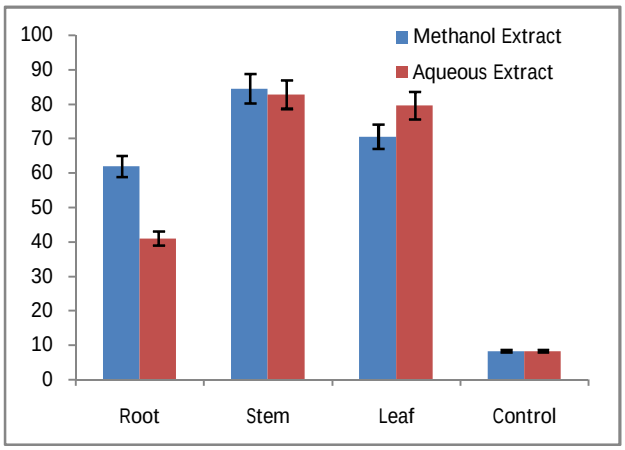

Figure 1: IC_{50} values of different parts of *R.cordifolia* in various extracts.

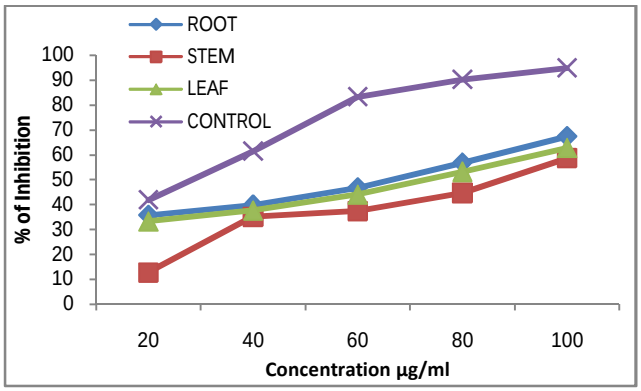

Figure 2: % of Inhibition of aqueous extracts of different parts of *R.cordifolia*

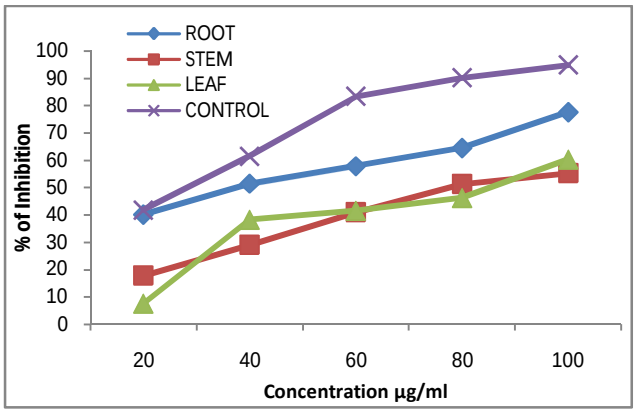

Figure 3: % of Inhibition of aqueous extracts of different parts of *R.cordifolia*

The free radical scavenging activity of the methanol and water extracts of root, stem and leaf carried out by DPPH method. The result obtained showed that the maximum reduction in absorbance in water extract of root followed by methanol extract of root. Among these results, the aqueous extract of root possess significant antioxidant potential and all the results of present investigation made the *R.cordifolia* that it possessed significant antioxidant potential. The previous studies revealed that, the methanol extracts showed antioxidant potential[22, 23] but our present studies on *in vitro* screening of antioxidant potential of *Rubia cordifolia* have shown that aqueous extracts possessed significant antioxidant potential than methanol.

CONCLUSION

Medicinal plants play an important role in curing various chronic diseases and were used in traditional medicine such as Ayurveda, Siddha, Unani etc. *In vitro* study of antioxidant potential in *Rubia cordifolia* reveals that all the extracts show potent antioxidant activity and this study supports its use in traditional medicine from times immemorial. It can be concluded that different parts of *R. cordifolia* also may be utilized in the preparation of drug formulations in modern medicines too in the treatment of different ailments caused due to the excessive production of free radicals.

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