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Original Article

Phytochemical screening, antioxidant and antimicrobial activities of leaf extracts of *Randia uliginosa*

Md. Solayman Hossain[1], Mir Monir Hossain*[2], Sinthia Zaman[1], Milon Mondal[1], Md. Sohel Rana[1]

[1]Laboratory of Natural Products Research, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh
[2]Assistant Professor, Department of Pharmacy, University of Science and Technology Chittagong (USTC), Foy's Lake, Chittagong-4202, Bangladesh

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ABSTRACT

Phytochemical consisting of phenols and flavonoid possess antioxidant properties, which are useful to scavenge reactive oxygen species (ROS).The present study was conducted to evaluate antioxidant and antimicrobial activities of leaf extracts of roots of *Randia uliginosa*, using DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging assay, cupric reducing antioxidant capacity, nitric oxide scavenging assay, ferric reducing antioxidant power, total antioxidant capacity, determination of total phenol and flavonoid contents and disc diffusion technique. Preliminary phytochemical study revealed the presence of alkaloid in the extracts. The tested fraction showed significant antioxidant activities in the assay compared to the reference ascorbic acid in a dose dependent manner. In DPPH radical scavenging assay, the IC50 value of the crude chloroform extract was 399.12 µg/mL, whereas IC50 value for the reference ascorbic acid was 8.77 µg/mL. In case of nitric oxide scavenging assay, the IC50 value of the crude chloroform extract was 58.27 µg/mL, whereas IC50 value for the reference ascorbic acid was 51.07 µg/mL. Moreover, at 200 µg/mL extract concentration, lower grade total antioxidant activity (36.27±1.39 mg/g equivalent to ascorbic acid) was observed. Furthermore, extract showed good cupric reducing power and reducing power capability. In addition, significant amount of flavonoids and low phenols content were obtained from the extract. In case of antimicrobial activity studied using different solvent like methanol, chloroform, petroleum ether against bacterial strains like *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, P.aeruginosa, E.coli, S.typhi, Serratia spp. and P.mirrabilis.* Varying concentration of each extracts 100 mg/ml, 50 mg/ml, 25 mg/ml prepared by using disc diffusion method. Among all the extracts used, methanol extract was found to be highly active against the entire organism when compared with amoxicillin 25 mg/ml. The results suggest that *Randia uliginosa* can be used as a medicament for various infections.

Key Words: Extracts, antioxidant, microorganisms, DPPH, nitric oxide, flavonoid

INTRODUCTION

Oxygen a wonderful gift of God for the mankind and other aerobes. Whenever oxygen is used by the cell for energy, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are created. These species can accelerate a deleterious process at a higher concentration which can results damage of cellular structures (Halliwell B, *2007;* Valko M *et al*, 2007). These can lead to many degenerative diseases, such as brain dysfunction, cancer, heart diseases, age-related degenerative conditions, declination of the immune system, cancer, coronary arteriosclerosis, ageing processes, carcinogenesis, gastric ulcer and DNA damage arise (Grzegorczyk *et al*, 2007; Kumaran and Joel, 2007; Shen *et al*, 2010; Kannan *et al*, 2010; Prakash *et al*, 2007, Slater, 1984). Antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (Soares *et al, 1997*). Antibiotics are getting resistant day by day as they are been used to treat a number of infectious disease irrationally and cross-resistance is also occurring. This excelled the search for new antibiotic principles in traditional medicinal plants. In recent years, antimicrobial properties of medicinal plants are being increasingly reported and the active principles are being approached to be isolated from the crude extracts (Grosvenor *et al.*, 1995; Ratnakar and Murthy, 1995; Saxena, 1997).These substances

***Corresponding Author Address:** Mir Monir Hossain, Assistant Professor, Department of Pharmacy, University of Science and Technology, Chittagong (USTC), Foy's Lake, Chittagong-4202, Bangladesh; E-mail: monir_pharm@yahoo.com*

serve as plant defence mechanisms against predation by microorganisms, insects and herbivores.

Randia uliginosa (Family: Rubiaceae), locally known as ''Piralu'' a medicinal herb, grows in dry deciduous forests, native to Bangladesh, India, Sri Lanka, and Thailand. *Randia uliginosa* is widely used in traditional medicine. Fruits of this plant are used as astringent, Cholera, diarrhea, dysentery, eye complaints, headache, pimples and sores, while the roasted pulp is used as a remedy in diarrhea and dysentery, especially during pregnancy and pulp is applied on boils. (Sudhakar K *et al.).* Roots are used as cooling, diuretics, tonic properties, biliousness, boil in children, diarrhea, aphrodisiac and dysentery (Sudhakar K *et al.).*The present research was aimed to investigate the antimicrobial activities of *Randia uliginosa* in order to understand the usefulness of this plant as medicine.

MATERIALS AND METHODS

Selection of plant: The plant *Randia uliginosa* was selected for study. Its leaves were collected from Jahangirnagar University campus, Savar, Dhaka, Bangladesh in September, 2013.The collected leaves were identified and authenticated by experts in Bangladesh National Herbarium, Mirpur, Dhaka, where a Voucher specimen (37959) has been deposited for future reference.

Leaf extract: The completely shade dried material was coarsely powdered and allowed soxhlet for successive extraction with methanol chloroform and petroleum ether. The obtained liquid extracts were subjected to Rotary evaporator and subsequently concentrated under reduced pressure (in vacuum at 40°C) and evaporated to dryness and stored at 4°C in air tight bottle.

Methanol extract: 50g of dried leaf powder were taken in a separate container. To this 250ml of methanol was added and kept for 24 h with periodic shaking then filtered and the filtrate was collected. The procedure was repeated three times with fresh volume of methanol. The filtrates were pooled.

Chloroform extract: 50g of dried leaf powder of *Randia uliginosa* were taken in a separate container. To this 250 ml of chloroform was added and kept for 24 h with periodic shaking. Filtered and the filtrate was collected. The procedure was repeated three times. The collected filtrates were pooled.

Petroleum ether extract: 50g of dried leaf powder of *Randia uliginosa* were taken in a separate container. To this 250 ml of petroleum ether was added and kept for 24 h with periodic shaking. Filtered and the filtrate was collected. The procedure was repeated three times. The collected filtrates were pooled.

Chemicals: DPPH (1, 1-diphenyl, 2-picrylhydrazyl) was purchased from Sigma Chemical Co., USA, potassium fericyanide [$K_3Fe(CN)_6$] from Loba Chemie Pvt. Ltd., Mumbai, India, Ascorbic acid from SD Fine Chem. Ltd., Biosar, India, Vincristine sulphate from Jayson Pharmaceuticals Ltd, Bangladesh and neocaproin ($C_{14}H_{12}N_2$), ammonium molybdate, Folin-Ciocalteu phenol reagent, gallic acid ($C_7H_6O_5.H_2O$), quercetin were purchased from Merck, Germany. All other chemicals and solvents for extractions were of analytical grade. All UV-Vis measurements were recorded on a Shimadzu UV-1601 (Kyoto, Japan) spectrophotometer.

Microorganisms: The antimicrobial activity assay of the extracts was carried out on both Gram-positive and Gram-negative bacteria.

Gram positive	***Gram negative***
Bacillus cereus	*E.coli*
Bacillus subtilis	*Serratia spp.*
S.aureus	*S.typhi*
	Pseudomonas spp.
	P.mirrabilis

These strains were obtained from Department of Microbiology, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Preliminary phytochemical screening: The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. Phytochemical screenings of the extract was performed using the following reagents and chemicals; alkaloids with Dragendroff's reagents, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions and saponins with ability to produce stable foam and steroids with Libermann- Burchard reagent. Gum was tested using Molish reagent and concentrated sulfuric acid; reducing sugars with Benedict's reagent. These were identified by characteristic color changes using standard procedures by Ghani A, 2005.

Tests for antioxidant activity

DPPH free radical scavenging activity: The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical, were determined by the method described by **Braca *et***

al., 2001. Plant extract (0.1 mL) was added to 3 mL of a 0.004 % ethanol solution of DPPH. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated from [(Ao-A1)/ Ao] ×100, where Ao is the absorbance of the control (DPPH solution) and A1 is the absorbance of the extract/standard. The inhibition curves were prepared and IC50 values were calculated.

Ferric reducing antioxidant power (FRAP): The ferric reducing antioxidant power was determinedaccording to the method previously described by **Oyaizu, 1986.** According to this method, the reduction of Fe3+ to Fe2+ isdetermined by measuring the absorbance of Perl's Prussian bluecomplex. Briefly, different concentrations of extracts (5-200 µg) in1 mL of distilled water were mixed with phosphate buffer (2.5 mL,0.2 M, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. An aliquot (2.5 mL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl3 (0.5 mL, 0.1 %) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the reference.

Cupric reducing antioxidant capacity (CUPRAC): The cupric reducing antioxidant activity of the pet ether and methanol extracts were determined by the method described by **Resat *et al*., 2004**. Different concentrations of the extract (5-200 µg) in 0.5 mL of distilled water were mixed with Cupric Chloride (1 mL, 0.01 M), Ammonium acetate buffer (1 mL, pH 7.0), Neocaproin (1 mL, 0.0075 M) and finally distilled water (0.6 mL). The mixture was incubated for 1 hour at room temperature. Then the absorbance of the solution was measured at 450 nm against blank. Distilled water (0.5 mL) in the place of extract is used as the blank. The molar absorptivity of the CUPRAC method for each antioxidant was found from the slope of the calibration line concerned. Ascorbic acid was used as the standard solution.

Nitric oxide Scavenging Assay: Based on the principle that the compound sodium nitroprusside (SNP) is known to decompose in aqueous solution at physiological pH (7.2) producing NO**.**Under aerobic conditions, NO**.**reacts with oxygen to produce stable products: nitrate and nitrite, the quantities of nitrate and nitrite can be determined using Griess reagent. The scavenging effect of *R.uliginosa* extract on nitric oxide was measured according to the method of (**Alisi and Onyeze, 2008**). Briefly 4ml of extracts olution at different concentrations were added (in the test tubes) to 1ml of sodiummnitroprusside (SNP) solution (25mM) and the tubes incubated at 29°C for 2 hours. A 2ml aliquot of the incubation solution was diluted with 1.2ml Griess Reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine-dihydrochloride). The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately read at 550nm.The percentage (%) inhibition activity was calculated from the following equation. $\{(A_0 - A_1)/A_0\}$ X 100.Where, A_0 is the absorbance of the Control and A_1 is the absorbance of the extract or standard.IC_{50} was calculated by linear regression method.

Determination of total antioxidant capacity: The antioxidant activity of the extracts were evaluated by the phosphomolybdenum method according to the procedure describe by **Prieto *et al***, 1999. A 0.3 mL extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95ºC for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer (UV visible spectrophotometer, Shimadzu, 1601) against blank after cooling at room temperature. Methanol (0.3 mL) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Determination of total phenol content: The total phenolic content of plant extracts were determined using Folin–Ciocalteu reagent (**Yu L *et al*, 2002**). Plant extract (100 µL) was mixed with 500 µL of the Folin–Ciocalteu reagent and 1.5 mL of 20 % sodium carbonate. The mixture was shaken thoroughly and made up to 10 mL using distilled water. The mixture was allowed to stand for 2 hour. Then the absorbance at 765 nm was determined. These data were used to estimate the phenolic contents using a standard curve obtained from various concentration of gallic acid.

Determination of total flavonoid content: The content of flavonoids compounds in the extracts was determined by the method described by **Chang *et al*., 2002**. 1.0 mL of extract was mixed with methanol (3 mL), aluminium chloride (0.2 mL, 10 %), potassium acetate (0.2 mL, 1 M) and distilled water (5.6 mL) and incubated the mixture for 30 min at room temperature. Then the absorbance was measured at 415 nm against blank. Methanol (1 mL) in the place of extract was used as the blank

and Quercetin was used as the standard solution. All determinations were carried out in triplicates. The amount of flavonoids in plant extracts in quercetin equivalents (QE) was calculated by the following formula: $X= (A \times m_0)/ (A_0 \times m)$, where X is the flavonoid content, mg/mg plant extract in QE, A is the absorption of plant extract solution, A_0 is the absorption of standard rutin solution, m is the weight of plant extract in mg and m_0 is the weight of quercetin in the solution in mg.

Antimicrobial activity by disc diffusion method: The antimicrobial activity of the plant extract was performed by the well accepted Bauer-Kirby disc diffusion method (**Bauer *et al.*, 1966; Drew *et al*., 1972**). The inoculums of microorganisms were spread over nutrient agar plates with a sterile swab.100 mg of each test samples were dissolved in 1 ml of respective solvent to obtain the concentration 100 µg/µl in an aseptic condition. Sterilized metrical filter paper discs (Whatman No. 1, 6 mm diam.) were soaked with 30 µl, 20 µl and 10 µl of solutions of test samples. Then the disks were placed on the previously marked agar plate and dried. Each extract was tested in triplicate and the plates were inoculated at 37°C for 24 h. Antimicrobial activities were evaluated by measuring inhibition zone diameters. Amoxicillin was used as positive control.

Statistical analysis: The results were expressed as mean ± standard deviation (SD) from triplicate experiments and evaluated with the analysis of student's t-test. Differences were considered significant at a level of $P<0.05$. IC50 was calculated using Sigma Plot 11.0 software.

RESULTS AND DISCUSSION

Preliminary phytochemical screening:
Preliminary phytochemical screening revealed the presence of various bioactive components like alkaloid, carbohydrate, saponin, steroid and tannin (Table 1).

Determination of total phenol content: Several reports have conclusively shown close relationship between total phenolic content and antioxidative activity of the fruits and vegetables. Phenolic compounds, as natural antioxidants exhibit therapeutic potential in multiple diseases including cardiovascular disease, aging and cancer (**Vinson *et al.*, 1998**). It has been reported that phenolic compounds with *ortho*- and *para*-dihydroxylation or a hydroxyl and a methoxy group are more effective than simple phenolics (**Frankel *et al.*, 1995**). Moreover, the antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (**Uritani *et al.*, 1994**).However, the methanol extract of the *R.uliginosa* was found to contain small amount of phenolics, 5.98 ± 1.53mg/g Gallic acid equivalent (GAE) using Folin-Ciocalteau method. **The result was represented in Table 2**. As the exact chemical nature of the Folin- Ciocalteu reagent is not known, but it is believed to contain hetero poly phosphotunstates molybdates. Sequences of reversible 1 or 2 electron reduction reactions lead to blue species, possibly PMoW11O40 (**Yu *et al.*, 2002**).

Determination of total flavonoids content:
Flavonoids play an important role in antioxidant system in plants. The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation (***Benavente-Garcia, 1997***). However, total flavonoid content of *R.uliginosa* extract is shown in Table 2. The result was exhibited as quercetin equivalent of flavonoids per gm of extracts of the sample. For the claimed extract, the total flavonoid content was found to be 52.42 ± 0.72 mg/g equivalent to quercetin. These results suggested that the antioxidant activities of *P.zelanica* might be due to its flavonoid content.

Determination of total antioxidant capacity content: The total antioxidant capacity of the root extract of the *R.uliginosa* is given in Table 2. Significant amount of total antioxidant activity was obtained from the chloroform extract (36.27±7.39 mg/g equivalent to ascorbic acid) at 200 µg/mL extract concentration. The phosphomolybdenum method was based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts (**Prieto *et al*, 1999**). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid.

$DPPH^-$ radical scavenging activity: In DPPH radical scavenging assay, as shown in Fig.1, here the extract exhibited a concentration dependent antiradical activity by inhibiting $DPPH^-$ radical. Ascorbic acid, which is a well known antioxidant, showed higher degree of free radical-scavenging activity than that of the plant extract at each

concentration points. The IC50 value of the crude chloroform extract was 399.12 µg/mL, while the IC50 value for the reference ascorbic acid was 8.77 µg/mL. The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants (**Kumarasamy *et al.*, 2007**). The method is based on the reduction of ethanolic $DPPH^{-}$ solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by reaction. The extracts were able to reduce DPPH radical (visible deep purple color) to the yellow-coloureddiphenylpicrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (e.g. hydroquinone, pyrogallol, gallic acid), and aromatic amines (e.g. p-phenylenediamine, p-aminophenol), reduce and decolorise 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability (**Blois, 1958**).

Ferric reducing antioxidant power (FRAP): Fig. 2 shows the reducing power capabilities of the plant extract compared to ascorbic acid. The extract displayed moderate reducing power which was found to rise with increasing concentrations of the extract. In reducing power assays, the presence of antioxidants in the root can reduce the oxidized form of iron (Fe^{3+}) to its reduced form (Fe^{2+}) by donating an electron. Thus, it can be assumed that the presence of reductants (i.e. antioxidants) in *R.uliginosa* extracts causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. A higher absorbance indicates greater reducing power ability (**Gordon, 1990**).

Cupric reducing antioxidant capacity: The reducing ability of a compound generally depends on the presence of reductants (**Pin-Der *et al.*, 1999**), which have been reported to exhibit antioxidative potential by breaking the free radical chain, donating a hydrogen atom (Gordon, 1990).

The CUPRAC method of reducing antioxidant capacity assay uses bis(2,9-dimethyl-1,10 phenanthroline: neocuproine) Cu(II) chelate cation as the chromogenic oxidant, which is reduced in the presence of n-electron reductant antioxidants to the cuprous neocuproine chelate [Cu(I)–Nc] showing maximum light absorption at 450 nm. Colour development in the CUPRAC method is based on the following reaction:

$n\text{-}Cu(Nc)_2^{2+}$ + n-electron reductant (AO) ↔ $nCu(Nc)^{2+}$ + n- electronoxidized product + n H+

Where, the electrons required for the formation of the Cu (I)–Nc chromophore are donated by the tested antioxidants. In this reaction, the reactive Ar-OH groups of polyphenolic antioxidants are oxidized to the corresponding quinones (Ar=O) (ascorbic acid is oxidized to dehydroascorbic acid) and Cu (II)-Nc is reduced to the highly colored Cu (I)-Nc chelate (**Resat *et al.*, 2008; Reşat *et al.*, 2007**).

As observed from Fig. 2, at concentration level of 200 µg/mL, the reducing capacity of chloroform extract and ascorbic acid is 0.412 and 0.744 respectively. According to changed concentration trend, we concluded that the reducing power of extracts were lower than that of ascorbic acid. The probable mechanism of Cupric reducing power of extracts, would be the resultant of having a good number of polyphenolics and flavonoids, as the reactive hydroxyl groups of polyphenolics, oligomeric flavonoids, is oxidized with the CUPRAC reagent to the corresponding quinines (**Resat *et al.*, 2004**).

NO Scavenging assay: Nitric Oxide (NO) scavenging assay is based on the scavenging ability of the extracts as well as ascorbic acid, which is used as standard. The scavenging of the NO generated from sodium nitroprusside in vitro indicates the possibility of preventing the peroxynitrite formation in the cell in vivo (**Joseph *et al.*, 2009**). Reducing the nitric oxide generation in the digestive tract was reported to be effective in preventing the reactions of nitrate with amines and amides to form carcinogenic nitrosamines and nitrosamides (**Boone *et al.*, 1990; Joseph *et al.*, 2009**).The scavenging of NO was found to increase in dose dependent manner. The IC50 value of the crude chloroform extract was 58.27µg/mL, while the IC50 value for the reference ascorbic acid was 51.07µg/mL. Based on these we speculate that nitric oxide scavenging activity of *R.uliginosa* may have great relevance in the prevention and control of disorders where NO is thought to play a key role.

Antimicrobial activity: Plants are important source of potentially useful sources for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (**Tona *et al.*, 1998**). Table: 1 reveals the antibacterial activity of different solvent extracts and also aqueous extract. A maximum zone of inhibition was observed against all the pathogens by methanol extracts of leaf of the plant *Randia uliginosa,* whereas petroleum ether, extract showed less zone of inhibition in comparison with the other extracts.

The standard, amoxicillin, exhibited significant zone of inhibition against all the test organisms.

CONCLUSION

The study clearly indicates that the chloroform extracts have the significant amount of antioxidants. This might be rationale behind the using of this plant extract as folk medicine. Since the chemical composition and structures of active extract components are important factors governing the efficacy of natural antioxidants, the extracts of *R.uliginosa* need their characterization. On the other hand the extracts **have poor cytotoxic property** compared to the standard. Therefore, further research is necessary for elucidating the active principles e.g. phenolic compounds and also in vivo studies are needed for understanding their mechanism of action as an antioxidant. In vitro studies in this work showed that the plant extracts inhibited bacterial growth but their effectiveness varied. Leaf Methanol extract of selected plants showed higher inhibition against tested bacteria at high concentration. The antibacterial activity has been attributed to the presence of some active constituents in the extracts. The demonstration of broad spectrum of antimicrobial activities by the plants used in this study may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. Therefore the effect of the plants on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out.

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Table 1: Zone of Inhibition produced by Leaf extracts of *Randia uliginosa* against some Gram positive and Gram negative bacteria.

Test microorganism	Zone of inhibition in mm									
	Extract Conc. µl/disc (RLME)			Extract Conc. µl/disc (RLPE)			Extract Conc. µl/disc (RLCL)			Standard Amoxicillin
	10	20	30	10	20	30	10	20	30	10
Bacillus subtilis	7.5	8	11	7	7.5	9	6.5	7	9	28.5
Bacillus cereus	10.5	12.5	13.5	7	7.5	9.5	ND	ND	ND	9.5
S.aureus	8.5	10.5	13	ND	ND	ND	ND	ND	ND	12.5
P.mirrabilis	11.5	11	12.5	8.5	9	9.5	9.5	11	11.5	9.5
E.coli	12.5	13.5	14.5	7	8.5	9	8	9	11	18
Serratia spp.	9	13	11	7	8.5	9	7.5	8.5	9	10.5
S.typhi	8.5	10	11.5	7	9.5	11	6.5	7	9	12
Pseudomonas spp.	9	9.5	12.5	7	9.5	9.5	6	6.5	7.5	20.5

*ND=Not Defined; Values are mean inhibition zone (mm) ± S.D of three replicates.

Table 2: Result of phytochemical screening of leaf extracts of *Randia uliginosa.*

EXTRACTS	Alkaloid test				Carbohydrate Test	Flavonoid Test	Glucoside Test	Glycoside Test	Saponin Test	Steroid Test	Tanin Test
RLME	+	+	+	-	+	-	+	-	+	+	-
RLPE	+	+	+	+	+	-	-	+	+	+	-
RLCF	-	+	+	+	+	-	+	-	+	+	-

RLME: *Randia uliginosa* leaf methanol, RLPE: *Randia uliginosa* leaf petroleum ethar, RLCF: *Randia uliginosa* leaf chloroform, Values are the average of triplicate experiments and represented as mean ± SD; (+): Present; (-): Absent.

Table 3: Total antioxidant capacity, total phenol and total flavonoid contents of leaf extracts of *Randia uliginosa.*

Extracts	Total antioxidant capacity equivalent to ascorbic acid mg/g plant extract	Total phenol (in mg/g, Gallic acid equivalents)	Total flavonoid (in mg/g, quercetin equivalents)
RLME	8.27±0.82	12.14±1.80	54.80± 2.16
RLPE	12.44±3.29	10.69±1.02	33.88± 2.16
RLCF	15.93±1.64	14.86±1.28	68.06±1.43

RLME: *Randia uliginosa* leaf methanol, RLPE: *Randia uliginosa* leaf petroleum ethar, RLCF: *Randia uliginosa* leaf chloroform, Values are the average of triplicate experiments and represented as mean ± SD

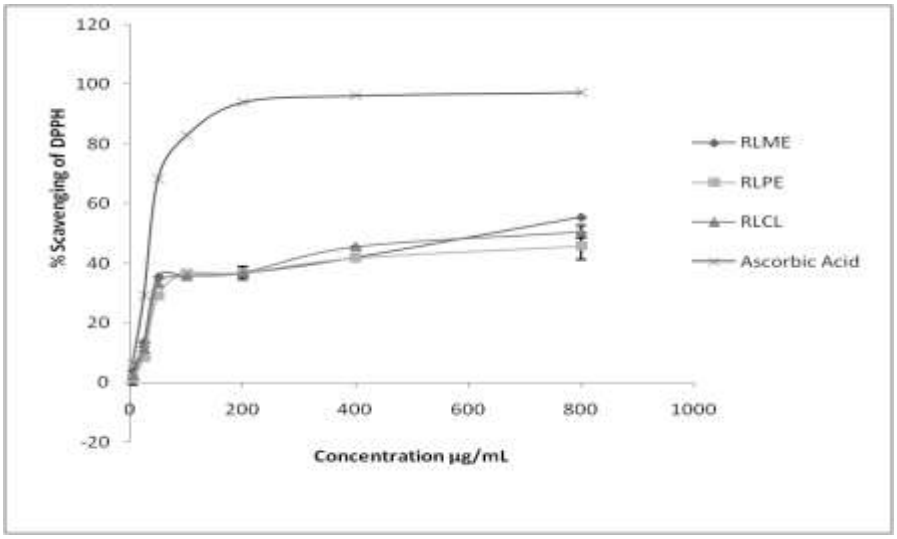

Fig. 1: DPPH scavenging activity of Leaf extracts of *Randia uliginosa* along with the standard ascorbic acid. Mean ± SD, n=3)

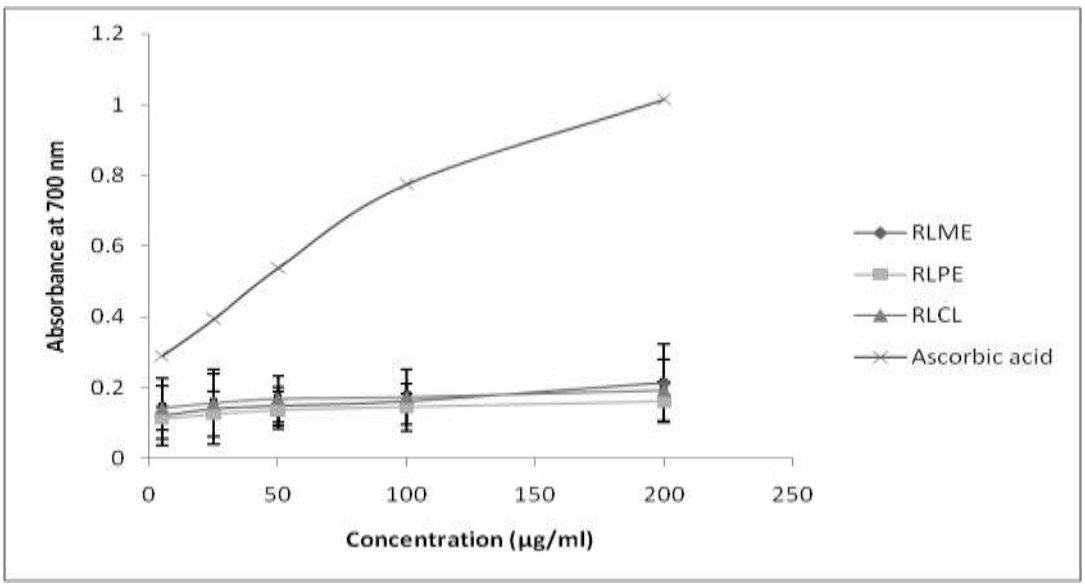

Fig. 2: Reducing power capacity of Leaf extracts of *Randia uliginosa* along with the standard ascorbic acid. Mean ± SD, n=3)

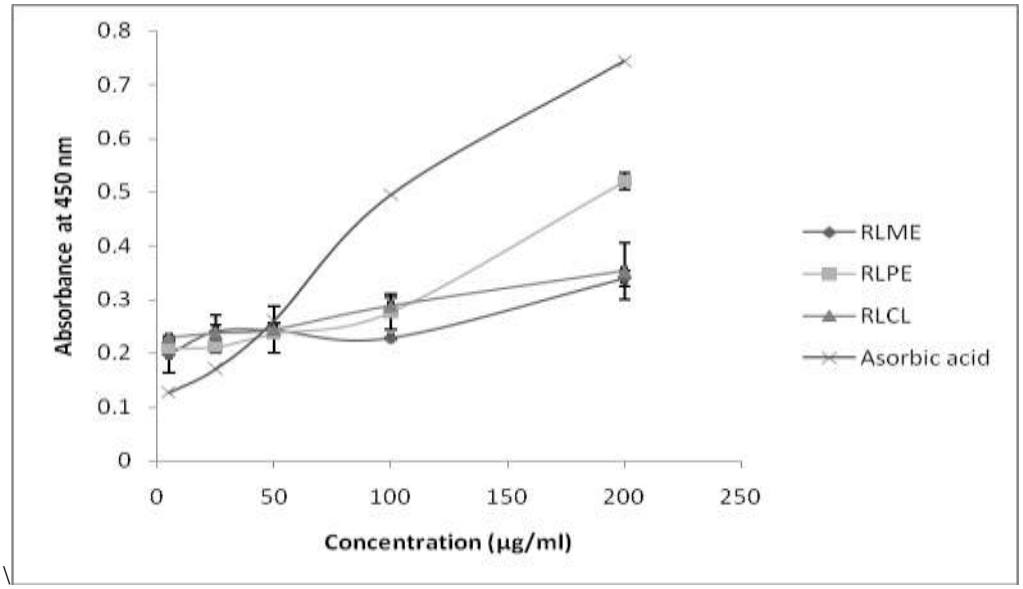

Fig. 3: Cupric reducing antioxidant activity of leaf extracts of *Randia uliginosa* along with the standard ascorbic acid. Mean ± SD, n=3)

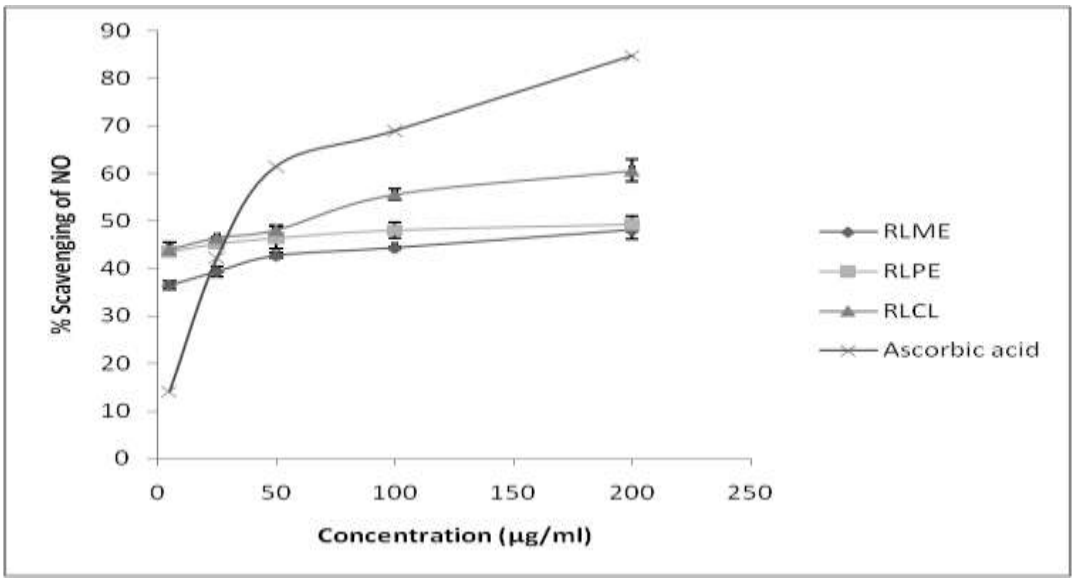

Fig. 4: NO scavenging activity of Leaf extract of *Randia uliginosa* along with the standard ascorbic acid. Mean ± SD, n=3)

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