

EVALUATION OF *IN VITRO* ANTIOXIDANT POTENTIAL OF AQUEOUS ROOT EXTRACT OF *CLERODENDRUM SERRATUM* L.RAJ KUMAR TIWARI^{1*}, UDAYABANU M², SILPI CHANDA³

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Received: 02 January 2017, Revised and Accepted: 27 January 2017

ABSTRACT

Objective: The intent of this report was to investigate the effect of aqueous root extract of *Clerodendrum serratum* L. for antioxidant activity using divergent models, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, superoxide scavenging assay, and ferric reducing antioxidant power (FRAP) assay.

Methods: The root of *C. serratum* was extracted using water. The yield of aqueous extract was 10% w/w. The outcome was examined statistically by the regression method.

Results: The inhibitory concentration 50% values are 85.43 and 107.59 µg/ml for DPPH radical scavenging assay and superoxide scavenging assay, respectively, whereas FRAP showed a significant reducing power activity with increased concentration of sample. The pilot study showed a significant correlation existed between concentrations of the extract and percentage grossment of free radicals.

Conclusion: The antioxidant property may be corresponding to the polyphenols and flavonoids adjacent in the extract. These results clearly revealed that *C. serratum* might be effective against diseases analogous with free radical mediated.

Keywords: *Clerodendrum serratum*, 2,2-diphenyl-1-picrylhydrazyl, Superoxide, Ferric reducing antioxidant power, Rutin, Antioxidant.

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INTRODUCTION

Human and plant relation is as old as its start of civilization and human being extensively depends on plant for food and cure [1]. India is known for its rich biodiversity and the use of herbal drugs since ancient time is well known and practiced as mentioned in Ayurveda [2]. Natural products play a foundation for drug discovery either in natural form or as lead for synthetic drug development. Isolation, extraction and modification of biologically active compounds lead toward novel and synthetic drugs from natural products by suitable biological or chemical means and had been significantly contributed for potent drugs [3].

Free radicals and reactive oxygen species (ROS) are generated in living cells as a result of physiological and biochemical processes which are associated with many chronic diseases, such as cancer, diabetes, aging, and other degenerative diseases in humans usually due to oxidative damage of proteins, lipids, and DNA [4]. Phenolic classes of secondary metabolites have antioxidant properties and are acknowledged for various ailments. They possess antiapoptosis and anti-inflammatory activities, and these biological connections are due to the potentiality of phenols [5]. ROS levels can dramatically increase, resulting in significant damage to cell structure [6]. Lifestyle disorders such as heart problems [7], inflammation, stroke, and metabolic disorders are common problems in human community [8]. Studies reveal that there is a correlation between high intake/high blood levels of antioxidants and low incidence of different types of cancer [9]. Antioxidant compounds that hunt free radicals help safeguard against different kinds of degenerative diseases [10]. Plants, as the source of medicine, have been playing an important role to promote our health. The search for potent natural antioxidants which also have traditional claim with medicinal value is still in progressing. One such plant is *Clerodendrum serratum* L. belonging to family Verbenaceae, commonly known as Bharangi. *C. serratum*

is a deciduous shrub widely distributed in Western Ghats [11]. In Ayurveda, the plant is popular as Bharangi (Sanskrit) and customarily known as Blue Glory (English). As per the traditional claims, the roots are the potential source of drug for ailments such as allergic disease, body soreness, respiratory illness, infectious disease, dropsy, eye diseases, fever, inflammations, malaria, ophthalmia, rheumatism, snakebite, tuberculosis, ulcers, and wounds [12]. The current study was focused on its antioxidant properties which may be the main reason of its traditional claim.

METHODS

Collection and authentication of plant material

Roots of *C. serratum* L. were purchased from Faizabad (Uttar Pradesh) in January. The taxonomical identification was done from Department of Agronomy, Acharya Narendra Dev Agriculture Technical University, Dist. Faizabad, Uttar Pradesh.

Preparation of extract

The collected brown-colored roots (100 g) were extracted (decoction) with water (500 ml) at a temperature not exceeding 110°C for 2 hrs. The extract was strained and lyophilized. The yield of obtained dried extract was 10% w/w.

Chemicals

Chemicals were procured from Sigma-Aldrich Co., St. Louis, USA. All other chemicals/solvent adopted was of analytical grade.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH assay of extract was determined using ultraviolet spectrophotometer [13,14], which involved the preparation of standard, test, and control.

Preparation of standard rutin solutions

Different solutions (20-100 µg/ml) of the rutin were prepared in methanol. 1.5 ml of each solution of rutin was mixed with 1.5 ml of 200 µM DPPH solution and fecundated for 30 minutes at room temperature in dark. Absorbance of each solution was taken after 30 minutes against methanol (as blank) at 517 nm.

Preparation of test solutions

Different solutions of the extract were prepared in methanol to give concentrations in the range of 20-100 µg/ml. 1.5 ml of each extract solution was blended with 200 µM DPPH solution and incubated for 30 minutes at room temperature. Optical density was measured after 30 minutes against methanol (as blank) at 517 nm.

Preparation of control solution

For control, 1.5 ml of methanol was mixed with 200 µM DPPH solution and incubated for 30 minutes at room temperature in dark. Absorbance of the control was taken after 30 minutes against methanol (as blank) at 517 nm. Percentage antioxidant activity of plant extract and rutin was calculated using the following formula:

$$\% \text{Inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Superoxide scavenging assay

Reagents and chemicals

1 ml of alkaline dimethyl sulfoxide (DMSO) contains 0.1 ml of 5 mM NaOH and 0.9 ml of DMSO. Nitroblue tetrazolium (NBT) solution was prepared by dissolving 25 mg of NBT in 25 ml DMSO to give concentration of 1 mg/ml.

Methodology

Different concentrations (20-100 µg/ml) of rutin and test sample were prepared. To the reaction mixture carrying 0.1 ml of NBT, 0.3 ml of extract and 1 ml of alkaline DMSO was added to give a final volume of 1.4 ml, and the absorbance was fixed at 560 nm. Plain DMSO used as blank and reaction mixture without extract (water in place of extract) used as control. Depreciate absorbance of the reaction mixture designated the increasing of superoxide anion scavenging activity. 50% inhibition of extract was determined by plotting a graph between absorbance and concentration [15].

$$\% \text{Inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Ferric reducing antioxidant power (FRAP) assay

The endowment to reduce ferric ions was deliberated using the method described by Benzie and Strain [16,17].

Reagent preparation

FRAP reagent was accomplished by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM tripyridyl triazine solution, and 20.0 mM FeCl₃·6H₂O solution in a proportion of 10:1:1 in volume.

Procedure

Rutin and test samples at divergent combinations (100-500 µg/ml) were added to 3 ml of FRAP reagent, and the counterpoise was incubated at 37°C for 30 minutes. The increase in absorbance at 593 nm was measured. The antioxidant capacity is based on the ability to reduce ferric ions of sample to ferrous ions.

RESULTS AND DISCUSSION

The main mechanism behind the antioxidation properties of any compound is inhibition of generation of free radicals. This can be achieved either by scavenging or decomposition of lipid peroxides,

breaking the autoxidative chain reaction or by reducing radicals. The antioxidant property of polyphenolic compound depends on the number and position of free OH groups present on the particular molecule. The antioxidant property of the extract was analyzed using DPPH, stable free radicals. Antioxidants are reacts with DPPH and reduce it to DPPH-H. Concentration ranges were used 20-100 µg/ml. At a concentration 100 µg/ml, the inhibition of the extract reached to 56.53%, whereas the standard rutin showed 68.57% of inhibition (Table 1). The inhibitory concentration 50% (IC₅₀) value for the extract was found to be 85.43 µg/ml. Figs. 1 and 2 showed the response curve of DPPH radical scavenging activity of rutin and aqueous extracts of the *C. serratum*, respectively.

Superoxide radicals were generated by the hypoxanthine-xanthine oxidase and the NBT system. The decrease in absorbance indicated the consumption of superoxide radicals. The superoxide scavenging activity of the extract and standard was shown in Table 2. The percentage of superoxide scavenging activity of the extract is 47.73% at a concentration 100 µg/ml. The IC₅₀ value was found to be 11.18 µg/ml. Figs. 3 and 4 show the response curve of superoxide scavenging assay where % superoxide scavenging inhibition is plotted against concentrations of aqueous root extract.

Fig. 5 shows the reducing power of the extract and the standard rutin, whereas Table 3 shows the FRAP in an increasing manner with increased concentration. The reducing power of the extract indicates that the extract must contain electron donors.

Table 1: DPPH radical scavenging assay

S.No	Concentration (µg/ml)	Inhibition (%)		IC ₅₀ (µg/ml)	
		Rutin	Sample	Rutin	Sample
1	20	52.86±0.089	31.42±0.399	10.10	85.43
2	40	55.50±0.358	36.42±0.224		
3	60	58.30±0.016	40.97±0.145		
4	80	61.68±0.092	45.81±0.083		
5	100	68.57±0.342	56.53±0.347		

IC₅₀: Inhibitory concentration 50%, DPPH: 2,2-diphenyl-1-picrylhydrazyl

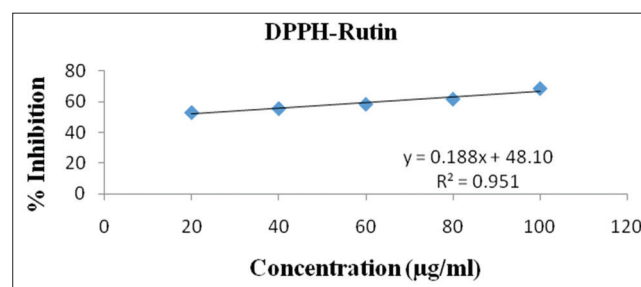


Fig. 1: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay whereas % DPPH inhibition is plotted against concentrations of rutin taken as standard

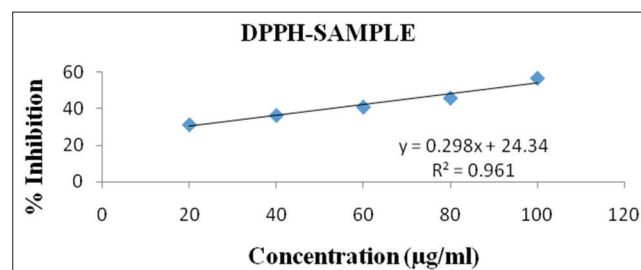


Fig. 2: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay whereas % DPPH inhibition is plotted against concentrations of aqueous root extract

Table 2: Superoxide scavenging assay

S.No	Concentration (µg/ml)	Inhibition (%)		IC ₅₀ (µg/ml)	
		Rutin	Sample	Rutin	Sample
1	20	51.95±0.026	25.61±0.377	11.18	107.59
2	40	57.30±0.226	34.05±0.038		
3	60	66.77±0.234	38.06±0.029		
4	80	72.42±0.261	42.28±0.290		
5	100	74.07±0.062	47.73±0.186		

IC₅₀: Inhibitory concentration 50%

Table 3: FRAP assay

S.No	Concentration (µg/ml)	Absorbance	
		Rutin	Sample
1	20	0.134±0.007	0.094±0.005
2	40	0.162±0.023	0.127±0.009
3	60	0.189±0.006	0.149±0.008
4	80	0.216±0.005	0.181±0.011
5	100	0.244±0.020	0.197±0.013

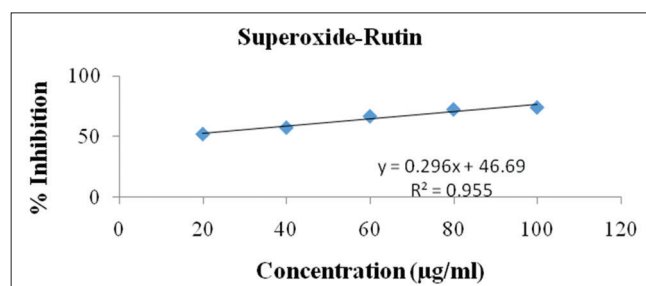


Fig. 3: Superoxide scavenging assay whereas % superoxide scavenging inhibition is plotted against concentrations of rutin

CONCLUSIONS

From the current study, we can conclude that *C. serratum* aqueous extract has significant antioxidant activity against DPPH scavenging assay, superoxide scavenging assay, and FRAP assay. This radical scavenging activity could be attributed to the presence of flavonoids, alkaloids, glycosides, and phenolic compounds. Plant exhibits the antioxidant activity through the scavenging of free radicals, and FRAP showed significant reducing power activity with increased concentration of the sample. The results suggest that the traditional value of the plant could be attributed to its antioxidant potential.

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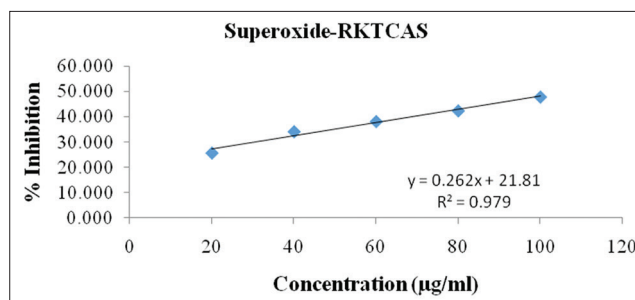


Fig. 4: Superoxide scavenging assay whereas % superoxide scavenging inhibition is plotted against concentrations of aqueous root extract

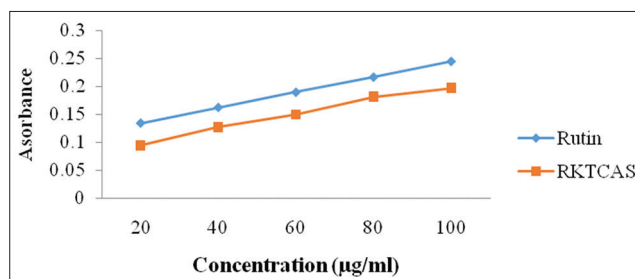


Fig. 5: Ferric reducing antioxidant power assay showing antioxidant capacity based on the ability to reduce ferric ions of sample with increase in absorbance of sample and standard

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