

Antioxidant Potential of Roots of *Clerodendrumserratum*(Linn.)

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ABSTRACT

The objective of this study was to determine the antioxidant capacity of polyphenols extracted from the roots of the *Clerodendrumserratum* plant. Extracts CSRC, CSRA and CSREA were found to have high percentage of total phenolic and flavonoid content and strong 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity. The results suggested that CSRC, CSREA and CSREAs has significant potential as a natural antioxidant to promote health and to reduce the risk of disease.

Keywords: Antioxidant, polyphenols, *Clerodendrumserratum*.

I. INTRODUCTION

The *Clerodendrumserratum*Linn.(CS) is a small perennial woody shrub of the family Verbenaceae and it is commonly known as Bharangi (Patel et al., 2014). It is native of India and growing in moist deciduous forests of Western Ghats of India (Manjunatha et al., 2004). Traditionally the various parts of this plant were used in the treatment of asthma, inflammation and infectious disorders (Patel et al., 2014). It has been reported that, this plant shows wide range of pharmacological activities including hepatoprotective (Vidya et al., 2007;Agrawal et al., 2013), Analgesic (Saha et al., 2012), Antioxidant (Bhujbal et al.,2009 b;Mohamed et al.,2012), antiinflammatory (Narayanan et al.,1999), antibacterial(Rashid et al., 2013; Vidya et al.,2010),anticancer(Zalke et al., 2010;Chinchali et al.,2011), and anti-asthmatic activity (Bhujbal et al.,2009a;Thalla et al.,2012).

The earlier study revealed the presence of pharmacologically active constituents include flavonoids(Bhujbal et al., 2010d; Fan et al., 2007), phenyl propanoids (Yang et al., 2000a; Fan et al., 2007; Wei et al., 2000a), Iridoids (Yang et al., 2000c; Wei et al., 2000a), terpenoids (Banerjee et al., 1969; Yang et al., 2000b; Raju et al., 2008; Vidya et al., 2007) and sterols (Banerjee et al., 1969;; Fan et al., 2007).A free radical is a very reactive species resulted from metabolic reaction at cellular level which leads to various types of diseases like ageing, neurodegenerative diseases, CNS

related disorders, mutagenic disorders etc. The antioxidants are the compounds which scavenge these radicals and prevent damage caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Kalita et al., 2013).The current study aims to identify the potent phytoconstituents present in CSRH, CSRC, CSREA and CSRA extracts of CS roots and further evaluation of their In- vitro antioxidant potential.

II. MATERIAL AND METHODS

Plant material

For this study, the roots of *Clerodendrumserratum*(CSR)were collected from the different localities of Paithan,They were identified by Dr. K. J. Salunke of the Department of Botany, PadmashriVikhe Patil College of Arts, Science and Commerce, Pravaranagar, India.The fresh roots of the plant were cleaned and powdered coarsely and stored for further use.

Extraction of crude drug

The 500 g powdered roots were subjected to the maceration in 4.5 L absolute ethanol (99.9%) at 50°C for 3days (1.5×3). The crude extract was filtered and then allowed to concentrate on rotary under reduced pressure. The obtained crude extract was dark brown in colour and the percentage yield was 5.53 %.

The crude extract (23 gm) was suspended in 10 ml distilled water and successively fractionated with n-

hexane, chloroform, ethyl acetate solvents and yields CSRH, CSRC, CSREA and residual aqueous extract CSRA respectively.

Estimation of total phenolic content

The total phenolic content (TPC) was estimated by Folin-Ciocalteu reagent using previously reported method (Khatoon et al., 2013). TPC of various extracts of CS roots were determined using calibration curve of standard gallic acid (GA). Methanol was used as blank and GA as a standard. All determinations were carried out in triplicates. TPC was determined from standard calibration curve produced with GA and it was expressed as GA equivalent per milligrams ($\mu\text{g GAE/mg}$) of extracts.

Estimation of total flavonoid content

The total flavonoid content (TFC) of different extracts were estimated by using aluminium chloride colorimetric method (Maddan et al., 2011; Saeed et al., 2012). TFC of various extracts was determined using calibration curve of standard rutin. All determinations were carried out in triplicates. The TFC was expressed in terms of rutin equivalent per milligrams ($\mu\text{g RE/mg}$) of extracts.

In-vitro antioxidant activity

DPPH radical scavenging activity

In the DPPH radical scavenging assay, the methods described in Khatoon et al. (2013) and Saeed et al. (2012) were followed with minor modifications. A 10 mg mL^{-1} stock solution of various extracts were prepared by dissolving the extract in DMSO and sample concentrations of 0.812 to $100 \mu\text{g mL}^{-1}$ were prepared in methanol. A sample solution was mixed with 0.004% freshly prepared DPPH methanol solution. The reaction mixture was shaken and kept in dark at room temperature. After that, the absorbance of the mixture was measured immediately at 517 nm using a Systronic 1203 UV/Vis Spectrometer. The DPPH radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_0 - A_s) / A_0 \times 100]$. Where A_0 and A_s are the absorbance of control and standard or tested sample respectively. The experiment

was performed in triplicates and the mean values were recorded

Statistical analysis

All experiments were carried out in triplicate and results are reported as mean \pm SD. Data was analyzed with one-way ANOVA.

III. RESULTS

Total phenolic and flavonoid content

The total phenolic content (TPC) of various extracts of CS roots were determined by using Folin-Ciocalteu reagent and it is reported as micrograms per milligrams ($\mu\text{g/mg}$) of gallic acid equivalent (GAE) by reference to gallic acid standard curve ($y = 0.1041x$ and $r^2 = 0.9917$). All extracts contained a considerable amount of phenolic content and it was found that, of all the extracts, the CSRC extract had the highest total phenolic content (139.74 ± 2.41) μg of GAE/mg followed by CSRA (99.35 ± 1.46), CSREA (53.20 ± 1.46) and CSRH (6.41 ± 2.22) μg of GAE/mg respectively.

The total flavonoid content (TFC) was expressed as $\mu\text{g/mg}$ of rutin equivalents (RE) by reference to rutin standard curve ($y = 0.0326x$ and $r^2 = 0.9976$). It was found that, the CSREA extract had the highest TFC (86.45 ± 4.77) μg of RE/mg followed by CSRA (72.91 ± 1.80), CSRC (15.72 ± 0.18) and CSRH (Nil) μg of rutin equivalent respectively.

Table 1. Estimation of total phenolic and flavonoid content of extracts of *Clerodendrum serratum*

Extract	TPC	TFC
CSRH	6.41 ± 2.22	0.0
CSRC	139.74 ± 2.41	15.72 ± 0.18
CSREA	53.20 ± 1.46	86.45 ± 4.77
CSRA	99.35 ± 1.46	72.91 ± 1.80

DPPH free radical scavenging activity

DPPH radical scavenging ability of various extract was determined using standard antioxidant ascorbic acid (AA). The % inhibition of standard and extract of *Clerodendrum serratum* roots are in the order of CSRC > CSRA > CSREA > CSRH (Fig. 1.). The IC_{50} values of standard Ascorbic acid and extracts are

presented in Table 2. The IC₅₀ values of AA and extracts (CSRA, CSREA, CSRC and CSRH) were 1.24, 3.36, 10.61, 165.58 μg/mL respectively.

Table 2. An effect of extracts of CS roots on free radicals by DPPH method

Extract code	IC ₅₀ (μg/ml) DPPH
AA	2.48
CSRH	165.98
CSRC	10.61
CSREA	3.36
CSRA	1.24

AA, Ascorbic acid; CSRH, hexane extract; CSRC, chloroform extract; CSREA, ethyl acetate extract; CSRA, alcohol extract.

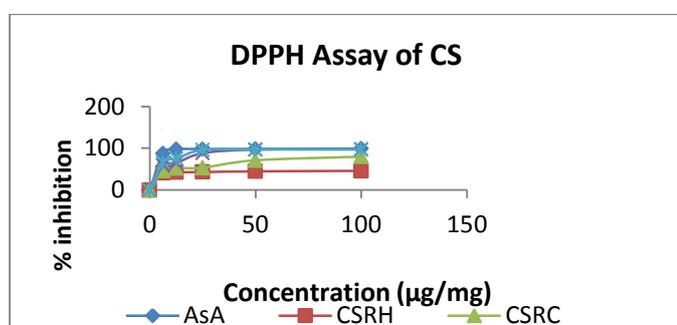


Figure 1. In vitro antioxidant activity of extracts of CS roots in DPPH scavenging assay

IV. DISCUSSION

The plant phenolic compounds and flavonoids have been reported to show strong antioxidant activity in biological systems, acting as oxygen radical and free radicals scavengers (Rice-Evans et al., 1997; Jorgensen *et al.*, 1999; Halliwell et al., 1995) due to ability of benzene rings to transfer electrons (Brown, 1995).

Present study revealed that relatively highest phenolic content are found in chloroform and hydro alcoholic residual extract, moderate in ethyl acetate extract, while lowest in n-hexane extract and highest flavonoid content in ethyl acetate and hydro-alcoholic extracts and absent in n-hexane extract. These results may arise due to higher solubility of phenolic and flavonoid compounds in ethyl acetate, chloroform and hydro-alcohol extract and thus it suggests the potent antioxidant property.

A number of way have been published in order to evaluate the antioxidant and the free radical scavenging properties of natural products out of which, the DPPH assay has provided information on a great deal of plants and is commonly used. DPPH• is a stable and organic nitrogen centered radical bearing no similarity to the highly reactive and transient peroxy radicals implicated in a variety of oxidative processes *in vivo* (Biapa et al., 2011; Basma et al., 2011). The scavenging ability of DPPH radical is related to the inhibition of lipid peroxidation and it was determined by decrease in intensity of violet colour. Lower IC₅₀ value indicates higher antioxidant activity (Rekka and Kourounakis, 1991). Among all extracts, CSREA and CSRA showed the lowest IC₅₀ value with highest antioxidant activity. Hence when more antioxidants occur in the extracts, the more DPPH reduction will occur and it relates to high scavenging capability of respective extracts. In this assay, the antioxidant activity of CSREA and CSRC extracts on DPPH radical may be attributed to a direct role in trapping free radicals by donating hydrogen atom.

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VI. REFERENCES

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