International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 8, 2016

Original Article

PHYTOCHEMICAL EVALUATION AND *IN VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY EFFECTS OF *CLERODENDRUM SERRATUM* ROOTS

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Received: 07 Apr 2016 Revised and Accepted: 20 June 2016

ABSTRACT

Objective: To evaluate *in vitro* free radical scavenging and anti-inflammatory effects of extract and fractions of *Clerodendrum serratum* roots along with its phytochemical analysis.

Methods: The crude 70% methanolic extract (MECSR) and ethyl acetate (EFCSR), *n*-butanol (BFCSR) and residual aqueous fractions (AQFCSR) of *C. serratum* roots were prepared and analyzed for qualitative and quantitative phytochemical study using reported methods. The *in vitro* anti-inflammatory effects were studied using protein denaturation and proteinase inhibitory assays whereas *in vitro* free radical scavenging effects were established using DPPH (1,1-diphenyl-2-picrylhydrazyl), modified 2,2'-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) and trolox equivalent antioxidant capacity (TEAC) assays.

Results: The qualitative and quantitative phytochemical studies of crude extract and fractions showed the presence of phenolics, flavonoids, saponins, and carbohydrates. All analyzed samples showed dose-dependent anti-inflammatory and free radical scavenging effects in the studied *in vitro* assays. The IC_{50} values of MECSR, EFCSR, BFCSR and AQFCSR for scavenging the DPPH and ABTS radical ranged from 12.52±2.21 to 200.47±2.84 µg/ml and 18.12±1.76 to 216.08±1.90 µg/ml, respectively, while that of protein denaturation and proteinase inhibition assays were ranged from 70.76±1.40 to 110.13±1.63 µg/ml and 76.66±2.02 to 116.55±2.19 µg/ml.

Conclusion: Among the analyzed samples, the EFCSR showed significantly higher free radical scavenging and anti-inflammatory effects in studied assays. The observed activities might be attributed to the higher content of polyphenols present in EFCSR fraction of roots. The present study will provide scientific data to reinforce the traditional claims of roots for treating inflammation, pain and other oxidative stress related diseases.

Keywords: *Clerodendrum serratum*, Quality issues, Phytochemical analysis, Phenolics, antioxidant, Anti-inflammatory

INTRODUCTION

Clerodendrum serratum (Linn.) Moon (family: Verbenaceae) is commonly known as bharangi (means which is glorious) in hindi and vatari (an enemy of *vata* dosa) and kasaghni (which alleviates a cough) in sanskrit. It is a perennial woody shrub up to 3-8 ft in height with blunt quadrangular stems. This plant is native to east India and Malaysia and found in moist deciduous forests and occasionally in plains of peninsular India and the Western and Eastern Himalayas up to 1,400 ft above sea level [1]. In India, it is distributed in lower Himalayas from kumaun eastwards, West Bengal and Bihar. *C. serratum* roots have been indicated in traditional systems of medicine like Ayurveda and Unani for the treatment of pain, inflammation, rheumatism, respiratory disorders and fever. Research reports available on chemical constituents of *C. serratum* roots showed the presence of saponins (triterpenoids and sterols), phenolics, flavonoids, and carbohydrates. A number of *in vivo* and/or *in vitro* studies have explained a wide spectrum of pharmacological properties of crude extract (mainly alcoholic) of roots including; anticancer [2, 3], hepatoprotective [4] anti-bacterial [5, 6] anti-inflammatory [7] and antioxidant [8-10]. However, systematic investigation along with biomarker analysis is required for roots of this plant.

Considering such traditional claims and documented pharmacological activities of the roots of *C. serratum*, it was designed to investigate anti-inflammatory and free radical scavenging effects of the crude 70% methanolic extract (MECSR) and ethyl acetate (EFCSR), *n*-butanol (BFCSR) and residual (AQFCSR) fractions using *in vitro* assays. *In vitro,* free radical scavenging effect of crude extract and fractions was investigated using DPPH (1,1-Diphenyl-2-picrylhydrazyl), modified 2,2'-Azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) and trolox equivalent antioxidant capacity (TEAC) assays whereas *in vitro* anti-inflammatory effect was studied using protein denaturation and inhibitory proteinase assays.

MATERIALS AND METHODS

Drugs and chemicals

Diosgenin, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), bovine serum albumin (BSA), tris-hydrochloric acid (HCl) buffer, trypsin were purchased from Sigma-Aldrich Chemical Co. (India). Folin-ciocalteau reagent was procured from Sisco Research Lab (India). Diclofenac sodium was gift sample from Astron Lab Chemicals, Ahmedabad, Gujarat, India. All the chemicals and solvents used were of standard analytical grades.

Collection and identification of plant material

The roots of *Clerodendrum serratum* Linn. were collected from Government Ayurvedic Udhyan, Gandhinagar, Gujarat (India) in the month of August 2011. The roots were authenticated and herbarium specimen (10EXTPHDP49CS11) was deposited in the department of pharmacognosy, an institute of pharmacy, Nirma University, Ahmedabad, Gujarat (India). The roots were washed and dried under sunlight for 15 d. The dried material was then subjected to pulverization and the powder sample was passed through 60# sieve. The powder sample was stored in airtight container at room temperature for further use.

Preparation of extract and fractions

Powdered roots of *C. serratum* (1 kg) were extracted thrice with 70% methanol (5 l) in soxhlet extractor at the boiling temperature for 24 h, followed by filtration using filter paper. Three percolates were mixed, concentrated and evaporated to dryness to give MECSR (yield: 12.9 %w/w). The dried extract was suspended in H_2O (500 ml) and subsequently extracted with ethyl acetate (3×250 ml) and *n*-

butanol (3×250 ml) by refluxing for 2 h with respective solvent. Both solvent fractions were evaporated to dryness under reduced pressure and designated as EFCSR (yield: 2.42 %w/w) and BFCSR (yield: 2.52 %w/w) fraction. The remaining aqueous extract was evaporated to obtain the residual fraction (AQFCSR, yield: 7.7 %w/w). The dried crude extract and fractions were stored in labeled bottles in the freezer at 4 °C for further use. Stock solution (1 mg/ml) of MECSR, EFCSR, BFCSR and AQFCSR was prepared by dissolving 100 mg of dried extract/fractions in 100 ml of methanol. Aliquots from these stock solutions were further diluted with methanol as per the concentrations required and used for quantitative estimation of phytoconstituents, anti-inflammatory and free radical scavenging studies.

Qualitative phytochemical analysis

Qualitative phytochemical analysis of MECSR, EFCSR, BFCSR and AQFCSR was performed using standard procedures for detection of various secondary metabolites [11].

Quantitative estimation of phytoconstituents

The total phenolics [12], flavonoids [13], saponins [14] and carbohydrate [15] content of crude extract and fractions were quantitatively estimated using reported methods. The amounts of phenolics, flavonoid, saponins and carbohydrates were expressed as gm/100 gm dry weight calculated as gallic acid equivalent, quercetin equivalent, diosgenin equivalent and glucose equivalent, respectively.

Anti-inflammatory activity (*In vitro*)

Protein denaturation assay

The method of Williams *et al.*, 2008 was employed for the determination of protein denaturation activity of extract and fractions. Briefly, a solution 5 ml of 0.2 %w/v of BSA (prepared in tris buffer saline) was reacted with different concentration of test sample (50 µl). The mixture was heated at 72 °C for 5 min and then cooled for 10 min. The diclofenac sodium was used as reference control. The turbidity was determined using UV-VIS spectrophotometer (UV 1800, Shimadzu, Japan) at 660 nm. The percentage inhibition of protein denaturation was determined, and IC_{50} value was calculated [16].

Proteinase inhibition assay

The proteinase inhibition assay was performed according to the method of Oyedepo *et al.*, 1985 [17]. The reaction mixture (2 ml;) consisted of 1 ml tris-HCl buffer (20 mM, pH 7.4) containing 0.06 mg trypsin and 1 ml test or standard (diclofenac sodium) solution of different concentrations. The mixture was incubated at 37 °C for 5 min followed by addition of 1 ml casein (0.8 %w/v) and 2 ml of perchloric acid (70%) to terminate the reaction. Turbidity (cloudy suspension) was read at 210 nm. The percentage inhibition was calculated with reference to diclofenac sodium, and IC_{50} values were determined from dose-response curve.

Free radical scavenging activity (*In vitro*)

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

The percentage of free radical scavenging capacity of crude extract and fractions was assessed using the method described by Brand-Williams *et al.*, 1995 employing DPPH (2, 2-diphenyl-1-picrylhydrazyl) [18]. Antioxidant capacity was measured by a decrease in absorbance at 517 nm of a solution of colored DPPH in methanol brought about by the sample. A stock solution of DPPH (0.5 mM in methanol) was prepared such that 30 µl of it in 3 ml methanol gave an initial absorbance of 0.9. Decrease in the absorbance in the presence of test or standard solutions at different concentrations was noted after 30 min. IC_{50} was calculated from percentage inhibition. A blank reading was obtained using methanol instead of the test samples, and ascorbic acid was used as positive control.

Modified 2, 2'-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) radical cation decolorization assay

The free radical scavenging activity of crude extract and fractions was also measured by modified ABTS radical cation decolorization assay according to the method of Nadeem *et al.*, 2008 [19]. ABTS cation radical was produced by reacting ABTS stock solution (in 20 mM sodium acetate buffer, pH 6.5) with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12-16 hr before use. The ABTS cation radical solution was prepared such that 35 µl of it in 3 ml double distilled water gave an initial absorption of 0.70±0.02 measured at 734 nm using an ultraviolet-visible spectrophotometer and equilibrated at 30 °C. Decrease in the absorbance in the presence of test or standard solutions at different concentrations was noted after keeping in the dark for 30 min at room temperature. Ascorbic acid was used as positive control. IC_{50} were determined from percentage inhibition.

Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay was performed according to the method described by Re *et al.*, 1999 [20]. In this assay, the ABTS cation radical solution (35 µl) as prepared in ABTS radical cation decolorization assay was mixed with 10 µl of test or standard solution and diluted with double distilled water or buffer (3 ml). The extent of decolorization as percentage inhibition of the ABTS radical cation was determined as a function of concentration (10 µl) and time (1, 4 and 6 min) and calculated relative to the reactivity of 1 mM trolox (prepared in 20 mM sodium acetate buffer, pH 6.5) as a standard, under the same conditions. The antioxidant capacity of studied extract and fractions were reported as TEAC.

Statistics

Experimental results were expressed as mean±standard error mean (SEM) of three parallel measurements. The IC_{50} values of analyzed samples were calculated from concentration-inhibition curves. Statistical significance of differences between means was established by analysis of variance (ANOVA) followed by dunnett's multiple comparisons test. *P* values below 0.05 were considered statistically significant. All the analysis was performed using GraphPad prism 6.02 software.

RESULTS

Qualitative phytochemical analysis

The result of qualitative phytochemical analysis of MECSR, EFCSR, BFCSR and AQFCSR is shown in table 1. The preliminary phytochemical analysis revealed the presence of saponins, steroids, terpenoids, flavonoids, phenolics and carbohydrates in the extract and fractions.

Table 1: Phytochemical screening of *C. serratum* roots

Phytoconstituents	MECSR extract	EFCSR fraction	BFCSR fraction	AQFCSR fraction
Reducing sugar	+	-	+	+
Anthraquinones	-	-	-	-
Flavonoids	+	+	-	-
Terpenoids	+	+	+	-
Steroids	+	+	+	-
Saponins	+	+	+	+
Alkaloids	-	-	-	-
Phenolics	+	+	+	+

+= present,-= absent

Quantitative estimation of phytoconstituents

The results of quantitative analysis of phenolics, flavonoids, saponins and carbohydrates in crude extract and fractions are reported in fig. 1. From the standard curve of gallic acid (y=0.0025x+0.0118, R^2=0.9978), total phenolic content was calculated and found in the range of 0.19±0.04 to 1.23±0.09 gm/100 gm dry extract.

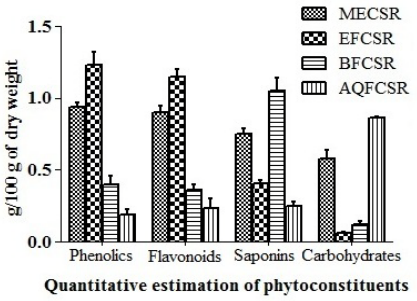

Fig. 1: Quantitative estimation of phytoconstituents of *C. serratum* roots (n=3, Values are represented as mean±SEM)

Total flavonoid content was calculated from the standard curve of quercetin (y=0.0014x+0.0128, R^2=0.9988) and found in the range of 0.23±0.07 to 1.15±0.05 gm/100 gm dry extract. Total saponin content was found in the range of 0.25±0.13 to 1.05±0.09 gm/100 gm dry extract (standard curve of diosgenin; y=0.002x+0.0369, R^2=0.9941). Total carbohydrate content was found in the range of 0.06±0.01 to 0.86±0.01 gm/100 gm dry extract calculated from the standard curve of glucose (y=0.0085X+0.05, R^2=0.991).

Anti-inflammatory activity (*In vitro*)

In present investigation, *in vitro* anti-inflammatory effect of crude extract and fractions of *C. serratum* roots was evaluated using protein denaturation and protease inhibitor assay. The present findings (fig. 2) exhibited statistically significant ($p<0.05$) and dose dependant anti-inflammatory activity of all analyzed samples (EFCSR>BFCSR>MECSR>AQFCSR) in both assays comparable to diclofenac sodium used as positive control.

The high protein denaturation and protease inhibitor activity of EFCSR were suggested by their IC_{50} values 70.76±1.40 and 76.66±2.02, respectively (fig. 3).

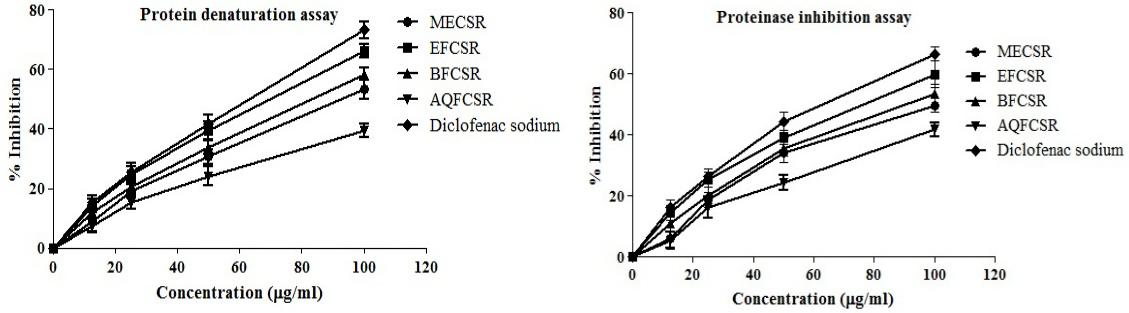

Fig. 2: *In vitro* anti-inflammatory effect of *C. serratum* roots (n=3, Values are represented as mean±SEM)

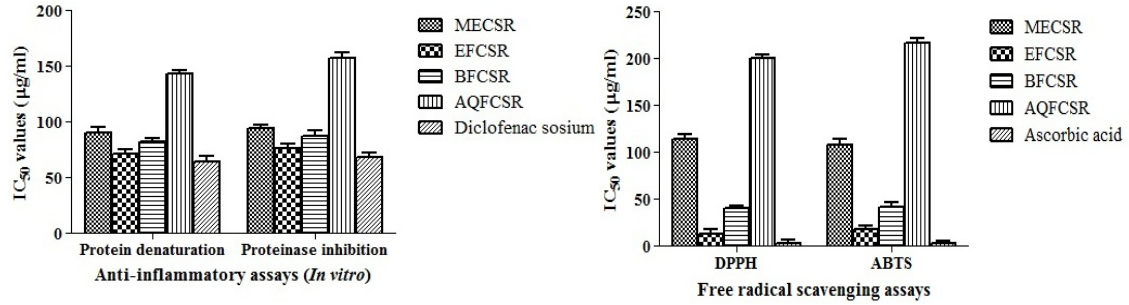

Fig. 3: IC_{50} values of *in vitro* anti-inflammatory and free radical scavenging effects of *C. serratum* roots (n=3, Values are represented as mean±SEM)

Free radical scavenging activity (*In vitro*)

Free radical scavenging assay is one of the most widely used methods in establishing the hydrogen donating ability as an index of antioxidant activity of herbal extracts and phytochemicals. The antioxidant activities of crude extract and fractions were carried out by performing DPPH, ABTS and TEAC assays.

A statically significant ($p<0.05$) and dose-dependent potential for DPPH and ABTS radical scavenging by MECSR extract, EFCSR and BFCSR fractions and ascorbic acid (reference standard) was observed at a studied concentration (table 2).

DPPH and ABTS radical scavenging effects of EFCSR (fig. 3) were found to be significantly higher (I_{50} value 12.52±2.21 µg/ml and 18.12±1.76 µg/ml) compared to others fractions at different concentrations.

However, the effect of radical scavenging was found to be lower than the ascorbic acid used as reference standard. The order of potency of analyzed extract and fractions was in the tune of EFCSR> BFCSR>MECSR>AQFCSR.

In order to determine the TEAC, the extent of inhibition of the absorbance of the ABTS radical was plotted as a function of concentration against time (1, 4 and 6 min). The gradient of the plot of the percentage inhibition of absorbance for the studied sample was divided by the gradient of the plot for trolox (1 mM) at each selected time points for the calculation.

The trolox equivalent antioxidant capacity of studied samples (table 3) at the dose of 10 µl was found in the range of 0.76±0.01 to 2.60±0.05, 0.87±0.07 to 2.53±0.06 and 0.93±0.05 to 2.36±0.10 mM at 1, 4 and 6 min, respectively.

Table 2: Free radical scavenging effect of *C. serratum* roots

Samples	DPPH assay		ABTS assay	
	Concentration (µg/ml)	Inhibition (%)*	Concentration (µg/ml)	Inhibition (%)*
MECSR	20	7.40±2.52	30	15.60±2.42
	40	15.54±3.19	60	29.43±2.59
	60	21.91µ±3.12	90	44.21±1.79
	80	34.64±1.95	120	54.96±4.51
	100	45.97±2.01	150	67.02±3.98
	120	56.93±2.95		
EFCSR	5	25.94±3.01	5	13.95±3.92
	10	43.07±2.85	10	27.78±3.09
	15	62.73±2.94	15	44.09±2.97
	20	77.81±2.36	20	57.33±3.86
	25	90.07±3.57	25	65.48±3.62
BFCSR	10	14.14±4.06	10	13.95±3.31
	20	25.47±3.48	20	24.47±2.99
	30	41.85±3.02	30	35.70±3.22
	40	50.00±2.99	40	48.11±2.19
	50	60.21±1.76	50	62.29±2.47
AQFCSR	30	8.43±3.75	50	12.77±2.71
	60	16.39±3.27	100	23.05±2.88
	90	20.97±2.39	150	33.69±3.52
	120	26.31±3.61	200	46.22±3.21
	150	35.21±2.78	250	58.39±2.12
	180	45.60±3.71		
	210	54.12±2.98		
Ascorbic acid	1	32.02±1.06	1	30.73±2.98
	2	37.73±2.99	2	48.11±2.84
	3	48.22±1.76	3	60.28±4.18
	4	64.89±2.43	4	73.05±3.29
	5	75.75±2.79	5	85.22±2.50

*n=3, Values are represented as mean±SEM

Table 3: TEAC of *C. serratum* roots at specific time points

Sample tested	% Inhibition*		
	At 1 min	At 4 min	At 6 min
Trolox	1.00	1.00	1.00
MECSR	1.37±0.05	1.29±0.02	1.24±0.09
EFCSR	2.60±0.05	2.53±0.06	2.36±0.10
BFCSR	1.97±0.02	1.91±0.06	1.89±0.03
AQFCSR	0.76±0.01	0.87±0.07	0.93±0.05

*n=3, Values are represented as mean±SEM

DISCUSSION

The root of *C. serratum* is popularly used in indigenous systems of medicine for the treatment of pain, inflammation, rheumatism, respiratory disorders and fever in India with a long history. The traditional knowledge led us to investigate antioxidant and anti-inflammatory effects of crude 70% methanolic extract (MECSR) and ethyl acetate (EFCSR), *n*-butanol (BFCSR) and residual (AQFCSR) fractions of roots of *C. serratum* along with phytochemical analysis.

The preliminary phytochemical analysis of studied extract and fractions revealed the presence of various phytochemicals such as saponins, flavonoids, phenolics, terpenoids, steroids and reducing sugars while alkaloids and anthraquinones were found absent (table 1). The presence of different phytochemicals of methanolic extract of a *C. serratum* roots have been previously reported [21];

However, our study is first ever reported to the best of our knowledge on qualitative and comparative quantitative analysis of crude extract and fractions. The findings conclude that amongst the studied test samples, EFCSR was found to contain high polyphenols including steroids, terpenoids and flavonoids while saponins were the main components of the BFCSR fraction. Reducing sugars were mainly observed in polar fractions i. e, MECSR extract, and AQFCSR fraction.

The subsequent quantitative phytoconstituents estimation (fig. 1) specifies that the EFCSR contain a high amount of phenolic (1.23±0.09 gm/100 gm) and flavonoids (1.15±0.05 gm/100 gm). However the BFCSR contains the highest amount of saponins (1.05±0.09 gm/100 gm), and AQFCSR showed the maximum content of carbohydrates (0.86±0.01 gm/100 gm). Among all test samples analyzed, the promising amount of polyphenols (phenolics and flavonoids) and saponins were found in a higher amount. Plant polyphenols are the widespread secondary metabolites and have been reported as a potential natural antioxidant in terms of their ability to act as efficient radical scavengers [22, 23] and anti-inflammatory agents [24, 25]. Thus, the present study was further directed to investigate anti-inflammatory and free radical scavenging effects of *C. serratum* roots.

Inflammation is a part of the immune system's response to the harmful stimuli. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the management of inflammatory conditions, but these are associated with many unwanted side effects such as gastric irritation, ulcer, etc. [26]. Medicinal plants used in traditional system of medicine to treat inflammatory conditions seem a viable and logical alternative as safe and effective anti-inflammatory agents. *C. serratum* roots are commonly used to treat inflammatory conditions; hence to support the traditional claims, a simple and viable protein denaturation and proteinase inhibition assay were selected to evaluate anti-inflammatory potential of crude extract and

fractions. Protein denaturation induced by physical or chemical agents is a well-documented cause of inflammation. Many scientists have reported the *in vitro* protein denaturation activity of NSAIDs as one of the mechanism. Here, heat-induced protein denaturation assay was studied to elucidate the *in vitro* anti-inflammatory effect of crude extract and fractions of roots. At temperature above 60 °C, rapid protein denaturation occurs, which is immediately cytotoxic and leads to coagulated necrosis [27]. All the analyzed samples showed inhibition of heat-induced denaturation of BSA in a dose-dependent manner (fig. 2). However, the strongest effect of protein denaturation was observed with EFCSR fraction comparable to standard, diclofenac sodium (fig. 3); indicating it's protein stabilization effect.

Proteinases are involved in a wide variety of biological processes, including inflammation and tissue injury. Various studies have focused on the role of proteinases in tissue injury, and it was thought that the balance between proteinases and proteinase inhibitors is a major determinant in maintaining tissue integrity. Serine proteinases from inflammatory cells, including neutrophils, are implicated in various inflammatory disorders, such as pulmonary emphysema. Pulmonary emphysema is a condition that forms part of chronic obstructive pulmonary disease (COPD) and involves the enlargement of the air sacs of lung leading to alveolar destruction and impairment in lung function. Both extracellular endogenous and exogenous proteinases play major roles in the pathophysiology of inflammatory diseases. Therefore, the study of proteinase inhibitory activity plays a pivotal role in aggravation of inflammatory response. The present study was aimed to elucidate the *in vitro* proteinase inhibition effect of crude extract and fractions of *C. serratum* roots using diclofenac sodium as a positive control. All the analyzed samples showed statistically significant and dose-dependent proteinase inhibition effect (fig. 2). However, the anti-protease activity of EFCSR was found to be significant amongst the analyzed samples (fig. 3), suggesting the role in protease induced inflammatory processes.

Present findings are further supporting the traditional claims of *C. serratum* as a remedy for pain, inflammation and fever as Narayanan *et al.*, 1999 have demonstrated anti-inflammatory activities of *C. serratum* roots in animal models using carrageenan-induced rat paw edema and cotton pellet implantation method [7]. The *in vitro* anti-inflammatory activity of analyzed samples can be attributed to polyphenols and saponins of the roots. Further definitive studies are necessary to ascertain the mechanisms and constituents behind its anti-inflammatory actions.

Free radicals of different forms are constantly generated for the specific metabolic requirement in the living system. When the generation of these species exceeds the levels of antioxidant mechanism, they cause extensive damage to the cells leading to oxidative damage of tissues and biomolecules, eventually leading to disease conditions, especially degenerative diseases and extensive lysis. Since the endogenous antioxidants acting as intracellular defense systems protecting cells from free radical damage and extensive lysis, scavenging and diminishing the formation of oxygen-derived species are not completely efficient, micronutrients or antioxidants taken as supplements are particularly important in diminishing the cumulative oxidative damages [28]. Herbal drugs containing free radical scavengers like phenolics and flavonoids are known for their therapeutic activity. In the present study, the antioxidant capacity of crude extract and fractions of *C. serratum* roots was evaluated using DPPH, ABTS and TEAC assays. Ascorbic acid was used as a standard antioxidant. Although standard antioxidant (ascorbic acid) had higher scavenging activity at all tested concentrations than the test samples, the crude extract and fractions still showed good free radical scavenging activity as suggested by their IC_{50} values (fig. 2). The scavenging activity of EFCSR against DPPH and ABTS radical was significantly higher or not significantly different than the activity of standard, ascorbic acid (table 2).

The results of TEAC assay demonstrated time-dependency of the reaction and the influence of the selected time point of measurement on the reported antioxidant activity. The high TEAC value of EFCSR, determined using decolorization of the ABTS radical cation is also in support of DPPH and ABTS assay findings (table 3). The higher scavenging effect of EFCSR is attributed to the high polyphenolic and saponins content; as determined by phytochemical content analysis. To our knowledge, any prior report on the antioxidant activity of all different extract and fractions of this plant has not been reported so far. The present study provided valuable preliminary data through demonstration of its high antioxidant capacity. However, antioxidant components in this plant have not been identified in the literature. Isolation and characterization of its individual active components and *in vivo* relevance of such activity awaits further comprehensive studies.

CONCLUSION

From the present investigation, it can be concluded that ethyl acetate fraction of *C. serratum* roots has potent antioxidant and anti-inflammatory effects exhibited through different mechanisms. The present findings will strengthen the traditional mention of *C. serratum* roots as a remedy for pain and inflammation as indicated by good antioxidant and anti-inflammatory *in vitro* effects. Phytochemical analysis revealed the presence of polyphenols and saponins in EFCSR, which was further confirmed by qualitative and quantitative phytochemical studies, and that might have contributed to the said effects. However, further studies are needed for the isolation and identification of bioactive from roots and its evaluation in *in vivo* models to understand their exact mechanism of action as an anti-oxidant and anti-inflammatory agent.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Patel JJ, Acharya SR, Acharya NS. *Clerodendrum serratum* (L.) Moon-a reviews on traditional uses, phytochemistry, and pharmacological activities. J Ethnopharmacol 2014;154:268-85.
2. Zalke AS, Kulkarni AV, Shirode DS, Duraiswamy B. *In vivo* anticancer activity of *Clerodendrum serratum* (L) Moon. Res J Pharm Biol Chem Sci 2010;1:89-98.
3. Chinchali JF, Sanakal RD, Kaliwal BB. Evaluation of the anti-carcinogenic activity of *Clerodendrum serratum* leaf extract on liver and kidney of 7, 12-dimethylbenz[a]anthracene (DMBA) induced skin carcinogenesis in mice. Eur J Exp Biol 2011;1:130-41.
4. Vidya SM, Krishna V, Manjunatha BK, Mankani KL, Ahmed M, Singh SDJ. Evaluation of hepatoprotective activity of *Clerodendrum serratum* L. Indian J Exp Biol 2007;45:538-42.
5. Narayanan N, Thirugnanasambantham P, Viswanathan S, Rajarajan S, Sukumar E. Comparative antibacterial activities of *Clerodendrum serratum* and *Premna herbacea* roots. Indian J Pharm Sci 2004;66:453-4.
6. Vidya SM, Krishna V, Manjunatha BK, Rao RB. Antibacterial activity of *Clerodendrum serratum* L. Electron. J Environ Agric Food Chem 2010;9:1059-63.
7. Narayanan N, Thirugnanasambantham P, Viswanathan S, Vijayasekaran V, Sukumar E. Antinociceptive, anti-inflammatory and antipyretic effects of ethanol extract of *Clerodendrum serratum* roots in experimental animals. J Ethnopharmacol 1999;65:237-41.
8. Bhujbal SS, Kewatkar SMK, More LS, Patil MJ. Antioxidant effects of roots of *Clerodendrum serratum* Linn. Pharmacogn Res 2009;1:294-8.
9. Ismail SM, Leelavathi S. Evaluation of antioxidant activity of *Anisolmeles malabarica* R Br and *Clerodendrum serratum* L. extracts against rheumatism. Res J Pharm Biol Chem Sci 2011;2:488-95.
10. Mohamed AJ, Mohamed EAH, Abdalrahim FAA, Ameer OZ, Ismail Z, Ismail N, *et al.* Antioxidant, antiangiogenic and vasorelaxant activities of methanolic extract of *Clerodendrum serratum* (Spreng.) leaves. J Med Plants Res 2012;6:348-60.
11. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. Chapman and Hall: London; 1998. p. 34-86.
12. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144-58.

13. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999;64:555-9.
14. Hiai S, Oura H, Nakajima T. Color reaction of some sapogenins and saponins with vanillin and sulphuric acid. Planta Med 1976;29:116-22.
15. Pawar HA, D'Mello PM. Spectrophotometric estimation of total polysaccharides in *Cassia tora* gum. J Appl Pharm Sci 2011;1:93-5.
16. Williams LAD, Connar AO, Latore l, Dennis O, Ringer S, Whittaker JA, *et al.* The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat-treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. West Indian Med J 2008;57:327-31.
17. Oyedepo OO, Femurewa AJ. Anti-protease and membrane stabilizing activities of extracts of *Fagra zanthoxiloides*, *Olax subscorpioides* and *Tetrapleura tetraptera*. Int J Pharmacogn 1995;33:65-9.
18. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebenson Wiss Technol 1995;28:25-30.
19. Nadeem AM, Zia KIU, Mukhtar IA, Ashraf QF. Modified 2,2'-azinobis (3-ethylbenzo thiazoline)-6-sulphonic acid radical cation decolorization assay for antioxidant activity of human plasma and extracts of traditional medicinal plants. Acta Chim Slov 2008;55:408-18.
20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay . Free Radical Biol Med 1999;26:1231-7.
21. Sharma M, Gupta A. Preliminary phytochemical investigations of methanolic roots extract of *Clerodendrum serratum*: antiulcer activity and histopathological study of stomach mucosa of wistar rats in ethanol-induced ulcer. J Pharm Res Opinion 2013;1:202-8.
22. Adesegun SA, Fajana A, Orabueze CI, Coker HAB. Evaluation of antioxidant properties of *Phaulopsis fascisepala* C. B. Cl. (Acanthaceae). J Evidence-Based Complementary Altern Med 2009;6:227-31.
23. Wong SP, Leong LP, Koh JHW. Antioxidant activities of aqueous extracts of selected plants. Food Chem 2006;99:775-83.
24. Garg VKR, Jain M, Sharma PKR, Garg G. Anti-inflammatory activity of *Spinacia oleracea.* Int J Pharm Professional's Res 2010;1:1-4.
25. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharm Sci 2010;2:146-55.
26. Amir M, Javed SA, Kumar H. Design and synthesis of 3-[3-(substituted phenyl)-4-piperidin-1-ylmethyl/-4-morpholin-4-ylmethyl-4,5-dihydro-isoxazol-5-yl]-1H-indoles as potent anti-inflammatory agents. Med Chem Res 2010;19:299-310.
27. Chu KF, Dupuy DE. Thermal ablation of tumours: biological mechanisms and advances in therapy Nat Rev 2014;14:199-208.
28. Anandjiwala S, Bagul MS, Parabia M, Rajani M. Evaluation of free radical scavenging activity of an ayurvedic formulation-*Panchvalkala*. Indian J Pharm Sci 2008;70:31-6.

How to cite this article

- Niyati S Acharya, Jagruti J Patel. Phytochemical evaluation and *in vitro* antioxidant and anti-inflammatory effects of *clerodendrum serratum* roots. Int J Pharm Pharm Sci 2016;8(8):158-163.