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Antioxidant, Antliglycation and Antimicrobial Activities of *Ziziphus oxyphylla* and *Cedrela serrata* Extracts

Rizwan Ahmad[1,2*], Atul Upadhyay[1], Mansoor Ahmad[2] and Luc Pieters[1]

[1]Department of Pharmaceutical Sciences, Natural Products & Food-Research and Analysis, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium.
[2]Department of Pharmacognosy, Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan.

Authors' contributions

This work was carried out in collaboration between all authors. Author RA designed the study and wrote the protocol. Authors RA and AU conducted experimental work and wrote the manuscript. Authors MA and LP managed the literature searches and analyzed the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To determine the antioxidant, antiglycation and antibacterial activity of two selected plants found wild in Pakistan (*Ziziphus oxyphylla* and *Cedrela serrata*).
Study Design: *In vitro* assessment of antioxidant assays, phenolic and flavonoid content, protein-glycation inhibition and antibacterial study.
Place and Duration of Study: Department of Pharmaceutical Sciences, University of Antwerp Belgium (February - April 2013; Antioxidant, Protein glycation). Department of Pharmacognosy, University of Karachi, Pakistan (March – June, 2012; antibacterial).
Methodology: *In vitro* laboratory experimental tests; preparation of plant extracts, antioxidant assays ($ABTS^{\cdot+}$, PMS-NADH radicals), total phenol, total flavonoid; protein glycation (fluorescence); susceptibility tests (zones of inhibition).
Results: The bark of *C. serrata* contained the highest amount of total phenol (0.35 ± 0.04 mg GAE/g extract) and exhibited significantly superior $ABTS^{\cdot+}$ and PMS superoxide radical scavenging activity with IC_{50} values of 0.043 ± 0.001 mg/ml and 0.18 ± 0.01 mg/ml, respectively (P=0.05). Similarly, the protein-glycation assay revealed that the bark of *C. serrata* had the best inhibitory property with a low IC_{50} value of 0.61 ± 0.02 mg/ml

**Corresponding author: Email: Rizwan.Ahmad@ua.ac.be; rizvistar_36@yahoo.com;*

(*P*=0.05), probably due to the presence of high amounts of total phenol. Furthermore, the various extracts showed considerable inhibition against both Gram–positive and –negative bacterial growth when compared against two standard drugs neomycin and doxycycline.
Conclusion: The results of this study substantiate a probable role for these plants to be utilized as a natural source of antioxidant having a wide range of bioactivities.

Keywords: Ziziphus oxyphylla; Cedrela serrata; antioxidant; protein-glycation; antimicrobial.

1. INTRODUCTION

The beneficial effects of plant are attributed to the variety of phytochemicals present, and polyphenols are considered to have a major role in having such bioactivity. Many phenolic compounds have revealed a remarkable spectrum of biochemical and pharmacological actions thought to be due to their antioxidative and free-radical scavenging properties [1]. Non-enzymatic glycation of protein has been a major factor responsible for the complication in diabetes and ageing [2]. Many biochemical pathways associated with hyperglycemia can increase production of free radicals [3]. Therefore, antioxidants may play a theoretical strategy for preventing diabetic complications. Furthermore, with the ever-present threat of diseases derived from various pathogenic organisms of clinical importance and the emergence of strains resistant to synthetic drugs, the importance of the search for novel medicinal plant intensifies.

Ziziphus oxyphylla (Rhamnaceae) is a small almost glabrous tree or shrub, which are commonly used in folklore medicine for the treatment of several diseases [4-5]. Previous studies on this plant have isolated cyclopeptide alkaloids with bioactivities [6-9]. *Cedrela serrata* (Meliaceae) is a hardy tree, usually found in moist shady places from 1000 m to 2500 m. The leaves and bark of the plant is used in Pakistan to treat fever, diabetes, dysentery, blood diseases, and skin diseases [10-11]. Recently, Praveen et al. [12] have reported antioxidant and DNA protection activity of the leaves of *C. serrata*.

In this study, we investigated the antioxidant property of the methanolic extracts of two plants and studied their antibacterial properties. Furthermore, we examined the anti-glycation activity of the extracts and found that the activity was associated with the phenolic constituents of the extract.

2. MATERIALS AND METHODS

2.1 Plant Materials and Extraction

The plants *Z. oxyphylla* and *C. serrata* were collected from Swat Valley, Pakistan in 2009 and the voucher specimen (0012-2009/AZ; 0013-2009/BC) is deposited at the herbarium of the University of Karachi. The extract was prepared by soaking different plant parts in methanol for 15 days with occasional shaking. The filtered extract was dried under vacuum (40 ºC) to obtain a semisolid mass of *Z. oxyphylla* root (Zr), *Z. oxyphylla* leaf (Zl), *C. serrata* bark (Cb), and *C. serrata* leaf (Cl).

2.2 Total Phenol Content

The total phenol content of the samples was determined using Folin reagent [13]. Briefly, to 0.2 ml of sample (500 µg/ml), 1 ml of 50% Folin-Ciocalteu reagent was added. After mixing, 0.8 ml of 7.5% sodium bicarbonate was added, and the mixture was allowed to stand for 30 min with intermittent shaking. The absorbance was measured at 760 nm using GENSYS spectrophotometer. Total phenolic content was expressed as Gallic acid equivalent (GAE) in mg per g extract.

2.3 Total Flavonoid Content

The amount of total flavonoids in the extracts was measured spectrophotometrically as reported [14]. Briefly, 1 ml of 500 µg/ml of methanolic extract solution was mixed with 1 ml of 2% AlCl3. After incubation for 15 min, the absorbance was measured at 430 nm using spectrophotometer. The flavonoids content was expressed as quercetin equivalents (QE) in mg per g extract.

2.4 Superoxide-Radical Scavenging Activity by PMS-NADH System

The superoxide radical scavenging ability of extracts was assayed as the method of Ao et al. [15]. Samples of different concentrations (0.1 ml) dissolved in methanol was added to a mixture containing 0.5 ml NADH (105.6 µM) and 0.5 ml NBT (66 µM) in 0.1 M phosphate buffer (pH 7.4). The reaction was initiated by the addition of 0.5 ml, 30 µM PMS and the absorbance was measured after 5 min in 560 nm. The superoxide radical scavenging activity was calculated as:

% radical scavenging = $(OD_c - OD_s)/ODc \times 100$,

where, OD_c and OD_s are the optical densities of control and samples, respectively.

2.5 Total Antioxidant Activity ($ABTS^{\cdot+}$ assay)

The total antioxidant activity of extracts was measured by the $ABTS^{\cdot+}$ method as described by Ao et al. [14]. In this test, $ABTS^{\cdot+}$ radical cation was generated by mixing 7 mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) after incubation at room temperature in dark. Before used, the $ABTS^{\cdot+}$ solution was diluted with 80% ethanol to get an absorbance of 0.700 ± 0.050 at 734 nm, the spectrophotometer was preliminary blanked with 80% ethanol. $ABTS^{\cdot+}$ solution (3.9 ml, absorbance of 0.700 ± 0.050) was added to 0.1 ml of the tested samples and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 6 min and the absorbance was immediately recorded at 734 nm. Appropriate solvent blanks were run in each assay and measurement was performed in triplicate. The percentage decrease of the absorbance at 734 nm was calculated as described above.

2.6 Anti-Glycation Assay

The inhibition of protein glycation was measured as described previously [16], with modifications. The 200 µL each of extract at different concentrations in DMSO were incubated with BSA (10 mg/ml), glucose (500 mM) in 50 mM phosphate buffer (pH 7.4) at 60ºC for 48 h. The change in fluorescence intensity (excitation 360 nm, and emission 450 nm) based on AGEs formations were monitored using a spectrofluorometer (TECAN Infinite

M 200). In order to reduce the interference in the fluorescence signal by the extracts, parallel incubation at 4ºC were performed. The AGEs inhibition was calculated as

% AGEs inhibition = $[1-(S-S_b)/(C-C_b)] \times 100$

where S and C represent relative fluorescence units (RFU) for test samples (in DMSO) and control (test mixtures containing only DMSO) incubated at 60 ºC, and S_b and C_b are RFU for samples incubated at 4 ºC.

2.7 Antibacterial Assay

Six pathogenic bacteria were selected to evaluate the antimicrobial activity of plant extracts. The antibacterial activities were tested against *Staphylococcus aureus, Bacillus subtilis* (Gram-positive) and *Proteus mirabilis, Salmonella typhi, Escherichia coli* and *Citrobacter* spp. (Gram-negative). Antibacterial activity was determined by the agar well diffusion technique, with minor modifications [17]. Briefly, 0.5 ml of 24-h test bacterial broth culture (10^8 cfu/ml) was mixed with 20 ml of antibiotic medium No. 1 (Merck, Germany) and poured in 9-cm dia Petri dishes. After the solidification, 6 wells at equidistant were prepared using 5 mm of sterile cork borer. To the wells, 0.1 ml of plant extracts of different concentrations were added. The plates were then incubated at 37ºC for 24 h and the zones of inhibition were measured in millimeter. Two different classes of drugs, Neomycin (Nm) (bacteriocidal) and doxycycline (Do) (bacteriostatic), at different concentrations were used as positive controls.

2.8 Statistical Analyses

All of the experiments were conducted in triplicates and repeated twice. The data represent the mean ± standard deviation (SD) of six results. The IC_{50} value was determined by linear regression using Excel, Microsoft Office, 2010. For significance analysis, the data were separated using Tukey's HSD range at P=0.05. All statistical analysis were performed using SPSS version 20.0 for Windows Vista.

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity of the Extracts

The total phenolic content of the extracts are shown in Fig. 1A. The results showed that Cb had the highest total phenolic content (0.35 ± 0.04 mg GAE/ g extract), followed by Zr, Cl, and Zl (0.17 ± 0.02, 0.12 ± 0.01, and 0.09 ± 0.01 mg GAE/ g extract, respectively). For flavonoid content, Cl with 113.58 ± 3.68 mg QE/ g extract exhibited the highest amount (Fig. 1B). The flavonoid content of Zl, Cb, and Zr were 37.41 ± 1.77, 20.55 ± 1.01, and 7.96 ± 0.40 mg QE/ g extract, respectively. The $ABTS^{\cdot+}$ radical scavenging activity showed that Cb had the significantly better activity than other extracts with IC_{50} value of 0.04 ± 0.01 mg/ml (Table 1; P =0.05). The IC_{50} values for Zr, Zl, and Cl were 0.73 ± 0.04, 1.39 ± 0.07, and 0.60 ± 0.03 mg/ml, respectively. Similarly, for the PMS superoxide radical inhibition, Cb exhibited significantly the highest scavenging activity with IC_{50} of 0.18 ± 0.02 mg/ml, followed by Cl, Zr, and Zb respectively (Table 1; P =0.05).

It is well established that the antioxidant power of plant extract is strongly related to the total phenolic and flavonoid content [18]. In this study, we found that Cb had the best radical scavenging activity with very high total phenol content. This indicates that the antioxidant activity of the extracts is related with the total phenol content.

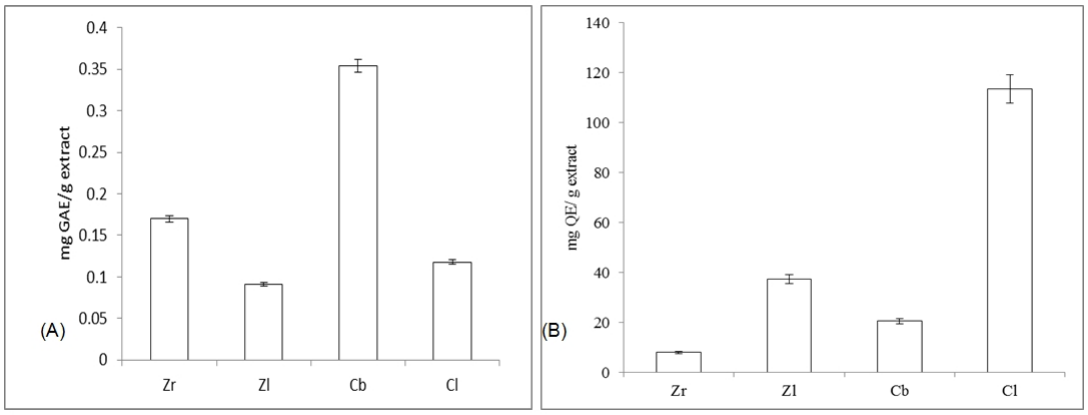

Fig. 1. Total phenolic content (A) and flavonoid content (B) of different plant extracts
Data represent Mean ± SD. See text for abbreviation.

Table 1. IC_{50} values (mg/ml) for antioxidant and antiglycation activities of plant extracts

Assays	Zr	Zl	Cb	Cl
$ABTS^{\cdot+}$ radical	0.72 ± 0.04^{b}	1.39 ± 0.07^{d}	0.04 ± 0.01^{a}	0.60 ± 0.03^{c}
PMS superoxide radical	0.70 ± 0.04^{c}	1.42 ± 0.11^{d}	0.18 ± 0.01a	0.57 ± 0.03^{b}
Antiglycation	1.31 ± 0.04^{c}	1.41 ± 0.04^{d}	0.61 ± 0.02^{a}	0.88 ± 0.03^{b}

The data represent mean ± SD of three replicates. See text for abbreviations. The same letters in rows mean no significant difference (P =0.05).

3.2 Inhibition of Protein Glycation

Advanced glycation endproducts (AGEs) are major factors responsible for the complication of diabetes [16]. It has been shown that antioxidants protect against glycation-derived free radicals and may have therapeutic potential [19]. Furthermore, it is reported that compounds with combined antioxidant and antiglycation properties are more effective in treating diabetes mellitus [20]. Since these extracts contained higher phenolic and flavonoid content, they were also subjected to antiglycation studies.

The prevention of AGEs formation by various extracts is shown in Table 1. It was found that Cb had the highest activity in barring AGEs formation with a low IC_{50} value of 0.61 ± 0.02 mg/ml (*P* =0.05), which was followed by Cl, Zl, and Zr with the respective IC_{50} values of 0.89 ± 0.03, 1.32 ± 0.04, and 1.41 ± 0.04 mg/ml. The high phenolic content of Cb and high flavonoid content of Cl may be the probable reasons for anti-glycation activity. This result is consistent with previous studies where concentration of phenolics has been related with inhibition of protein glycation [21].

3.3 Antimicrobial activity of the extracts

The results showed that all extracts have inhibited the growth of bacteria to different degrees in a dose-dependent manner. A typical plate with different zones of inhibition is shown in Fig. 2. At the highest concentration (150 µg/ml) used, the bark extract of *C. serrata* (Cb) showed significantly better antimicrobial activities than the root extract of *Z. oxyphylla* (Zr) against all bacteria except against *S. aureus* and *S. typhi* (*P* =0.05; Table 2). The results indicated that Zr exhibited a significantly higher zone of inhibition of 22.29 ± 0.04 mm against *S. aureus*, whereas, Cb had greater suppression against *Citrobacter spp.* growth with an inhibition zone of 22.33 ± 0.05 mm when used at 150 µg/ml (*P* =0.05; Table 2). At the same dose of standard antibiotics used in this assay, neomycin (Nm) and Doxycycline (Do) had better zones of inhibition (33.16 ± 0.07 and 27.1 ± 0.05 mm, respectively.

However, the leaf extracts of *Z. oxyphylla* (Zl) and *C. serrata* (Cl) showed considerable activity only at doses higher than 300 µg/ml. On carrying out statistical analysis, it was found that Cl had significantly higher activity than Zl against *Citrobacter*, *B. subtilis*, and *S. aureus* (*P* =0.05; Table 3). The highest inhibitory activity for Zl was against *S. typhi* (22.15 ± 0.01 mm), whereas Cl showed greater inhibition against *Citrobacter* spp, with a zone of inhibition of 21.31 ± 0.01 mm (*P* =0.05; Table 3). The standard drugs, Nm and Do, showed higher inhibition zones (28.6 ± 0.04 and 28.11 ± 0.04 mm, respectively) for the same organisms at the same dose. Previous studies on methanolic extracts of leaf and stem parts of *Z. oxyphylla* have also reported antibacterial properties [22-23].

The antimicrobial assays showed varied results with different extracts and organisms. These variations may reflect differences in cell surface structures between different microorganisms. The higher antibacterial activities of Cb and Zr may owe to the higher amounts of total phenols. Previous studies have reported that the inhibition of growth of microorganisms could be attributed to phenolic compounds present in the extracts [13].

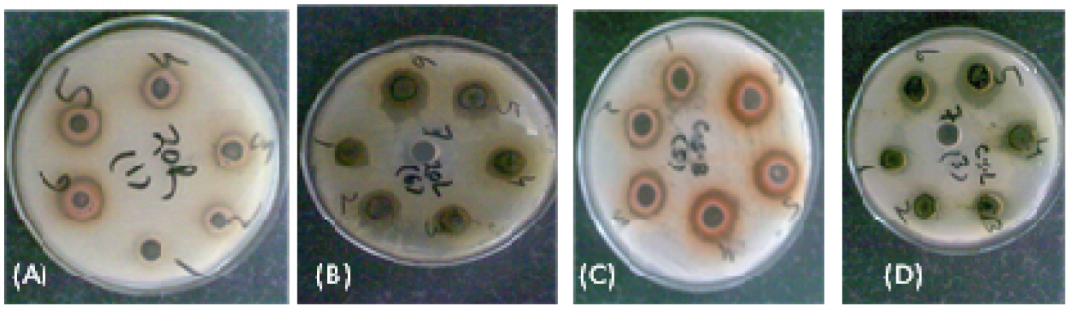

Fig. 2. Typical plates showing zones of inhibition by various extracts Zr (A), Zl (B), Cb (C), and Cl (D)

Table 2. Antibacterial activities of *Z. oxyphylla* roots and *C. serrata* bark

µg/ml	Extract	*Citrobacter*	*E. Coli*	*B. subtilis*	*S. aureus*	*P. mirabilis*	*S. Typhi*
	Zr	12.84±0.01	12.73±0.00	12.51±0.06	0.00±0.00	0.00±0.00	0.00±0.00
10	Cb	17.30±0.01	17.15±0.07	16.74±0.01	16.73±0.01	16.18±0.01	17.12±0.05
	Nm	24.59±0.01	22.09±0.08	21.16±0.05	27.22±0.03	18.75±0.15	23.71±0.05
	Do	21.84±0.20	25.48±0.05	21.02±0.02	18.01±0.09	20.22±0.10	22.55±0.05
	Zr	13.20±0.06	13.94±0.06	13.51±0.01	12.82±0.01	11.25±0.01	14.63±0.01
25	Cb	17.31±0.01	17.15±0.01	16.75±0.01	15.85±0.08	16.19±0.01	17.13±0.01
	Nm	24.59±0.08	22.49±0.06	22.85±0.07	29.19±0.02	21.09±0.03	25.42±0.08
	Do	24.42±0.01	25.52±0.05	25.39±0.09	22.80±0.07	22.84±0.03	24.87±0.03
	Zr	14.99±0.01	15.62±0.01	14.72±0.01	15.99±0.01	11.94±0.01	15.92±0.01
50	Cb	17.43±0.01	17.15±0.01	17.85±0.01	18.11±0.01	17.30±0.05	17.13±0.01
	Nm	26.37±0.10	25.65±0.09	25.71±0.11	31.76±0.08	24.04±0.02	25.40±0.05
	Do	24.53±0.02	25.73±0.05	26.78±0.07	24.12±0.08	32.36±0.05	25.61±0.06
	Zr	16.79±0.01	16.17±0.06	15.26±0.08	16.53±0.09	12.86±0.06	15.93±0.07
75	Cb	19.08±0.01	18.71±0.02	19.12±0.08	18.41±0.01	19.25±0.06	18.33±0.01
	Nm	26.38±0.05	28.86±0.10	26.54±0.09	32.76±0.05	25.44±0.06	26.04±0.05
	Do	26.22±0.03	26.29±0.04	27.16±0.06	25.13±0.06	24.83±0.05	27.42±0.07
	Zr	17.39±0.01	17.06±0.06	15.96±0.05	17.89±0.01	15.16±0.06	17.53±0.06
100	Cb	21.89±0.01	19.46±0.05	19.68±0.07	19.83±0.05	19.26±0.07	19.81±0.05
	Nm	26.47±0.02	28.79±0.10	27.05±0.02	33.00±0.09	25.45±0.08	26.04±0.02
	Do	27.62±0.05	26.55±0.02	30.79±0.03	25.40±0.04	24.87±0.05	28.20±0.03
	Zr	$18.29±0.05^{a,3}$	$17.62±0.03^{a,2}$	$17.00±0.05^{a,1}$	$22.29±0.04^{b,5}$	$17.21±0.05^{a,1}$	$19.86±0.06^{a,4}$
150	Cb	$22.33±0.05^{b,5}$	$21.46±0.06^{b,4}$	$20.55±0.05^{b,3}$	$20.57±0.02^{a,3}$	$19.58±0.05^{b,1}$	$19.82±0.32^{a,2}$
	Nm	$29.53±0.14^{d}$	$29.04±0.11^{d}$	$28.44±0.02^{c}$	$33.16±0.07^{d}$	$25.45±0.02^{c}$	$28.60±0.01^{c}$
	Do	$28.11±0.02^{c}$	$26.81±0.02^{c}$	$31.40±0.01^{d}$	$27.13±0.05^{c}$	$25.11±0.02^{c}$	$29.94±0.03^{d}$

Values are means of 3 replications ± SD. See text for abbreviations. The same letter in a column indicate no significance difference (P =0.05). The same numbers in the same row indicate no significant difference (P =0.05).

Table 3. Antibacterial activities of *C. serrata* and *Z. oxyphylla* and leaves

µg/ml	Extract	*Citrobacter*	*E. Coli*	*B. subtilis*	*S. aureus*	*P. mirabilis*	*S. typhi*
300	Cl	0.00 ±0.00	16.22±0.06	13.17±0.01	0.00±0.00	12.42±0.02	16.02±0.05
	Zl	14.78±0.04	17.69±0.05	12.78±0.06	0.00±0.00	12.33±0.05	12.96±0.02
	Nm	24.59±0.01	22.09±0.08	21.16±0.05	27.22±0.03	18.75±0.15	23.71±0.05
	Do	21.84±0.20	25.48±0.02	21.02±0.02	18.01±0.09	20.22±0.10	22.55±0.05
	Cl	13.58±0.05	16.21±0.02	13.17±0.05	0.00±0.00	13.11±0.01	16.66±0.03
500	Zl	14.78±0.06	17.69±0.04	13.72±0.05	11.74±0.05	12.62±0.01	12.96±0.02
	Nm	24.59±0.81	22.49±0.61	22.85±0.02	29.19±0.02	21.09±0.03	25.42±0.08
	Do	24.41±0.12	25.53±0.50	25.39±0.93	22.80±0.02	22.84±0.03	24.87±0.02
	Cl	14.93±0.01	16.21±0.03	14.19±0.05	0.00±0.00	13.10±0.01	16.68±0.01
600	Zl	14.79±0.01	17.72±0.23	13.72±0.07	14.63±0.06	13.16±0.01	12.97±0.01
	Nm	26.37±0.10	25.65±0.91	25.72±0.12	31.76±0.08	24.04±0.02	25.40±0.01
	Do	24.53±0.02	25.73±0.52	26.78±0.60	24.12±0.08	32.36±0.05	25.62±0.01
	Cl	14.94±0.01	14.94±0.01	14.45±0.05	13.72±0.03	13.12±0.01	17.68±0.01
800	Zl	15.14±0.02	17.71±0.07	14.62±0.07	14.63±0.06	13.17±0.05	13.63±0.01
	Nm	26.38±0.05	28.86±0.10	26.54±0.09	32.76±0.05	25.44±0.06	26.04±0.05
	Do	26.21±0.02	26.29±0.04	27.16±0.06	25.13±0.06	24.83±0.05	27.42±0.07
	Cl	19.22±0.03	17.23±0.05	20.34±0.07	14.49±0.05	14.86±0.07	17.69±0.01
1000	Zl	15.52±0.05	17.82±0.06	14.62±0.03	14.64±0.05	13.18±0.01	17.86±0.01
	Nm	26.47±0.02	28.79±0.10	27.05±0.05	33.00±0.09	25.45±0.08	26.04±0.05
	Do	27.62±0.05	26.55±0.07	30.79±0.01	25.40±0.04	24.87±0.05	28.20±0.06
	Cl	$21.31\pm0.01^{b,5}$	$18.23\pm0.05^{a,3}$	$20.35\pm0.05^{b,4}$	$15.52\pm0.07^{b,1}$	$15.57\pm0.01^{a,1}$	$17.71\pm0.01^{a,2}$
1500	Zl	$19.13\pm0.05^{a,3}$	$20.42\pm0.02^{b,4}$	$14.62\pm0.08^{a,1}$	$14.65\pm0.05^{a,1}$	$16.72\pm0.01^{b,2}$	$22.15\pm0.01^{b,5}$
	Nm	29.53 ± 0.12^{d}	29.04 ± 0.13^{d}	28.44 ± 0.05^{c}	33.16 ± 0.07^{c}	25.45 ± 0.08^{c}	28.60 ± 0.04^{c}

Values are means of 3 replications ± SD. See text for abbreviations. The same letter in a column indicate no significance difference (P =0.05). The same numbers in the same row indicate no significant difference (P =0.05).

4. CONCLUSION

It was found that methanolic extracts of *C. serrata* had higher contents of phenolic acid and flavonoids with better anti-glycating and anti-bacterial activities, although these properties are not correlated. Our results provide a pharmacological explanation of using these plants in folk medicine in Pakistan. From the above study, it can be inferred that the main constituents responsible for the antioxidant, antiglycation, and antibacterial activities of the extracts were phenolic and flavonoid groups. However, further studies on isolation and structure elucidation of active components from the extract as well as investigations in their inhibitory mechanism is indispensable.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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