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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *ZALEYA PENTANDRA*

SAMINA AFZAL[1], BASHIR AHMAD CHAUDHRY[1], JAVARIA SAEED[1], KHURRAM AFZAL[1], BILAL AHMED[2] and MUHAMMAD IMRAN QADIR[3]*

[1]Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan
[2]College of Medicine, Nursing and Health Sciences, Fiji National University, Fiji Island
[3]Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan, Pakistan

Abstract: The objective of this study was to evaluate the antibacterial and antioxidant activity of methanolic extract of *Zaleya pentandra*. It inhibited the growth of *S. typhi,* with zone of inhibition 13 mm at concentration 3 mg/100 µL and 11 mm at concentration 1.5 mg/100 µL. It also showed zone of inhibition against *S. aureus* with 17.5 mm and 12.5 mm at concentration 3 mg/100 µL in comparison to erythromycin with 15.6 mm. It showed 73% radical scavenging at concentration 161 µL/mL. The extract was fractioned by column chromatography using eluents (chloroform : methanol : H_2O). The isolation and purification afforded amorphous solid which was subjected to physical, chemical and spectral techniques UV, IR, ^{1}H-NMR, ^{13}C-NMR and HREI-MS for the structure elucidation of the isolated compound. The compound was named pentandraol. From the present study, it was concluded that the methanolic extract of *Zaleya pentandra* has antibacterial and antioxidant activity and contains a novel compound named as pentandraol.

Keywords: *Zaleya pentandra*, extraction, antibacterial activity, antioxidant activity

Antibiotic resistance is a common issue in the whole world and it has become a serious problem in case of treatment of many diseases which are caused by resistant strains of bacteria. Therefore, for the survival of human population and better quality of health, we need some other antimicrobials which are highly effective against such dangerous resistant strains. Antioxidant compounds boost immune system making the human body to fight against the infection (1). Recently many plants have been proved to have antibacterial activities: *Vicia sativa*, *Asparagus racemosus*, *Amberboa divaricata* (2-4).

Zaleya pentandra L. (synonym *Trianthema pentandra* L.), belonging to family Aizoaceae, is widely distributed prostrate and branched herbs. The genus has 6 species found in Africa, Asia and Australia, only one species, *Zaleya pentandra* is found in Pakistan (5). Traditionally, *Zaleya pentandra* is used for stomach ailment, influenza and phlegmatic cough (6).

The objective of this study was to evaluate the antibacterial and antioxidant activity of methanolic extract of *Zaleya pentandra*.

MATERIALS AND METHODS

Plant collection

The whole plant of *Zaleya pentandra* was collected from Peruwal (District Khanewal) in May 2005 and identified by Professor Dr. Altaf Ahmed Dasti, Plant Taxonomist, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan; voucher specimen No. FLP-235/5 for *Zaleya pentandra* was deposited.

Extraction

The freshly collected whole plant material of *Zaleya pentandra* (1000 g) was shade dried, ground and extracted successively with methanol (3 × 6 L) at room temperature for 24 h. The combined methanolic extract was concentrated under vacuum on Buchi-rotavapor model no. R.200 to yield dark brown crude extract (35 g).

Spectral analysis

Column chromatography has been used for the purpose of isolation with silica gel of 70-230, 230-400 mesh along with Sephadex LH-20. For TLC

* Corresponding author: e-mail: mrimranqadir@hotmail.com

purpose, aluminium sheets pre-coated silica gel 60 F_{254} (20 × 20 cm, 0.2 mm thick; E-Merck) have been used to check the percentage purity of the compounds. The visualization of components was observed under ultraviolet light (254 and 366 nm) followed by Godine reagent and 10% sulfuric acid used as spraying reagents. IR spectrum was recorded using Bruker vector-200 spectrophotometer (ν in cm^{-1}). EI-MS spectrum was recorded on Jeol JMS-600H spectrometer and HREI-MS was recorded on MET-95-XP apparatus. The ^{1}H-NMR spectrum was recorded by Bruker Avon-300 MHz instrument by using TMS as internal standard. The values of chemical shift were reported in ppm (δ) units and the coupling constants (*J*) were recorded in Hz. The ^{13}C-NMR spectrum was also recorded on Bruker Avon-300 MHz instrument.

Antibacterial assay

Assay was carried out using agar well diffusion method. Mueller Hinton agar medium was prepared by suspending Mueller Hinton agar 38 g/L of distilled water and then autoclaved. After autoclaving, it was cooled up to 50°C then, 75 mL of medium was poured in each 14 cm Petri plates and after solidifying it was incubated at 37°C for 24 h to test the sterility. Then, the plates were seeded with inoculums of different strains of bacteria with the help of sterile cotton swabs. Ten wells per plate were made using the sterile steel borer (8 mm). Hundred microliters of each test solution and their different dilutions were poured in respective well. Erythromycin (30 µg/100 µL) was used as positive control. DMSO was used as negative control. Finally, each plate was poured with four dilutions of two different plant extract samples one positive control and one negative. Then, the Petri plates were incubated for 24 h at 37°C. After 24 h incubation, detected zone of inhibition (clear zones) were measured by using vernier caliper. The experiment was performed in triplicate and the final results were calculated.

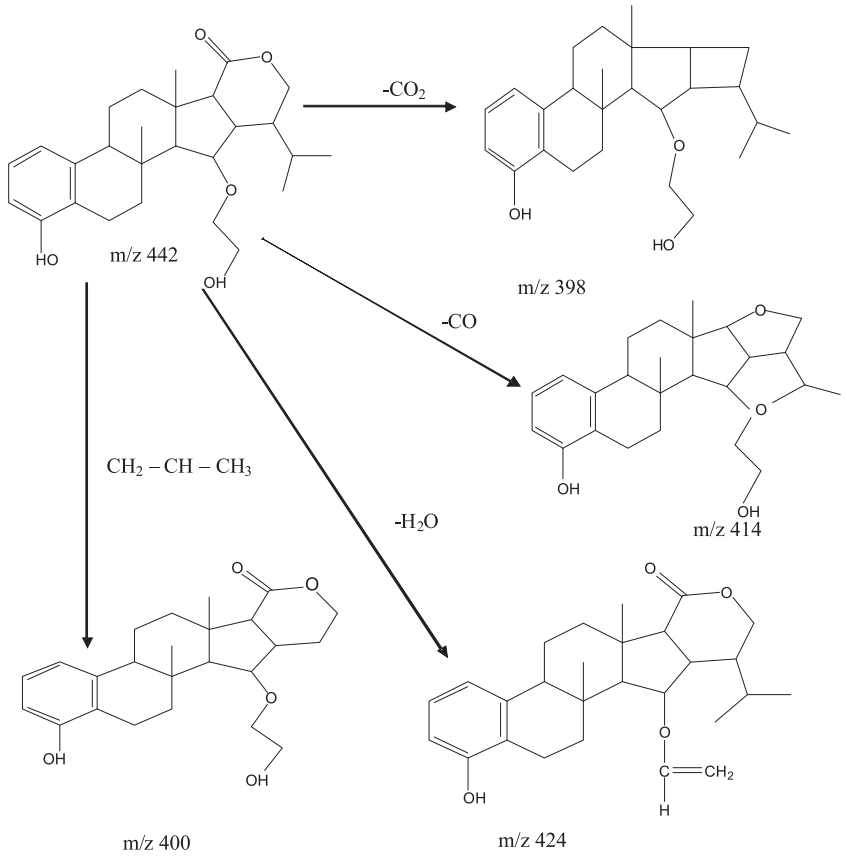

Figure 1. Mass fragmentation pattern of the isolated compound

Table 1. Zone of inhibition (in mm) by different concentrations of methanolic extract of *Zaleya pentandra* as compared to erythromycin (30 µg/100 µL).

Concentration (mg/100 µL)	*E. coli*	*S. typhi*	*B. spizizenii*	*S. aureus*	*S. epidermidis*
Standard	15	13	16.3	15.6	15.3
0.375	0	0	26	0	0
0.75	0	0	34	0	0
1.5	0	11˘	39	12.5˘	0
3	0	13˘	30	17.5˘	0

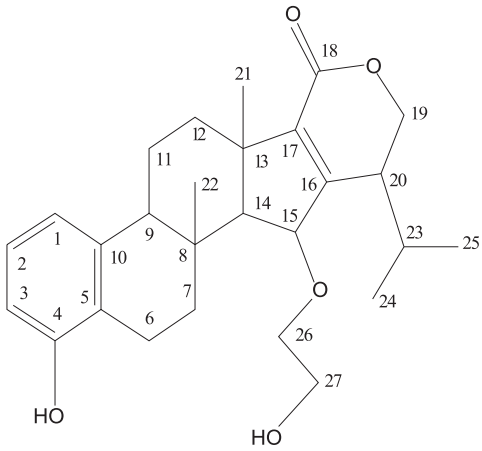

Figure 2. Structure of pentandraol ($C_{27}H_{37}O_5$: m.w. 442)

Antioxidant activity

The free radical scavenging activity was measured with 1,1-diphenyl-2-picrylhydrazyl (DPPH). The DPPH stock solution was prepared by dissolving 20 mg DPPH in 100 mL 95% methanol. This stock solution was stored at 20°C until needed, not more than 10 days. DPPH working solution was prepared by diluting the stock of DPPH solution by adding methanol and absorbance was adjusted about 0.980 ± 0.02 at 517 nm wavelength using the spectrophotometer. Three milliliter aliquot of this working solution mixed with 100 µL of the plant samples at five different varying concentrations (4–322 µg/mL). The solutions in the test tubes were shacked well and put in dark for 15 min at room temperature. Then again, the absorbance was measured at 517 nm. The percentage scavenging activity was determined based on the percentage of DPPH radical scavenged by using the following equation:

Scavenging effect (%) = [control absorbance – sample absorbance/ control absorbance] × 100

RESULTS

Spectral analysis

The compound was isolated as amorphous solid from aerial part of methanolic extract of *Zaleya pentandra*. The IR spectrum of compound showed the absorption band at 1725 cm^{-1} (C=O). The absorption at 1599 cm^{-1} showed the presence of (C=C), the absorption at 3339 cm^{-1} was due to hydroxyl function and stretching bands at 2856 cm^{-1} and 2926 cm^{-1} were due to sp^3 C-H and sp^2 C-H, respectively. The absorption at 1664 cm^{-1} showed the presence of aromaticity. UV spectroscopy gave the result of (MeOH) λ_{max} log nm = 230 (0.20), 255 (0.08).

The ^{1}H-NMR spectrum of the compound showed aromatic hydrogen at δ 7.60 ppm (1H, d, *J* = 3.4 Hz, H-1), 7.61 ppm (1H, dd, *J* = 2.2, 3.3 Hz, H-2), 7.62 ppm (1H, dd, *J* = 0.6, 3.5 Hz, H-3). The phenolic proton gave singlet at δ 8.53 ppm (1H, s, H-4) and alcoholic proton gave triplet at δ 3.41 ppm (2H, t, *J* = 1.2, Hz, H-27). The compound displayed signals at δ 0.94 ppm (6H, d, *J* = 7.4 Hz, H-24, 25) showing the presence for isopropyl proton. The signal showed lactone hydrogen at δ 4.60 ppm (2H, d, *J* = 4.5 Hz, H-19).

The ^{13}C-NMR spectra (BB and DEPT) of compound disclosed 27 carbon signals for four methyls, ten methines, seven methylenes and six quaternary carbon moieties. The downfield signal at δ 169.3 ppm singlet showed the presence of carbonyl carbon. The aliphatic carbon appeared at δ 21.0 to 49.4 ppm. The signals at δ 71.6, 69.0 ppm showed the presence of lactone carbon. The signals at δ 127.0, 128.6, 129.8, and 132.4 ppm indicated the presence of aromatic ring. The signal at δ 132.4 ppm showed the presence of phenolic carbon. HR-EI-MS *m/z* gave the result of 442 $[M]^+$ (calculated for $C_{27}H_{37}O_5$: 442). HREI-MS pattern of the compound is given in Figure 1. By comparing the data with literature it was found a novel compound,

Table 2. DPPH radical scavenging activity of different extracts of *Zaleya pentandra* using ascorbic acid as standard.

	Percentage radical scavenging at conc.			
	32 µg/mL	16 µg/mL	8 µg/mL	4 µg/mL
Extracts of *Zaleya pentandra*	73.79734	26.20266	22.72262	21.69908
Control	-	93.69919	89.12602	56.09756

Table 3. ^{13}C- (300 MHz) and ^{1}H-NMR (300 MHz) spectral data of compound "pentandraol".

Carbon no.	Multiplicity (DEPT)	C^{13}-NMR (δ, ppm)	^{1}H-NMR (δ, ppm)	*J value*
C – 1	CH	127.0	7.60 d	(*J* = 3.4, Hz, H-1)
C – 2	CH	128.6	7.61 dd	(*J* = 2.2, 3.3 Hz, H-2)
C – 3	CH	129.8	7.62 dd	(*J* = 0.6, 3.5 Hz, H-3)
C – 4	CH	132.4	8.53 s	(*J* = 1H, s, H-4)
C – 5	C	133.6	-	-
C – 6	CH_2	11.4	3.41, 3.43 t	(*J* = 1.0, Hz, H-6)
C – 7	CH_2	30.1	1.66, 1.65 dt	(*J* = 5.9, 6.0, Hz, H-7)
C – 8	C	40.1	-	-
C – 9	CH	48.5	3.29 t	(*J* = 1.3, Hz, H-9)
C – 10	C	142.9	-	-
C – 11	CH_2	24.0	1.68, 1.70 dt	(*J* = 7.8, 6.1 Hz, H-11)
C – 12	CH_2	24.9	1.42, 1.44 t	(*J* = 7.0, Hz, H-12)
C – 13	CH	49.0	-	-
C – 14	CH	49.5	1.67 dd	(*J* = 6.2, 5.9, Hz, H-14)
C – 15	CH	71.6	4.20 dd	(*J* = 4.9, 5.8, Hz, H-15)
C – 16	C	129.8	-	-
C – 17	C	132.4	-	-
C – 18	C	169.3	-	-
C – 19	CH_2	71.6	4.60, 4.22 d	(*J* = 4.5, Hz, H-19)
C – 20	CH	49.0	3.30 dt	(*J* = 1.8, 0.6, Hz, H-20)
C – 21	CH_3	30.1	0.92 s	(*J* = 3H, s, H-21)
C – 22	CH_3	30.1	0.91 s	-
C – 23	CH	24.9	1.68 m	-
C – 24	CH_3	24.0	0.94 d	(*J* = 7.4, Hz, H-24)
C – 25	CH_3	24.0	0.94 d	(*J* = 7.4, Hz, H-25)
C – 26	CH_2	69.0	4.21 t	(*J* = 4.8, Hz, H-26)
C – 27	CH_2	71.6	3.41 t	(*J* = 1.3, Hz, H-27)

s = singlet, dd = doublet of doublets, d = doublet, dt = doublet of triplets, t = triplet, td = triplet of doublets, q = quartet, br = broad signal, quint = quintet, m = multiplet (denotes complex pattern).

which name was given on the basis of species as "pentandraol" (Fig. 2).

Antibacterial activities

Antibacterial activity was determined against the five different strains of bacteria, two of them were Gram negative bacteria (*Escherichia coli, Salmonella typhimurium*) and three were Gram positive bacteria (*Bacillus spizizenii, Staphylococcus aureus, Staphylococcus epidermidis*) (Table 1). The zones of inhibition of *Zaleya pentandra* methanolic extract (ZPM) against Gram negative bacterium *S.*

typhi were found 13 mm and 11 mm at extract conc. 3 mg/100 µL and 1.5 mg/100 µL, respectively. ZPM also showed zones of inhibition against Gram positive bacteria *S. aureus* were reported as 17.5 mm and 12.5 mm with conc. 3 mg/100 µL and 1.5 mg/100 µL, respectively. The activity against Gram positive bacterium *B. spizizenii* was observed as large bacteriostatic zones: 30 mm, 39 mm, 34 mm 26 mm at different concentrations.

Antioxidant activity

In the antioxidant activity test (DPPH), it showed the highest inhibitory percent reaching 73% radical scavenging at conc. 161 µL/mL (Table 2).

DISCUSSION

The genus *Zaleya* has diverse medicinal application that prompted us to carry out the phytochemical investigations on this species. Herein we report the isolation and characterization of a novel steroidal compound named pentandraol. The genus is enriched with pharmacological properties. *Trianthema decandra* showed hepatoprotective, antidiabetic, antioxidant, antibacterial, antimicrobial, antipyretic, analgesic and anti-inflammatory activities (7). *Trianthema portulacastrum* also displayed hepatoprotective activity, anti-cancer property, hypoglycemic, anti-hyperglycemic, hypolimidemic and anthelmintic activity (8). Literature survey of genus revealed phytochemical constituents: trianthemine, trianthenol, and ecdysteroid along with flavonoids and phytoserolines and ketone (9). We have isolated a novel steroidal compound, pentandraol. The steroids have already been confirmed to have antibacterial and antioxidant activity (10). Therefore, it seems that antibacterial and antioxidant activity of methanolic extract of *Zaleya pentandra* is due to this steroidal constituent.

From the present study, it was concluded that the methanolic extract of *Zaleya pentandra* has antibacterial activity against *B. spizizenii* and *S. aureus* along with antioxidant activity. It contains a novel compound named as pentandraol.

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Conflicts of interests

No conflicts of interests exist.

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