Antioxidant Potential of Cyclopeptide Alkaloids Isolated from *Zizyphus oxyphylla*

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Summary: The present study reports on the antioxidant potential of five cyclopeptide alkaloids isolated from *Zizyphus oxyphylla* including Oxyphylline-D **1**, Nummularin-C **2**, Nummularin-R **3**, Oxyphylline-B **4**, Oxyphylline C **5** using DPPH free radical assay, nitric oxide radical assay and reducing power assay. The isolated alkaloids demonstrated marked antioxidant potential in a concentration dependent manner. Among the tested molecules, the compounds, **2** was most potent with IC_{50} values of 27.23, 32.03 and 22.45 µg/ml in DPPH free radical assay, nitric oxide radical assay and reducing power assay respectively.

Keywords: *Zizyphus oxyphylla,* cyclopeptide alkaloids, antioxidant activity.

Introduction

Zizyphus is a genus of about 40 species belonging to family Rhamnaceae. Mostly spiny shrubs and small trees, these species are distributed in warm-temperate and subtropical regions of the world. *Zizyphus oxyphylla* Edgew is a small or medium sized tree growing in northern areas of Pakistan. It is used in these areas as a folk medicinal remedy in the treatment of inflammatory conditions, pain, especially of rheumatic origin, fever, microbial infections, allergy and diabetes [1]. *Z. oxyphylla* has been shown to possess analgesic and antipyretic activities in animal models [1]. The analgesic and antipyretic activities of some other species of the genus *Zizyphus* have also been reported [1, 2]. Cyclopeptide alkaloids are a large group of compounds, which are present in a several plant families. Some of the representative families are *Asteraceae, Celastraceae, Euphorbiaceae, Menispermaceae, Pandaceae, Rubiaceae, Sterculiaceae* and *Urticaceae* along with *Rhamnaceae*. These compounds may be defined as basic compounds having a structure 10 or 12 member peptide type bridge span that is attached to benzene at 1,3 or 1,4 positions. These alkaloids have been reported to have antibacterial, antifungal and sedative activity [3].

This article deals with the antioxidant activities of five cyclopeptide alkaloids isolated from *Z. oxyphylla* against DPPH free radical, nitric oxide scavenging and reducing power assay.

Experimental

Plant material, extraction and isolation

The plant material was collected from Swat Valley (Khyber Pakhtunkhwa, Pakistan). Plant was identified by Dr. Hassan Sher, Jehanzaib College Swat and voucher specimen has been deposited in the national herbarium Islamabad with voucher no NH-012 (2004). The air-dried root powder of *Z. oxyphylla* (8 kg) was macerated in methanol (3 × 7 days × 20 L). After removal of the solvent under vacuum at 35 °C–40 °C, the crude extract (372 g) was obtained. This extract was partitioned between water and dichloromethane. Dichloromethane extract (2.5 g) was subjected to column chromatography over silica gel with hexane/acetone/diethyl amine (75:25:0.1, 10 L) mixture to afford eight fractions (A–H). Compounds **1** and **2** were obtained from fractions D (41.3 mg) and E (53.4 mg) by preparative TLC (hexane/acetone/diethyl amine, 15:10:1).

The air-dried stem powder of *Z. oxyphylla* (8 kg) was macerated in methanol (3 × 7 days × 20 L) solvent was removed under reduced pressure at 35–40°C to give the crude extract (375 g), which was

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partitioned between water and chloroform. Chloroform extract (11.0 g) was purified by column chromatography over silica gel with hexane/acetone/diethyl amine mixture (75:25:0.1, 10 L) to give eight fractions (A–H). Fraction C (202 mg) was subjected to preparative TLC (hexane/acetone/diethyl amine, 15:10:1) to obtain compound 3. Fraction D (1.89 g) was subjected to column chromatography over silica gel using chloroform/methanol (89:11) gradient. Compounds 4 (Oxyphylline B) and 5 (Oxyphylline C) were recovered [1].

DPPH radical scavenging assay

The antioxidant activity was performed by DPPH radical scavenging assay. The electron donation abilities of the corresponding compounds and standards drug (qurcetrine) were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). All Analysis was carried out in triplicate according to the standard procedure [4 - 6]. Briefly, a 1 mM solution of DPPH radical solution in methanol was prepared and 1mL of this solution was mixed with 3 mL of sample solutions in methanol (containing 5-100 µg/mL) and control (without sample). The solution was stand for 30 min, in dark the absorbance value was monitored by using spectrophotometer at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated as follows

$$\% \text{ DPPH} = \frac{(\text{OD Control} - \text{OD sample})}{\text{OD control}} \times 100$$

where, OD control is the absorbance of the blank sample, and OD sample is the absorbance of samples or standard sample. IC_{50} was the determined as 50% scavenging of test compounds on DPPH.

Nitric oxide scavenging assay

The activity of compounds was measured according to the already reported method [6]. Briefly, different concentrations of test compounds (10-100 µg/mL) was added to 1 mL of sodium nitroprusside (SNP) solution (5 mM) and incubated for 2 h at 27 °C. An aliquot (2 mL) of the incubation solution was taken and diluted with 1.2 mL of Griess reagent (1% sulfanilamide in 5% H3PO4 and 0.1% naphthylethylene diamine dihydrochloride). The absorbance of the chromophore was observed at 550 nm compared with standard BHT.

Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide.

$$\text{Activity (\%)} = \frac{(\text{OD Control} - \text{OD sample})}{\text{OD control}} \times 100$$

where, OD control is the absorbance of the blank sample, and OD sample is the absorbance of samples or standard sample.

Reducing power assay

For reducing power assay, compounds (10-100 µg/mL), phosphate buffer (2 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2 mL, were mixed, and then incubated at 50°C for 20 min. Trichloroacetic acid (2 mL) was added to the mixture. A volume of 2 mL from each of the aforementioned mixtures was mixed with 2 mL of distilled water and 0.4 mL of 0.1% (w/v) ferric chloride in a test tube. After 10 min reaction, the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated a high reducing power. Butylated hydroxytoluene (BHT) was used as reference standard. IC_{50} was the determined as 50% scavenging of test compounds [7].

Results and Discussion

The effects of isolated compounds (**1-5**) against DPPH are shown in Fig. 1. Compounds exhibited concentration dependent scavenging effect on DPPH. Of the compounds, **2** was most potent scavenger with IC_{50} values of 27.23 µg/mL followed by compound **3** with 47.89 µg/mL (Table-1). It is a popular spectrophotometric method for evaluation of antioxidant potential of test compounds in which the stable free radical, DPPH changes color from violet to yellow upon reduction by either the process of hydrogen- or electron- donation [8,9]. All the tested compounds except **5** showed potential scavenging effect.

Table-1: IC_{50} values of isolated compounds (**1-5**) in various antioxidant assays.

Conc (µg/mL)	IC_{50} µg/mL		
	DPPH assay	NO assay	Reducing power assay
1	**98.35±0.34**	**106.58±0.67**	**89.22±0.88**
2	**27.23±0.45**	**32.03±0.78**	**22.45±1.10**
3	**47.89±0.23**	**55.14±0.96**	**41.22±1.04**
4	**92.82±0.31**	**99.07±1.21**	**88.45±1.05**
5	–	–	–

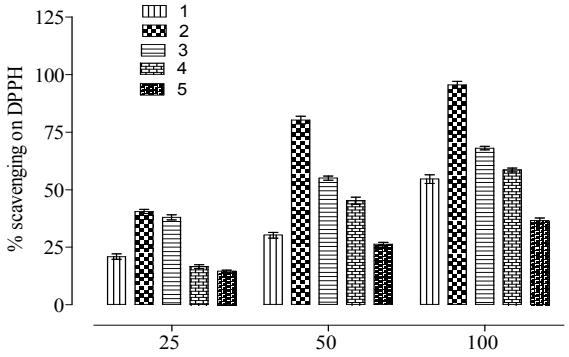


Fig 1: Percent Effect of isolated compounds (**1-5**) in DPPH scavenging assay at 25, 50 and 100 µg/mL. Values are mean ± SEM (n = 3).

The results of isolated compounds (Fig. 4) in nitric oxide scavenging assay are illustrated in Fig. 2. Scavenging on NO was increased with increased in concentration. Compound **2** was observed as the most potent scavenger of NO with IC_{50} values of 32.03 µg/mL followed by compound 3 with 55.14 µg/mL as shown in Table-1. In the anaerobic conditions, the NO molecule is very unstable and reacts with the oxygen that lead to the production of intermediates such as NO_2, N_2O_4, N_3O_4 the stable products nitrate and nitrite. These products progenitors are highly genotoxic, the deamination of guanine, cytosine and adenine is mediated primarily by the N_2O_3 [10]. The tested compounds demonstrated marked effect on NO free radical except compound **5**.

The isolated compounds (**1-5**) showed most promising activity in reducing power assay in a concentration dependent manner as shown in Fig. 3. The potency was expressed in terms of IC_{50} in which **2** was most potent (22.45 µg/mL) followed by **3** (41.22 µg/mL). Reducing power assay is a significant tool for the assessment of antioxidant potential of test articles. Compounds with reducing power reflecting their tendency of electron donation and thus can reduce the oxidized intermediates of lipid peroxidation process [11].

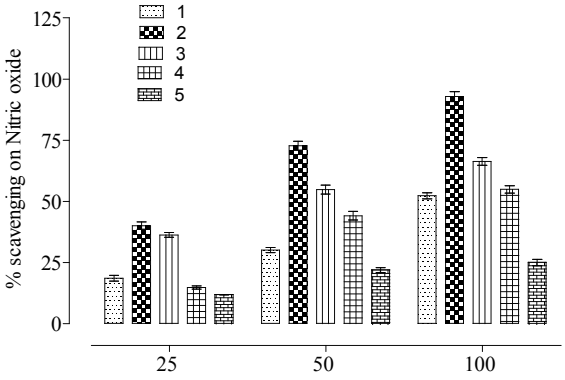


Fig. 2: Percent Effect of isolated compounds (**1-5**) in NO scavenging assay at 25, 50 and 100 µg/mL. Values are mean ± SEM (n = 3).

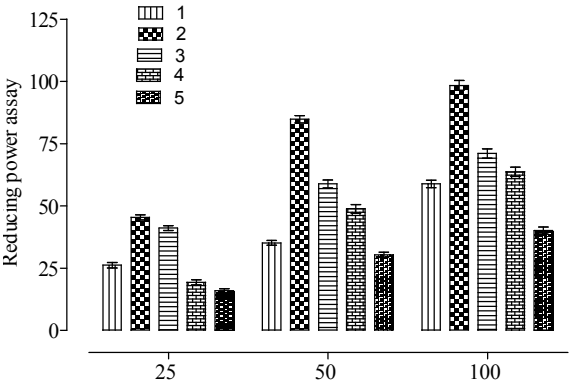
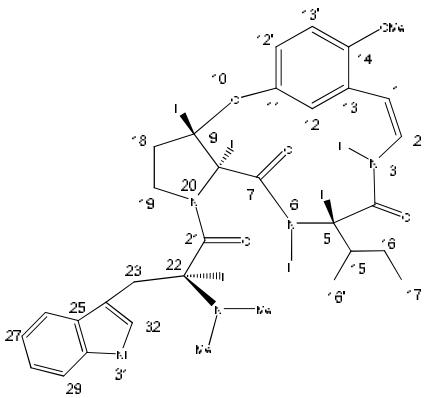
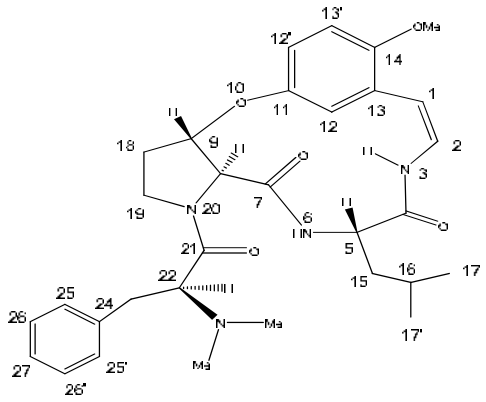


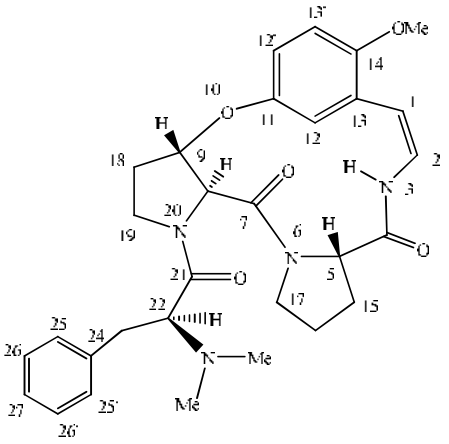
Fig 3: Percent Effect of isolated compounds (**1-5**) in reducing power assay at 25, 50 and 100 µg/mL. Values are mean ± SEM (n = 3).



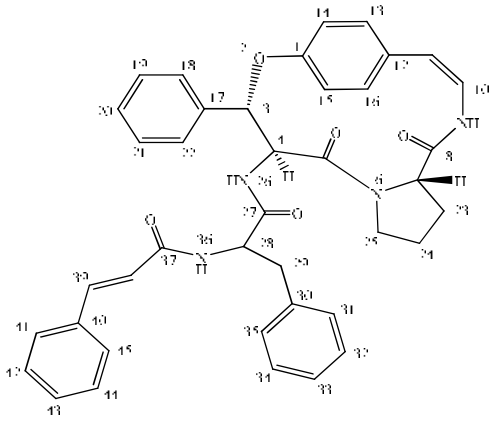
Nummularin-R



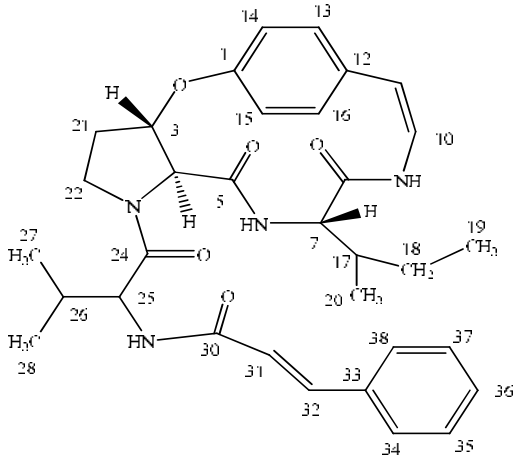
Nummularin-C



Oxyphylline D



Oxyphylline C



Oxyphylline B

Fig. 4. Structures of isolated compounds.

Conclusion

Our test compounds showed profound reducing power except compound **5** like in other assays.

In short, it is concluded that the cyclopeptide alkaloids (**1-5**) isolated from *Zizyphus oxyphylla* possessed strong antioxidant potential in three different *in-vitro* paradigms. Further detail studies on clinical level could lead to the discovery of new antioxidant(s) for the effective management of various pathological conditions.

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