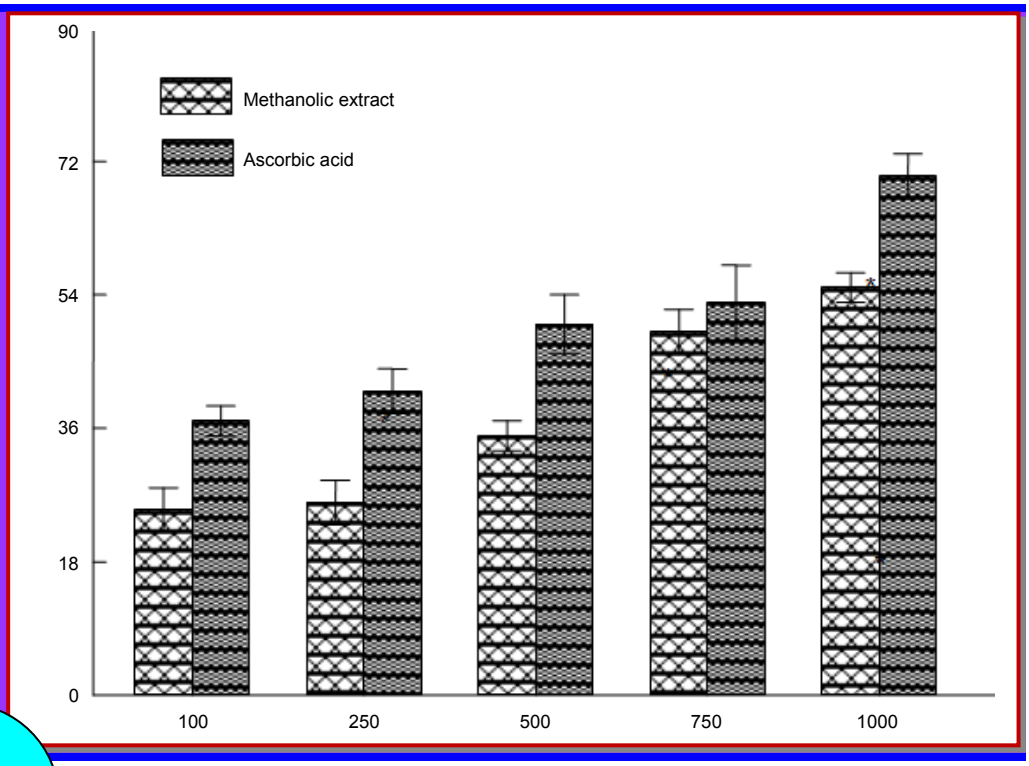

BJP

Bangladesh Journal of Pharmacology

Research Article

In vitro anti-oxidant, antifungal and cytotoxic activity of methanolic extract of *Calligonum polygonoides*

A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
Bangladesh J Pharmacol 2015; 10: 316-320
ISSN: 1991-0088

In vitro anti-oxidant, antifungal and cytotoxic activity of methanolic extract of *Calligonum polygonoides*

Arif Khan, Rahmat Ali Khan, Mushtaq Ahmed and Nadia Mushtaq

Department of Biotechnology, University of Science and Technology, Bannu 28 100, Khyber Paktun Khawa, Pakistan.

Article Info

Received: 5 March 2015
Accepted: 30 March 2015
Available Online: 10 April 2015

DOI: 10.3329/bjp.v10i2.22448

Cite this article:
Khan A, Khan RA, Ahmed M, Mushtaq N. *In vitro* anti-oxidant, antifungal and cytotoxic activity of methanolic extract of *Calligonum polygonoides*. Bangladesh J Pharmacol. 2015; 10: 316-20.

Abstract

Present study is aimed at the pharmacological characterization of methanol extract of *Calligonum polygonoides* from District Bannu. Dried plant was grounded and extracted with methanol to prepare methanol crud extract. *In vitro* biological assays were conducted using this methanolic extract according to standard protocol. Cytotoxic activity of plant methanolic extract against brine shrimps while antifungal activity was also measured. Eighty percent death rate of brine shrimp was observed at 1,000 µg/mL of plant extract. 70 ± 0.0% growth inhibition of *Aspergillus niger* was measured during the present study. Significant scavenging results were observed during scavenging of free radicles viz; 78.1% against DPPH, 83.1% to ABTS and 36% against superoxide at 500 µg/mL were obtained. The results obtained in this study indicate that *C. polygonoides* possess significant anti-oxidant, antifungal and cytotoxic bioactive compounds.

Introduction

Natural products have been in practice for the treatment of free radicals (Kokate et al., 2004). The oxidants or free radicals are those substances which having short life span and highly reactivity towards the other substances while those compounds and reaction which suppress their formation, scavenge them or oppose their action are called anti-oxidants. When the reactive oxygen species are increased or in the body the anti-oxidants level decreased thus the balance changes towards the pro-oxidants and this conditions is known as oxidation stress and cellular damage occur in prolong oxidative stress. To solve this problem we can add the anti-oxidants in a proper amount in our nutrition (Gupta et al., 2004; Ku and Mun, 2007). According to Rababah et al. (2004) different parts of the plants such as leaves, fruits, oil seed roots and vegetables have the capacity as natural anti-oxidants. According to Bajpai et al. (2005) and Sun et al. (2002) some nutrients and non-nutrient molecules of the medicinal and automatic plants show antimicrobial properties these can protect us from different specific pathogens. Medicinal plants are also used in the treatment of cancer and as well as play an important role as a source of effective anti-cancer agents (Crabbe, 1979; Mitscher et al., 1987; Cook et al., 1996; Marino et al., 2001). The cytotoxicity screening gives an important data to select the plant extracts having probable anti-neoplastic properties for the future work (Dikic, 2005; Duraipandiyan et al., 2009). *Calligonum polygonoides* are used as a fodder for camels (Goyel and Sharma, 2006). Recent literature findings shows that flavonoids, alkaloids, tannins, steroids, phenols, carbohydrates and terpenoids are present in different parts of *C. polygonoides* (Samejo et al., 2011). According to literature survey, calligonolides, tetracosan-4-olide, steroidal ester and ursolic acid isolated from *C. polygonoides* (Yawer et al., 2007).

Materials and Methods

Plant collection

The *C. polygonoides* medicinal plant was collected from area of Domel District Bannu and was identified by Prof. Sultan Mehmood Wazir, Dean, Faculty of Biological Sciences UST Bannu. Collected plant sample was dried under shadow at a room temperature and ground mechanically up to mash size 0.1 mm.

Plant extraction

Fine powder (600 g) of *C. polygonoides* was socked in 3 L of the 80% methanol with gentle shaking and then placed it at room temperature for 7 days, thus after the seven days the plant is extracted and filtered by using what man filter paper and concentrated with the help of the rotary evaporator, after the concentration the extra methanol was evaporated at 37°C to obtain crude extract having the weight of 8 grams.

Cytotoxic brine shrimp lethality test

Cytotoxic brine shrimp lethality test was carried out according to standard protocol. Sub solutions of plant extract were prepared of 50, 100, 250, 500 and 1,000 µg/mL from stock solution by using the formula $M_1V_1 = M_2V_2$. Media for Shrimp-hatching was prepared by dissolving 5 g of sea salt is in 250 mL distilled water and put the magnetic stirrer for nearly about 2 hours. Brine shrimps were hatched in two compartment rectangular tray containing sea salt saline. Eggs were sprinkled in dark compartment of tray and after 24 hours of shrimps hatching larvae was collected by pipette from the lightened side. Solution (0.5 mL) was taken in vial and evaporated the solvent. Residue was resolved in saline of 2 mL. Shrimps (n=10) were transferred to each vial and raised the volume up to 5 mL and incubate at 25–28°C. After 24 hours of incubation survivors were counted with help of 3x magnifying glass and calculation was done using Abbot's formula;

% Death = (Sample-control/control) × 100

Antifungal bioassay

The antifungal activity of the plant extract was screened through the agar tube dilution method by using the protocol by Duraipandiyan and Lgnacimuthu (2009).

DPPH radical scavenging activity

Procedure was used for determination of DPPH scavenging capacity of various fractions. DPPH **(**2.4 mg) was dissolved in 100 mL methanol to prepare stock solution (Duraipandiyan and Lgnacimuthu, 2009). The stock solution was further diluted with methanol until attaining an absorbance less than 1.00 using the spectrophotometer at 517 nm. Solution (3 mL) was mixed with 100 µL sample solution (1-100 µg/mL) and measured absorbance at 517 nm. % Inhibition was calculated as;

Scavenging effect (%) = [(OD of control-OD of sample) / (OD of control)] × 100

While IC_{50} value was obtained by using graph prism pad software.

ABTS radical scavenging assay

Equal volumes of 7 mM ABTS solution and 2.45 mM potassium per sulfate solution were mixed to prepare stock solution and incubated in the dark for 12 hours at room temperature to yield a dark colored solution consisting of $ABTS^{\bullet+}$ radicals. 50% methanol and stock solution were mixed to prepare working solution for an initial absorbance of about 0.700 (± 0.02) at 745 nm, with control temperature set at 30°C. Free radical scavenging activity was determined by mixing 300 µL of different concentrations (50 to 500 µg/mL in methanol) with 3.0 mL of ABTS working standard. When the solutions were mixed then after 1 min and 6 min of the decrease in absorbance was measured. Experiment was done on six concentrations. Ascorbic acid was used as positive controls in this experiment. The scavenging activity was determined based on the percentage of ABTS radicals scavenged by the formula given below:

Percent scavenging = $[(A_0 - As) / A_0] \times 100$

Where A_0 = absorption of control; A_S = absorption of sample solution

Determination of superoxide radical scavenging assay

The reaction mixture was prepared by mixing 1 mL of nitro blue tetrazolium (NBT) solution (l M NBT in 100 mM phosphate buffer, pH 7.4), 0.1 mL of different fractions and ascorbic acid (50 mM phosphate buffer, pH 7.4) and 1 mL NADH solution (l M NADH in 100 mM phosphate buffer, pH 7.4). 100 µL of (PMS) solution (60 µM PMS in 100 mM phosphate buffer, pH 7.4) was added in the mixture and there action was started. All The tubes were illuminated evenly with an incandescent visible light for 15 min and before and after the illumination the optical density was measured at 530 nm. The percentage inhibition of superoxide production was assessed by comparing the absorbance values of the control and experimental tubes. Superoxide radical scavenging ability was determined by following formula:

% scavenging = $(1 - A_e / A_o) \times 100$

Where A_e = Absorbance with sample; A_o = The absorbance without sample

Results

Primary screening of the plant through cytotoxicity provides helpful information about the anti-tumor and anti-cancer activity of the plant's extract for the future use. Cytotoxic effect of the *C. polygonoides* methonolic crude extract (CPME) was measured and noted against brine shrimps growth. After complete hatching the shrimps were transferred into the glass test tubes containing saline solution of sea salt and extract of

Table I

Survival and death of brine shrimps in the presence of various concentration of plant extract

Concentration of plant extract (µg/mL)	Total number of brine shrimps	Survived brine shrimps	% Survival	% Death
50	10.0 ± 0.0	9.0 ± 1.0	90.0 ± 1.0	10.0 ± 0.0
100	10.0 ± 0.0	7.4 ± 1.2	70.0 ± 1.5	30.0 ± 0.5
250	10.0 ± 0.0	5.1 ± 1.0	50.4 ± 2.0	50.0 ± 0.9
500	10.0 ± 0.0	3.4 ± 1.6	30.2 ± 2.5	70.0 ± 0.5
1,000	10.0 ± 0.0	2.3 ± 1.8	20.1 ± 1.8	80.0 ± 0.8

Data are mean ± SD

Table II

Antifungal activity of *Calligonum* polygonoides methanolic extract (% inhibition)

Strain	Terbinafine	Calligonum polygnoids	DMSO
Aspergillus niger	99.4 ± 5.5	70.0 ± 0.1	2.0 ± 0.6
Aspergillus flavius	98.1 ± 3.7	50.7 ± 0.1	10.0 ± 0. 5
Aspergillus fumegatus	99.0 ± 2.0	50.0 ± 0.1	10.5 ± 0.8

Data are mean ± SD

Table III

Comparison between *Calligonum* polygonoides methanolic extract and ascorbic acid scavenging activity for DPPH free radicals

Concentration (µg/mL)	%CPME scavenging	% Ascorbic acid scavenging
50	24.4 ± 0.1	58.0 ± 0.0
100	48.2 ± 0.2	78.1 ± 0.1
150	57.2 ± 0.1	81.0 ± 0.1
200	67.2 ± 0.1	82.0 ± 0.0
250	71.3 ± 0.1	85.1 ± 0.1
500	78.1 ± 0.1	89.6 ± 0.1

Data are mean ± SD

different concentrations of the plant *C. polygonoides*. After 24 hours the effects of different concentrations of the plant's extract was noted and found that the brine shrimps survival was inversely proportional to the concentration of the plant extract while death of the brine shrimps was noted directly proportional to the concentration of the plant extract (Table I). From the Table I, it was very much clear that at 50 µg/mL, 90% survival and 10% death were noted, similarly at 100, 250, 500 and 1,000 µg/mL, 70, 50, 30 and 20% survival and 30, 50, 70 and 80% death occurred respectively.

For the screening of antifungal activities of the plant *C. polygonoides*; 67 µL (200 µg/mL) of the CPME, 67 µL (200 µg/mL) of the terbinofine and 67 µL of the DMSO (99.9%) were used (Table II). The *C. polygonoides* methanolic extract (CPME) shows its antifungal activities up to some extant against *Aspergillus niger* (0198), *Aspergillus flavius* (0064), *Aspergillus fumigatus* (66) strain. The *C. polygonoides* methanolic extract (CPME) showed activity against *Aspergillus fumugatus* i.e. % followed by *flavis* (50.7%) while the highest activity was shown against *Aspergillus niger* (70%). Similarly the terbinofine, a positive control was indicated highly active against this fungal strains, While the DMSO (negative control) indicate zero percent (0%) inhibition activity against all the used three fungal strains.

To compare the anti-oxidant activity we used DPPH radical scavenging assay. The 1,1-diphenyl-2-picryl–hydrazyl (DPPH) free radical scavenging capacity of the sample extract *C. polygonoides* along with the standard ascorbic acid was recorded. It is found that the scavenging ability of the sample extract is some less than the standard ascorbic acid as given in Table III.

To compare the anti-oxidant activity we used ABTS radical scavenging assay which are applicable for both lipophilic and hydrophilic anti-oxidants. The ABTS (2,2, -azo-bis-(3-ethyl benzothiazoline-6-sulphonic acid) free

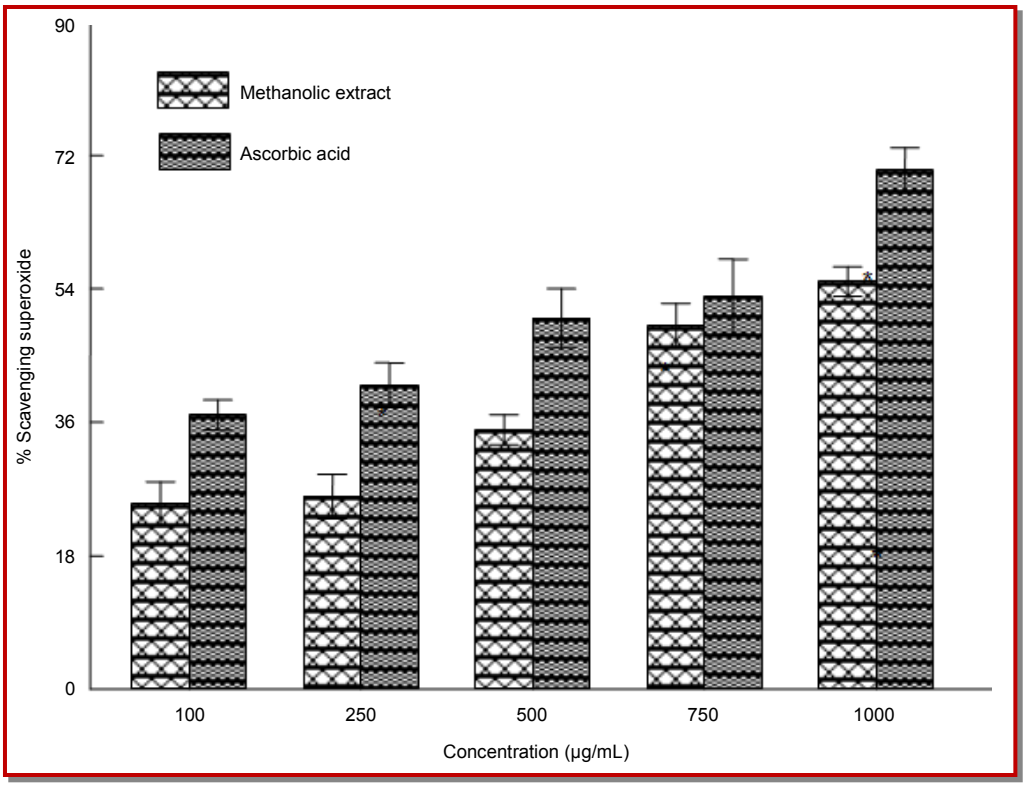

Figure 1: O_2 free radical scavenging of *Calligonum polygonoides* extract and ascorbic acid

Table IV

Comparison between *Calligonum* polygonoides methanolic extract and ascorbic acid scavenging activity for ABTS free radicals

Concentration (in µg/mL)	% COME scavenging	% Ascorbic acid scavenging
50	62.7 ± 0.1	78.3 ± 0.0
100	68.3 ± 0.1	82.3 ± 0.1
150	74.3 ± 0.2	87.6 ± 0.2
200	79.3 ± 0.1	89.6 ± 0.1
250	81.9 ± 0.0	81.6 ± 0.0
500	83.1 ± 0.1	94.5 ± 0.1

Data are mean ± SD

radical scavenging capacity of the sample extract along with the standard ascorbic acid was recorded. It was found that the scavenging ability of the sample extract was some less than the ascorbic acid (Table IV).

Superoxide radical are the reactive oxygen species (ROS), cause very harmful and toxic effects to cellular components, as well as contributing in many fetal diseases. The Figure 1 shows scavenging of the various fractions of *C. polygonoides* methanolic extract possessed the most potent superoxide radical scavenging activity (56.4 ± 3.2 µg/mL) showed the scavenging effect near to standard compounds.

Discussion

Our results shows some similarities with the investigation of Hogerman et al. (1998) reported that the medicinal plants have highly scavenge the free radicals. The anti-oxidants potential of methanolic extract of this plant could be due to the presence of phenolic and polyphenolic compounds in this medicinal plant which reduce the free radicals which cause the oxidative stress. The results obtained by Kilaniet al. (2008) also support the results obtained from our experiments.

In our present study, the antifungal activity of *C. polygonoides* result shows that the antifungal strain are inhibited by these samples. Due to the presence of phenolic compounds in medicinal plants, showed the antimicrobial activity or antifungal activity Baydar et al. (2004) and the antifungal activity of the medicinal plants are also due to the presence of bioactive compounds saponines (Mothan et al., 2007).

The cytotoxic activity of the plant extract provides information about the anti-cancer and anti-tumor potential of *C. polygonoides*. Cytotoxic effect of the methanolic extract of *C. polygonoides* was determined by using brine shrimps lethality test. The order of the cytotoxicity was 1,000>500>250>100>50 µg/mL. The result showed that the brine shrimps survival is inversely proportional to the methnolic extract of the *C. polygonoides* plant. It was reported that methanolic fraction of *Arceuthobium oxycedri* showed 100% cytotoxicity at high dose for brine shrimps which are related to the present result. The result of our present study supports the traditional usage of the studied plant and suggests that methanolic extract possess some bioactive constituents with antimicrobial and as well as anti-cancer disease caused by the pathogens.

References

Ali N, Shah I, Shah SWA, Ahmed G, Shoaib M, Junaid M, Ali W, Ahmed Z. Anti-oxidant and relaxant activity of fractions of crude methanol extract and essential oil of *Artemisia macrocephala* jacquem. BMC Complem Altern Med. 2013; 13: 1-8.

Bajpai M, Pande A, Tewari SK, Prakash D. Phenolic contents and anti-oxidant activity of some food and medicinal plants. Int J Food Sci Nutr. 2005; 56: 287-91.

Baydar NG, Ozkan G, Sagdic O. Total phenolic contents and antibacterial activities of grapes (*Vitis vinifera* L.) extracts. Food Control. 2004; 5: 333-35.

Cook NC, Saman S. Flavonoids: Chemistry, metabolism, cardioprotective effects and dietary sources. Nutr Biochem. 1996; 7: 66-76.

Crabbe P. Some aspects of steroid research based on natural product from plant orgin. Bull Soc Chim Belg. 1979; 88: 5-7.

Dikic M. Allelopathic effect of aromatic medicinal plants on the seed germination of *Galinsoga parviflora*, *Echinochloa crus-galli* and *Galium molugo*. Herbologia 2005; 6: 51-57.

Duraipandiyan V, Ignacimuthu S. Antibacterial and antifungal activity of flindersine isolated from the traditional medicinal plant, *Toddalia asiatica* (L.) Lam. J Ethanopharmacol. 2009; 123: 494-98.

Gupta S, Zhang D, Yi J, Shao J. Anti-cancer activities of *Oldenlandia diffusa*. J Herbal Pharmacother. 2004; 4: 21-33.

Goyal M, Sharma SK. Prospects and dimension for utilization of arid foods. Bikaner, Yash Publishing House, 2006, pp 101-03.

Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford University Press, 1999, pp 617-783.

Hogerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW. High molecular weight plant polyphenolice (tannins) as biological anti-oxidants. J Agric Food Chem. 1998; 46: 1887-92.

Kilani S, Sghaier MB, Limem I, Bouhlel I, Boubaker J, Bhouri W, Skandrani I, Neffatti A, Ammarb RB, Dijoux-franca MG, Ghedira K, Chekir-Ghedira L. *In vitro* evaluation of antibacterial, anti-oxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of *Cyperus rotundus*. Bioresour Technol. 2008; 99**:** 9004-08.

Kokate CK, Purohit AP. Text book of pharmacognosy. 2004; 29: 317-18.

Ku CB, Mun SP. Anti-oxidant activities of ethanol extracts from seeds in fresh Bokbunja (*Rubus coreanus* Miq) and wine processing waste. Bioresour Technol. 2007; 99: 4503-09.

Marino M, Bersani C, Comi G. Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. Int J Food Microbiol. 2001; 67: 187-95.

Mitscher LA, Drake S, Gollapudi SR, Okwute SK. A modern look at folkloric use of anti-infective agents. J Nat Prod. 1987; 50: 1025-40.

Mothana RAA, Gruenert R, Lindequist U, Bednarski PJ. Study of the anti-cancer potential of Yemeni plants used in folk medicine. Pharmazie 2007; 62: 305-07.

Rababah TM, Hettlarachchy NS, Horex R. Total phenolics and anti-oxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola and ginkgo extracts, vitamin E and tert. butylhydroquinone. J Agric Food Chem. 2004; 52: 5183–86.

Sun J, Chu Y, Wu X, Liu R. Anti-oxidant and anti-proliferative activities of common fruits. J Agric Food Chem. 2002; 50: 7449-54.

Samejo MQ, Memon S, Bhanger MI, Khan KM. Preliminary phytochemicals screening of *Calligonum polygonoides* Linn. J Pharm Res. 2011; 4: 4402-03.

Yawer MA, Ahmed E, Malik A, Ashraf M, Rasool MA, Afza N. New lipoxygenase-inhibiting constituents from *Calligonum polygonoides*. Chem Biodivers. 2007; 4: 1578–85.

Author Info
Rahmat Ali Khan (Principal contact)
e-mail: Rahmatgul_81@yahoo.com