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Pharmacological evaluation of methanolic extract of *Cyperus scariosus*

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Abstract

The present study is aimed to investigate the phytochemical screening and biological activities of methanolic extract of *Cyperus scariosus* roots. Dried plant was grounded and extracted with methanol to prepare methanol crud extract. *In vitro* biological tests were conducted using this methanolic extracts according to the standard procedure. 100% death rate of brine shrimp was perceived at 3 mg/mL of plant extract after 72 hours. The extract showed action against *Aspergillus flavius* i.e. 90% followed by *A. niger* (91%) while the highest activity was shown against *A. fumegatrus* (94%). Important scavenging results were detected during scavenging of free radicals viz; 92.2% against DPPH, 82.2% to ABTS, 75.8% to hydrogen peroxide, 88.1% to β-carotene, 86.1% to hydroxyl radical and 89.4% against phosphomolybdate at 3 mg/mL were obtained. The results obtained in this study point out that extract showed significant biological activities which might be due to the presence of bioactive constituents.

Introduction

In the previous decade, there has been a rising interest in the medical consequences of free radicals. Free radicals are harmful and cause oxidative stress which may cause very serious human diseases (Vani et al., 1997; Jadhav and Bhutani, 2002). Many natural products have been using in practice for the cure of free radicals (Kokate, 2004). Those compounds which destroy the formation of ROS, scavenge them or face their action are called anti-oxidants (Khan et al., 2015). Already some natural anti-oxidant are sold on business trade either as anti-oxidant flavors or nourishing food supplements (Schuler, 1990). Bajpai et al. (2005) and Sun et al. (2002) reported that some nutrients and non-nutrient molecules of the medicinal and aromatic plants have anti-microbial properties. Medicinal plants are also used in the treatment of cancer and play a significant part as a source of active anticancer agents (Crabbe, 1979; Mitscher et al., 1987; Khan et al., 2010). Nearly more than 60% anticancer agents are used in different ways from the natural source (Chopra et al., 1992).

The plant *Cyperus scariosus* is an angiosperm belonging to family *Cyperaceae*, contains almost 3,000 species out of which about 220 species are recognized as weeds. The rhizomes of *C. scariosus* used as an anti-microbial, anti-inflammatory, antifungal agent and it is also used as in many formulations for the Ayurvedic systems of medicine (Srivastava et al., 2014). The present study is aimed to evaluate anti-oxidants, antifungal, cytotoxicity activity and phytochemical analyses.

Materials and Methods

Plant collection

C. scariosus was collected from the District Bannu and was recognized by Prof. Abdul Rahman, GPGC Bannu. Collected plant sample was dried under shadow at a room temperature and ground mechanically up to

mash size 0.1 mm.

Plant extraction

100 g fine powder of *C. scariosus* was socked in 500 mL methanol with temperate shaking and then placed it at room temperature for 5 days. After five days the plant was extracted and filtered by using Whatman filter paper. It was concentrated with the help of the rotary evaporator. Then the concentration the extra methanol was evaporated at 37°C to obtain crude extract.

Qualitative phytochemical screening

Phytochemical screening was performed to recognize the existence of bioactive compounds in plant part by using standard phytochemical methods as described by Khan et al. (2010).

Cytotoxic brine shrimp lethality test

Cytotoxic brine shrimp lethality test was carried out according to Meyer-Alber et al. (1992) with some modification as described by Khan et al. (2015).

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

Method was used for determination of DPPH scavenging ability of various fractions. 3 mg DPPH was dissolved in 30 mL methanol to prepare stock solution (Duraipandiyan and Lgnacimuthu, 2009). The stock solution was further diluted with methanol until reaching an absorbance less than 1.00 using the spectrophotometer at 517 nm. Scavenging calculated through the following formula.

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

ABTS radical scavenging assay

With certain modifications the ABTS radical scavenging assay was measured using the method developed by Re et al. (1999). 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 100 µL of different concentrations (3 mg/mL, 1.5 mg/mL, 0.75 mg/mL and 0.37 mg/mL in methanol) with 1 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 four 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

Hydrogen peroxide scavenging assay

With certain modifications the hydrogen peroxide scavenging activity was assessed using the method developed by Ruch et al. (1989).

Phosphomolybdate assay

The anti-oxidant activity of samples was calculated by the phosphomolybdenum method according to the procedure of Umamaheswari and Chatterjee, (2008). An aliquot of 0.1 mL of sample solution was mixed with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes were capped with silver foil and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 765 nm against a blank. Ascorbic acid was used as standard. The anti-oxidant capacity was estimated using following formula:

Anti-oxidant effect (%) = [(control absorbance-sample absorbance) / (control absorbance)] × 100

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of extracts was examined by the method of Halliwell and Gutteridge, (1981). The reaction mixture contained 50 mL of 2-deoxyribose (2.8 mM) in phosphate buffer (50 mM, pH 7.4), 10 mL of premixed ferric chloride (100 mM) and EDTA (100 mM) solution (1:1; v/v), 10 mL of H_2O_2 (200 mM) without or with the extract solution (10 mL). The reaction was triggered by adding 10 mL of 300 mM ascorbate and incubated for 1 hour at 37°C. A solution of TBA in 1 mL (1%; w/v) of 50 mM NaOH and 1 mL of 2.8% (w/v; aqueous solution) TCA was added. The mixture was heated for 15 min on a boiling water bath and then cooled. The absorbance was measured at 532 nm. The scavenging activity on hydroxyl radical was calculated as follows:

Scavenging activity (%) = (1- absorbance of sample / absorbance of control) × 100

β-Carotene bleaching assay

The assay was performed as given by Elzaawely et al, (2007) and modified slightly. First, 1 mg of β-carotene dissolved in 5 mL of chloroform was mixed with 10 mg of linoleic acid and 50 mL of Tween 80 followed by chloroform removing under nitrogen and 25 mL of distilled water adding with vigorous shacking to pre-

Table I

Antifungal activity of *Cyperus scariosus* methanolic extract

		% Inhibition	
Fungal strain	Concentrations (mg/mL)	*Cyperus scariosus*	Terbinofine
Aspergillus flavius	3	90.0 ± 2.7	100.0 ± 0.7
	1.5	84.1 ± 2.5	98.0 ± 0.5
	0.75	81.3 ± 2.4	91.1 ± 0.3
	0.37	76.4 ± 2.1	85.3 ± 0.2
Aspergillus niger	3	91.0 ± 3.1	100 ± 0.7
	1.5	85.1 ± 2.9	96.1 ± 0.4
	0.75	81.2 ± 2.5	87.3 ± 0.2
	0.37	78.7 ± 2.2	88.2 ± 0.3
Aspergillus fumegatus	3	94.0 ± 3.4	100.0 ± 0.7
	1.5	79.3 ± 2.3	97.1 ± 0.6
	0.75	64.5 ± 2.2	89.2 ± 0.4
	0.37	62.1 ± 2.0	85.3 ± 0.2

Data are Mean ± SD

pare β-carotene linoleate emulsion. An aliquot of sample (200 µL) was mixed with 800 µL of the suspension, and then the absorbance was resolute at 470 nm at 45°C for 2 hours. β-Carotene bleaching inhibition was estimated as the following equation:

Bleaching inhibition (%) = (β-carotene content after 2 hours of assay/initial β-carotene content) × 100

Results

Phytochemical composition

Phlobatannins, tannins and terpenoids were present in the extract while flavonoids, anthraquinone and saponins were absent.

Antifungal activity

C. *scariosus* methanolic extract shows antifungal activities up to some extant against *Aspergillus niger*, *A. flavius*, and *A. fumigatus* strain. The extract showed activity against *A. flavius* i.e. 90% followed by *A. niger* (91%) while the highest activity was shown against *A. fumegatrus* (94%). Likewise the terbinofine, a positive control was shown highly active against this fungal strains, while the DMSO (negative control) shows zero percent (0%) inhibition activity against all the used three fungal strains (Table I).

Cytotoxic activity (%death)

After 24, 48 and 72 hours incubation the cytotoxic effects of extract of different concentrations were noted and found that the brine shrimp survival rate was inversely proportional to the concentrations of the plant extracts and the period of incubations. The results of the present study show that *C. scariosus* had significant cytotoxic activity (Figure 1).

ABTS scavenging activity

The ABTS (2,2, azo-bis-(3-ethyl benzothiazoline-6-sulphonic acid) free radical scavenging capacity of the sample extract was less than the standard ascorbic acid (Table II).

DPPH free radical scavenging activity

The 1,1-diphenyl-2-picryl–hydrazyl (DPPH) free radical scavenging capability of the sample extract was less than the standard ascorbic acid (Table II).

Hydrogen peroxide-scavenging activity

The methanolic extract showed distinct scavenging activity, markedly scavenged the free radial and were the most potent than reference chemical compound (Table II).

Phosphomolybedate scavenging activity

The phosphomolybedate free radical scavenging ability

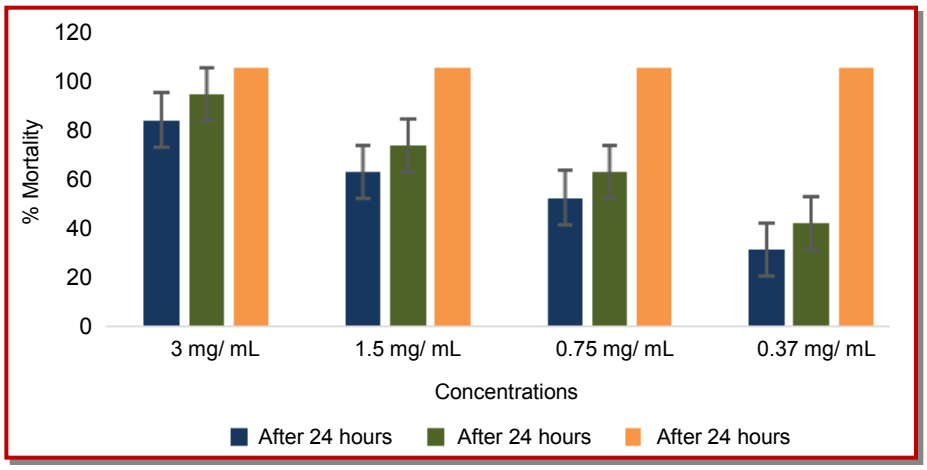

Figure 1: Cytotoxic effects of *Cyperus scariosus* methanolic extract (% death)

of the sample extract along with the standard ascorbic acid recorded on different concentrations. It was found that the scavenging capacity of the sample extract was to some extent less than the standard (Table II).

β-Carotene bleaching assay

With concern to the β- carotene bleaching assay, the anti-oxidant activity of sample can be classified as 3 mg/mL > 1.5 mg/mL > 0.75 mg/mL > 0.37 mg/mL. At 3 mg/mL, β- carotene bleaching inhibitions were 88.32, 1.5 mg/mL 82.67, 0.75 mg/mL 78.45 and 0.37 mg/mL 72.69. β-carotene bleaching assay showed the dose response graph for all the concentrations ranging from 3 mg/mL-0.37 mg/mL. It was found that the scavenging capacity of the sample extract was less than the standard (Table II).

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity can be categorized as 3 mg/mL > 1.5 mg/mL > 0.75 mg/mL > 0.37 mg/mL. All results showed anti-oxidant activity in dose-dependent manner at concentration 3-0.37 mg/mL. However, the scavenging activity at all the concentrations less than the standard (Table II).

Discussion

The *C. scariosus* revealed the presence of phlobatannins, tanninsand terpenoids, while anthraquinone, flavonoids, saponins were absent.

Reactive oxygen species are recognized to play a definite role in a wide variety of pathological expressions. Anti-oxidants combat free radicals and protect us from various diseases. They utilize their action either by scavenging the reactive oxygen species or defending the anti-oxidant defense mechanisms (Umamaheswari and Chatterjee, 2008). The obtained results shows that *C. scariosus* have the ability to scavenge all this free radicals that is due to the existence of some bioactive compounds. The results which are drawn from the extract shows some resemblances with the study of Hogerman et al. (1998) described that it is the quality of medicinal plants that have highly scavenge the free radicals. The anti-oxidants potential of methanolic extract of this plant might be due to the occurrence of phenolic and polyphenolic compounds in this medicinal plant which decrease the free radicals which cause the oxidative stress. The results that were obtained by Kilani et al. (2008) also support the results obtained from the experiments.

The cytotoxic activity of the plant extract offers information about the anticancer and anti-tumor possible of *C. scariosus*. Cytotoxic effect of the methanolic extract was resolute by using brine shrimps lethality test. The order of the cytotoxicity 3 mg/mL > 1.5 mg/mL > 0.75 mg/mL and 0.37 mg/mL. From the experiments, the results showed that the brine shrimps survival is inversely proportional to the methanolic extract. It was reported that methanolic fraction of *Arceuthobium oxycedri* showed 100 % cytotoxicity at high dose for brine shrimps which are similar to the current result. It is obvious from the extract that the results of the present study helps the traditional usage of the studied plant and recommends that methanolic extract possess some bioactive compounds with antifungal as well as anticancer ailment caused by the pathogens.

Literature shows that synthetic drugs have side effects and there is some strains of microorganism which are antibiotic resistant so due to this reason examination of effective anti-microbial drugs obtained from natural resources has been an objective of researchers and scientists. In our present, study the anti-microbial activities of *C. scariosus* results shows that the fungal strain are inhibited by these samples. The existence of phenolic compounds in medicinal plants also showed antimicrobial activities (Baydar et al., 2004) and the antimicrobial activities of the medicinal plants are also due to the existence of bioactive compounds saponins (Mothana et al., 2007).

Table II

Ani-oxidant activities of *Cyperus scariosus* methanolic extract

Conc, (mg/mL)	DPPH		ABTS		H_2O_2		Phospho molybedate		β - Carotene		Hydroxyl radical	
	Ascorbic Acid	Extract	Ascorbic Acid	Extract	Ascorbic Acid	Extract	Ascorbic Acid	Extract	Ascorbic Acid	Extract	Ascorbic Acid	Extract
3	94.5 ± 5.4	92.2 ± 5.2	84.2 ± 4.5	82.2 ± 4.4	80.5 ± 4.8	75.8 ± 3.9	97.1 ± 5.7	89.4 ± 4.9	93.1 ± 4.9	88.1 ± 4.3	90.1 ± 4.9	86.1 ± 4.3
1.5	91.3 ± 5.2	88.7 ± 4.9	74.7 ± 4.3	72.8 ± 4.1	77.2 ± 3.9	73.3 ± 3.5	96.2 ± 5.6	84.7 ± 4.5	87.9 ± 4.6	83.2 ± 4.5	87.2 ± 4.5	82.2 ± 4.1
0.75	89.1 ± 4.9	86.2 ± 4.4	68.1 ± 3.9	65.7 ± 3.6	74.1 ± 3.6	70.4 ± 3.4	94.2 ± 5.3	80.3 ± 4.3	84.1 ± 4.3	80.5 ± 4.2	84.9 ± 4.1	77.1 ± 3.7
0.37	87.5 ± 4.8	84.4 ± 4.2	61.1 ± 3.6	59.8 ± 3.3	77.6 ± 3.4	67.2 ± 3.2	92.1 ± 5.1	77.6 ± 3.9	76.4 ± 3.9	72.4 ± 3.2	81.1 ± 3.5	73.3 ± 3.1

Data are mean ± SD

Conclusion

C. scariosus showed significant cytotoxic and anti-oxidant activities as well as inhibitory effects on fungus which are might be due to the presence of bioactive constituents.

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