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Research Article

PRELIMINARY PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT, AND ANTIMICROBIAL EVALUATION OF *CARTHAMUS LANATUS*

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ABSTRACT

Objective: This study is aimed at examining alkaloids, tannins, saponins, flavonoids, and steroids contents in methanolic extract of *Carthamus lanatus* and their antimicrobial (antifungal and antibacterial) and antioxidant activities.

Methods: The conventional phytochemical screening tests were used for the identification of secondary metabolites. Antioxidant, antifungal, and antibacterial activities were carried out using 2, 2-diphenylpicrylhydrazyl (DPPH) free radical scavenging method, agar tube dilution, and agar well diffusion methods, respectively.

Results: Phytochemical screening showed the presence of alkaloids, tannins, saponins, flavonoids, coumarins, steroids, and betacyanins. The antimicrobial activities of *C. lanatus* in methanol solvent were tested against Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis,* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli*) organisms as well as the fungus (*Aspergillus niger, Aspergillus flavus,* and *Alternaria solani*). The plant showed 100% activity against *A. flavus*, 90% activity against *A. solani* and *A. niger* each. For the antimicrobial test, well diffusion technique was used, and the zone of inhibition of microorganisms was measured in mm. Antioxidant assay measures by a spectrophotometer the ability of antioxidants to reduce DPPH, another radical not commonly found in biological systems. *C. lanatus* showed 96.2% activity at 10 ppm concentration and as concentration is increased to 100 ppm its activity is enhanced to 98.2%.

Conclusion: *C. lanatus* is a very good antioxidant and antimicrobial plant. The presence of various secondary metabolites makes it a very significant plant for various activities.

Keywords: *Carthamus lanatus*, Phytochemical screening, Antimicrobial activities, Antioxidant assay, 2, 2-diphenylpicrylhydrazyl.

INTRODUCTION

Carthamus has an Arabic origin signifying floral color [1], but some report this name is originated from the safflower plant, which is known as *Carthamus tinctorius* in Arabic [2]. *Carthamus lanatus* comprises of small flowers with a short pedicel. Petals may be present or not, but usually, 2 or 4 stamens are present. Seeds are reddish brown. *C. lanatus* is a shallow-rooted annual that produces upright wiry stems, usually 0.1-1 m tall. The average mature height of the plant varies from 0.6 m in McClain County, about 2 m in Australia [3], 0.5-2 m in Tasmania. C. lanatus is used as sedative and antitumor agent with interferon-inducing activities [4]. It can be used for anti-inflammatory and analgesic purposes [5,6]. Its radical scavenging [7], antimicrobial activity and cytotoxicity[4,8,9] make the plant very beneficial.

METHODS

Plant material

Stem and leaves of *C. lanatus* were collected from Takht Bhai, Mardan, KPK Pakistan.

Plant identification

The plant was identified by Ghulam Jelani Department of Botany, University of Peshawar Pakistan.

Extraction

The plant with stem and leaves was soaked in methanol for 3-4 days. After 3-4 days, the methanolic extract was obtained by rotary evaporator and concentrated [10].

Phytochemical screening

The crude extract was processed to phytochemical tests to recognize bioactive secondary metabolites using standard methods [11-17].

Alkaloids

About 1 g of crude extract was warmed with 2% H_2SO_4 for 2 minutes. The reaction mixtures were cooled and filtered and added a few drops of Dragendorff's reagent to each filtrate. Orange red precipitate is the indication for the presence of alkaloids.

Tannins

About 1.5 g of each extract was mixed with water and heated on water bath and filtered. A few drops of ferric chloride were added to each of the filtrate. A dark green solution indicates the presence of tannins.

Anthraquinones

About 0.75 g of each extract was boiled with 10% HCl for few minutes on water bath. The reaction mixtures were cooled and filtered. An equal volume of $CHCl_3$ was added to each filtrate. Few drops of 10% ammonia were added to each mixture and heated. Rose pink formation signifies the presence of anthraquinones.

Glycosides

Each extract was hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling's Solutions A and B were added to each mixture. Formation of red precipitate signifies the presence of glycosides.

Reducing sugars

Each extract was shaken with distilled water and filtered. The filtrates were boiled with few drops of Fehling's Solutions A and B for few minutes. An orange-red precipitate signifies the presence of reducing sugars.

Saponins

About 2 ml of each extract was shaken with 6 ml of distilled water and heated to boiling. Frothing (appearance of creamy miss of small bubbles) signifies the presence of saponins.

Flavonoids
About 0.5 ml of each extract was dissolved in diluted NaOH, and few drops of HCl were added. A yellow solution that turns colorless implies the presence of flavonoids.

Phlobatannins
About 0.5 ml of each extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate implies the presence of phlobatannins.

Steroids
To 1 ml of each extract, few drops of acetic acid were added. It was gently warmed and then cooled, and then, a drop of H_2SO_4 was added. The color changes to green which shows the presence of steroids.

Terpenoids
About 2 ml of each extract was mixed with 5 ml of chloroform and concentrated H_2SO_4 (6 ml) was carefully added to form a layer. The formation of a reddish brown coloration at the interface signifies positive results for the presence of terpenoids.

Coumarin
Exact 3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow indicates the presence of Coumarin.

Emodins
Exact 2 ml of NH_4OH and 3ml of benzene were added to the extract. Appearance of red indicates the presence of emodins.

Anthocyanin and betacyanin
To 2 ml of plant extract, 1 ml of 2 N NaOH was added and heated for 5 minutes at 100°C. Formation of bluish green indicates the presence of anthocyanin and formation of yellow indicates the presence of betacyanin.

Carbohydrates
Few drops of Molisch's reagent were added to each of the portion dissolved in distilled water; this was then followed by addition of 1 ml of concentration H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for 2 minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet at the interphase of the two layers was a positive test.

Monosaccharides
About 0.5 g each fraction was dissolved in distilled water and filtered. 1 ml of the filtrate was then mixed with 1 ml of Barfoed's reagent in a test tube and then heated on a water bath for 2 minutes. Reddish precipitate of cuprous oxide was considered as a positive test.

Antibacterial activity of extract
Three strains of Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, and Bacillus subtilis*) were used for this activity. These organisms were kept in Mueller-Hinton agar in the refrigerator at 4°C.

The tests for responsiveness were done using modified agar well diffusion method to tryout the antibacterial activity of various fractions. To assess antibacterial activity, standard protocol was used [18]. The Mueller-Hinton agar was the medium. The cultures were taken in triplicates at cultivation temperature of 37°C for 3 days. The broth culture of the test organism was placed in a sterilized Petri-dish to which 20 ml of the sterilized molten Mueller-Hinton Agar was added. Wells were bored into the medium using 0.2 ml of the extracts. The standard antimicrobial agent used was streptomycin (2 mg/ml). Inoculation was done for 1 h to make possible the diffusion of the antimicrobial agent into the medium. The inoculation plates were incubated at 37°C for 24 hrs, and the diameter of the zone of inhibition of bacterial growth was measured in the plate in millimeters.

Antioxidant activity using 2, 2-diphenylpicrylhydrazyl (DPPH) radical scavenging assay (RSA)
DPPH RSA was used to perform the antioxidant activity using the standard protocol as discussed earlier [19,20]. The oxidation abilities of the mentioned fractions and standards were measured from the bleaching of the DPPH purple-colored solution in methanol. Concisely, a 1 mM solution in methanol was prepared by dissolving DPPH in it, and 1 ml of this solution was mixed with 3 ml of each fraction solutions in methanol (containing 10-100 µg) and blank (without sample). Each solution was kept in dark for 30 minutes, and then, absorbance was measured at 517 nm. Decline in the DPPH solution absorbance confirms an increase in the DPPH RSA. As (%RSA) using DPPH, the scavenging of free radicals was calculated as:

%DPPH = (OD blank/control-OD sample) × 100/OD blank

Where, OD blank indicates the absorbance of the blank solution, and OD sample shows the absorbance of samples or standard sample.

Antifungal activity
The most used method for screening antifungal agents is the agar tube dilution method [21]. 22 mg of each extract was taken in clean and sterile vials. These weighed samples were then dissolved in 1 ml sterile DMSO. They were then properly homogenized. The growth media used for fungus in this bioassay was Sabouraud dextrose agar (SDA). 9.75 g of the powdered SDA was taken and was dissolved in 150 ml distilled water. It was homogenized. The media and micropipette tips were sterilized by autoclave. The tubes were allowed to cool at 50°C after autoclaving, and test samples were loaded into the non-solidified SDA in a biological safety cabinet. Each sample was loaded in test tubes for the given fungal cultures and for negative control. Tubes were arranged with their corresponding samples in slanting positions on the table at room temperature for 24 hrs to form slants. Next day, slants were checked for their sterility and each sample tube was inoculated with 4 mm diameter of fungus removed from 7 day old cultures of fungus. The tubes were incubated in fungal incubators at 27°C-29°C for 3-7 days. The following formula is used to calculate inhibition of fungal growth [22].

Percentage inhibition = 100-linear growth in test sample (mm)/linear growth in control (mm) ×100

RESULTS AND DISCUSSION

Acquaintance of the phytochemical constituents of plants is advantageous for the discovery of therapeutic agents as well as to get valuable information in disclosing new sources of such economic materials such as alkaloids, tannins, oils, gums, flavonoids, saponins, and essential oils which are precursors for the synthesis of complex chemical substances. Phytochemical analysis of methanolic crude of *C. lanatus* showed the presence of alkaloids, tannins, saponins, flavonoids, steroids, coumarins, and betacyanins (Table 1).

Antioxidant and antimicrobial properties of various extracts of many plants are of great interest in both fundamental science and alternative medicine since their potential use as natural extracts has emerged from a growing tendency to replace synthetic antioxidants by natural ones. The present study confirmed the antimicrobial and antioxidant activities of methanolic extract of *C. lanatus*. The tests were performed thrice to confirm the activities. The obtained results suggest that the extract shows antimicrobial activity under *in vitro* conditions against the test bacteria and fungi (Tables 2 and 3).

The flavonoids and tannins present in all the plants are likely to be responsible for the free radical scavenging effects. The DPPH test is used to gather information on the reactivity of the test compounds with a stable free radical. Absorption band for DPPH is at 517 nm in the visible region. Absorption at this wavelength reduces when the odd electron becomes paired off in the presence of a free radical scavenger,

Table 1: Phytochemical screening of methanolic crude extract of *C. lanatus*

Type of test	Result
Alkaloids	+
Tannins	+
Anthraquinones	-
Glycosides	-
Reducing sugars	-
Saponins	+
Phlobatannins	-
Steroids	+
Terpenoids	-
Coumarin	+
Emodin	-
Anthocyanin	-
Betacyanin	+
Flavonoids	+

C. lanatus: Carthamus lanatus

Table 2: Antibacterial activity of methanolic crude extract of *C. lanatus*

Micro-organism	Gram	1	2	3	Result
Staphylococcus aureus	+	16	17	15	16
Staphylococcus epidermidis	+	14	13	15	14
Bacillus subtilis	+	16	18	14	16

C. lanatus: Carthamus lanatus, S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, B. subtilis: Bacillus subtilis

Table 3: Antifungal activity of crude extract of *C. lanatus*

Fungus	% activity	% activity	% activity
Aspergillus flavus	100	100	100
Aspergillus niger	90	90	90
Alternaria solani	90	90	90

C. lanatus: Carthamus lanatus, A. flavus: Aspergillus flavus, A. niger: Aspergillus niger, A. solani: Alternaria solani

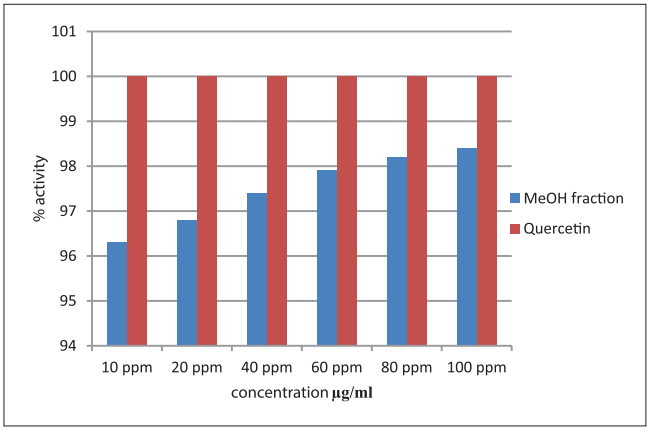

Fig. 1: Graphical representation of antioxidant activity of *Carthamus lanatus*

which, in turn, decolorizes the DPPH solution from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract (Fig. 1).

CONCLUSION

In the present research work, it is clear that the plant has very good pharmaceutical activities and metabolites. The said plant can be used as antifungal agent significantly. The plant is a very active antioxidant moiety.

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