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Antibacterial and antifungal study of *Cichorium intybus*

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ABSTRACT

Objective: To elucidate the antibacterial and antifungal activities of *Cichorium intybus* (*C. intybus*).**Methods:** The antibacterial and antifungal activities of *C. intybus* crude extract and its different solvent soluble fractions were carried out by using standard methods. Six bacterial strains like *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus*, and *Bacillus subtilis* were used for antibacterial activities while four fungal strains like *Aspergillus flavus*, *Fusarium solani*, *Aspergillus fumigatus* and *Aspergillus niger* were subjected to various fractions for antifungal activities.**Results:** Potentail activities of *C. intybus* were measured against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Water and ethyl acetate fractions showed promising activity against *Fusarium solani* and *Aspergillus niger*. Likewise, chloroform fraction was also found highly active against *Fusarium solani*.**Conclusions:** The findings suggest that the plant has good antibacterial and antifungal properties and can be used for infection control and treatment. Therefore, it is recommended that *C. intybus* is an significant medicinal plant and can be very useful for further biological assays.

1. Introduction

Therapeutic or medicinal plants are very beneficial for economic and therapeutic purposes. These plants contain active constituents which are used for the rehabilitation of many human diseases. These constituents are isolated and used against various pathogens^[1,2].

Cichorium intybus (*C. intybus*) seeds have been successfully used in Ayurvedic and Unani systems of medicine. It is one of the important medicinal plant belonging to Asteraceae family. *C. intybus* is a small scented biennial or perennial herb containing a number of medicinally imperative compounds, such as insulin, esculin, volatile compounds (sesquiterpenes and monoterpenes), vitamins, flavonoids and coumarins^[3]. It contains number of medicinally important phytochemicals like carbohydrate, alkaloids, flavonoids, terpenoids, tannins, saponins, steroids

and volatile oils. The root contains sesqui-terpene lactones like lactucin and lactucopicrin, flavonoids like quercetin-3-galactose, inulin, phlobaphenes, caffeic acid, cichoric acid, pectin, fixed oils, cholin and reducing sugar. The seed contains triterpenes cichoridiol and intybusoloid along with 11 known compounds such as lupeol, fridelin, beta sitosterol, sigmasterol, betulinic acid, betunaldehyde, syringic acid and vanilic acid. The volatile components include octane, *n*-nanodecane, pentadecanone, hexadecane and pentasalicylate. The pharmacological actions revealed that it possesses anticarcinogenic, hypoglycemic, hepatoprotective and anti-ulcer potentials^[4].

Currently, multiple drug resistance has been urbanized due to the random and frequent use of cost-effective antimicrobial drugs in the handling of contagious diseases. Besides, antibiotics are occasionally allied with harmful effects on the host, including allergic reactions, hypersensitivity and immune suppression. These circumstances enforced scientists to investigate for novel antimicrobial compounds. Accordingly, there is a demand to extend substitute antimicrobial drugs for the treatment of diseases from medicinal plants. There are numerous screening investigations on the antimicrobial activity of

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various plant extracts in diverse parts of the globe^[5].

The present study was also carried out on the basis of need to develop alternative antimicrobial agents for the handling of various diseases caused by microbes.

2. Materials and methods

2.1. Sample collection

C. intybus plant was collected from Mardan District, Khyber Pakhtunkhwa, Pakistan and was authenticated by plant taxonomist, Nisar Ahmad, Department of Botany, Kohat University of Science and Technology, Kohat Khyber Pakhtunkhwa, Pakistan. Then the same plant was parched and grinded to obtain fine powder of plant.

2.2. Extract preparation

The dried plant sample (1 kg) was macerated to obtain ethanolic extract according to well established reported protocols. After filtration, crude extract was evaporated under low pressures by rotary evaporator at 40 °C to give crude extract (60 g), which was further fractionated with various solvents on the basis of polarity *i.e.*, *n*-hexane, chloroform, ethyl acetate and aqueous fractions to get subsequent solvent soluble fractions with 20 g (*n*-hexane fraction), 15 g (chloroform fraction), 8 g (ethyl acetate fraction) and 16 g (aqueous fraction), respectively. The crude ethanolic (3 mg/mL) as well as all the subsequent fractions (4 mg/mL each) were evaluated for antibacterial and antifungal activity respectively.

2.3. Test compound preparation

A total of 30 mg of the extract and fractions were dissolved in dimethylsulfoxide for test compound preparation. Dimethylsulfoxide was used as a solvent in the current study^[6].

2.4. Antibacterial activities

Antibacterial activities of crude extract and its solvent soluble fractions of *C. intybus* were evaluated by using well assay method. Six bacterial strains like *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), *Staphylococcus epidermidis* (*S. epidermidis*), methicillin resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Bacillus subtilis* (*B. subtilis*) were tested for the antibacterial activities. Nutrient agar media was prepared in conical flask in accordance to the guidelines provided by the company. The media and related apparatus were uncontaminated in autoclave for 15 min at 121 °C and 15 pounds per square inch pressure. The media was poured into Petri dishes under aseptic environment (laminar flow hood)^[7].

The modified well assay method of Khan *et al.* was adopted^[8]. Bacterial strains like *E. coli*, *K. pneumoniae*, MRSA, *P. aeruginosa*, *S. epidermidis* and *B. subtilis* were

obtained from Biotechnology Department, Kohat University of Science Technology Kohat, Khyber Pakhtunkhwa Pakistan. Prepared nutrient agar media in Petri dish was inoculated with a selected bacterial culture equivalent to 10⁶ CFU. The bacterial strains were stretched on the solidified agar media. By using sterile metallic borer, 7 mm wells were punched in the nutrient agar media. Then 200 µL from each stock solution prepared in dimethylsulfoxide at concentration of 20 mg/mL was added into respective wells. The Petri dishes were incubated at 37 °C for 24 h. Doxycyclin (µg) was used as a positive control. Antibacterial activities were evaluated by measuring the diameter of the zones of inhibition after 24 h and compared with the zone of inhibition of standard drug (doxycyclin).

2.5. Antifungal activities

2.5.1. Sabouraud dextrose agar (SDA)

SDA is composed of dextrose, peptone and agar (5:40:15 g/L). The pH of agar ranged from 5.5 to 5.6. Then agar media was prepared by dissolving SDA (32.5 g) in distilled water by heating and volume was then step to 500 mL. A total of 4 mL of medium was then transferred into autoclaved and screw-capped test tubes for 15 min at 121 °C.

2.5.2. Preparation of medium and antifungal activities

For antifungal activities, fungal strains like *Fusarium solani* (*F. solani*), *Aspergillus flavus* (*A. flavus*), *Aspergillus niger* (*A. niger*) and *Aspergillus fumigatus* (*A. fumigatus*) were selected. The antifungal bioassay was determined by agar tube dilution method by using method of Imtiaz *et al.* with some modifications^[9]. In a conical flask, 28 g of nutrient agar media was dissolved in 1 L of distilled water. The flask was sterilized. Gentamycin was used as a standard and dissolved in 1.5 mL of distilled water. About 7 mL of media was taken into sterilized test tubes. Crude extract and subsequent fractions solution were prepared, each was at 4 mg/mL concentration. About 1 mL of sample (4 mg/mL) was also taken in a test tube; the test tube was reserved in inclined position to make a slant. Alike method was repeated for all kinds of test tubes. When it becomes cool and solidify, the fungi were added into test tubes and kept in incubator for 3 d at 25 °C. The test tubes were observed for fungal growth after 3 d.

3. Results

In the current investigations, the antibacterial activities of *C. intybus* were evaluated. The antibacterial activity was screened against six bacterial strains *i.e.*, *K. pneumoniae*, *P. aeruginosa*, MRSA, *S. epidermidis*, *E. coli* and *B. subtilis*. Results showed that *n*-hexane and chloroform fractions showed potential activity against *P. aeruginosa*, *K. pneumoniae*, *B. subtilis* and *S. epidermidis*. The solvent soluble fractions of *C. intybus* showed significant inhibitory action against MRSA. Ethanol and aqueous fractions were active against *P. aeruginosa* while ethyl acetate fraction was active up to some extent against *B. subtilis* as shown in Table

1.

Table 1Antibacterial activities of extracts and fractions of *C. intybus*.

Bacterial strains	Zone of inhibition (mm)					
	Ethanol extract	<i>n</i> -Hexane fraction	Chloroform fraction	Ethyl-acetate fraction	Aqueous fraction	Doxycycline (30 µg)
<i>E. coli</i>	7.4	4.5	6.2	7.0	0.0	30
<i>P. aeruginosa</i>	10.5	18.0	11.5	9.3	11.4	33
<i>K. pneumoniae</i>	0.0	14.1	9.0	9.7	7.8	31
MRSA	12.0	17.8	16.0	17.0	15.6	35
<i>S. epidermidis</i>	8.0	15.7	14.1	9.5	9.4	30
<i>B. subtilis</i>	6.5	16.4	12.1	11.0	6.7	33

Fungal strains like *F. solani*, *A. flavus*, *A. niger* and *A. fumigatus* were tested for antifungal potential and the results are tabulated in Table 2. Chloroform and *n*-hexane fractions were found very vigorous against all tested fungal strains. Ethanol and aqueous fractions showed activity against *A. niger*, *A. fumigatus* and *A. flavus*. The inhibitory effect of ethyl acetate fraction was prominent against *F. solani* but approximately motionless against other fungal strains. Likewise, ethyl acetate fraction showed best activity against *F. solani* but completely inactive against other fungal strains.

Table 2Antifungal activities of crude extract and different fractions of *C. intybus*.

Fungal strains	Inhibition				
	Ethanol extract	<i>n</i> -Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Aqueous fraction
<i>A. niger</i>	+	++	++	–	++
<i>A. fumigatus</i>	+	+++	++	–	+
<i>A. flavus</i>	++	++	+	–	+
<i>F. solani</i>	–	+	++	++	–

–: No growth; +: 25% growth; ++: 50% growth; +++: 75% growth.

4. Discussion

Herbal plants are main source of potentially valuable constituents for the development of new chemotherapeutic agents. The first stairway towards this target is the *in vitro* antimicrobial activity assay. Reports are available on the antibacterial, antiviral, antifungal, antimolluscal, anthelmintic and anti-inflammatory properties of plants. Some of these observations have helped in identifying the active principle responsible for such activities and in developing drugs for the therapeutic use in human beings^[10]. *C. intybus* is used to treat AIDS, diabetes, cancer, insomnia, dysmenorrhoea and tachycardia. Recent pharmacological investigation on the root and leaf fraction of this plant exposed immunomodulator, antitumor and anticancer properties. The sesquiterpene lactones such as lactucin and lactucopicrin were isolated from *C. intybus* and reported for its antibacterial and antimalarial activity. Literature shows that the roots and leaves of this plant possess strong antibacterial and nematocidal effect^[11]. However, very few reports are available on antibacterial properties of *C. intybus* root and leaf against the important human pathogens so far. The present study reports the antimicrobial activity of various extracts of *C. intybus* against some important pathogenic bacteria and fungi. The results of present investigation clearly indicate that

the antibacterial and antifungal activity vary with the extracts of the plant. The findings suggest that the plant has good antibacterial and antifungal properties and can be used for infection control and treatment. Therefore, it is recommended that *C. intybus* is a significant medicinal plant and can be very useful for further biological assays.

Conflict of interest statement

We declare that we have no conflict of interest.

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