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ORIGINAL ARTICLE

Antimicrobial screening of *Cichorium intybus* seed extracts

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KEYWORDS

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Abstract Medicinal plants play an important role in the field of natural products and human health care system. Chemical constituents present in the various parts of the plants can resist to parasitic attack by using several defense mechanisms. One such mechanism is the synthesis of antimicrobial compound. *Cichorium intybus* is one of the important medicinal plants which belong to Asteraceae family. In the present work, antimicrobial screening of *C. intybus* seed extract was studied by agar well diffusion assay by using aqueous and organic extracts. The pathogenic microorganisms tested include *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli*. All the seed extracts showed antimicrobial activity against tested microorganisms whereas *S.* aureus was found to be most sensitive against aqueous extract and had the widest zone of inhibition. Ethyl acetate and ethanol extract were found to be significant against *P.* aeruginosa and *S. aureus*. The results obtained from antimicrobial screening scientifically support the effectiveness of the medicinal plant.

1. Introduction

In India, the use of medicinal plant to cure specific ailments has been in vague from ancient times. Ayurveda, Siddha and Unani system of medicine have been in existence since centuries. These systems of medicine contribute need of nearly seventy percent of the population residing in the villages. Medicinal plants which form a backbone of traditional medicine have in the last few decades been the subject of very intense pharmacological studies. In the past few years, the development of resistance by pathogens to many of the commonly used antibiotics provides sufficient impetus for further attempts to search for new antimicrobial agent (Kamatou et al., 2007). This worldwide interest in medicinal plant reflects recognition of the validity of many traditional claims regarding the value of natural product in health care and development of microbial resistance to the available antibiotics. Since ancient time, numbers of herbal drugs have been used in the treatment of bacterial disease and several studies were carried out in search of a suitable plant drug which can effectively treat this disease.

Cichorium intybus seeds have been successfully used in ayurvedic and unani system of medicine. It is one of the important medicinal plants which belongs to family Asteraceae. It

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contains number of medicinally important phytoconstituents which belongs to carbohydrate, alkaloids, flavonoids, triterpenoids, tannins, saponins, fatty acids, volatile oils etc. (Nandagopal and Ranjitha, 2007). The root contains sesquiterpene lactones like lactucin and lactucopicrin, flavonoids like quercetin 3 galactose (Kirtikar andBasu, 2006), up to 60% inulin (Pushparaj et al., 2007), phlobaphenes caffeic acid, cichoric acid, pectin, fixed oils, cholin and reducing sugar (Wealth of India, 1950). The seed contains triterpenois cichoridiol and intybusoloid along with 11 known compound lupeol, fridelin, beta sitosterol, sigmasterol, betulinic acid, betunaldehyde, syringic acid, vanilic acid (Rahman et al., 2008) etc. The volatile component includes octane, *n*-nanodecane, pentadecanone, hexadecane and pentasalicylate (Asta and Jurga, 2008). The pharmacological actions revealed that chicory possesses anticarcinogenic (Hazra et al., 2002), hypoglycemic, hepatoprotective (Gadgoli and Mishra, 1997), anti-ulcer (Nadkarni, 1976), etc.

Candida albicans is a diploidfungus (a form of yeast) and a causal agent of opportunisticoral and genital infections in humans. *C. albicans* is commensal and is among the gut flora, many organisms that live in the human mouth and gastrointestinal tract. Under normal circumstances, *C. albicans* lives in 80% of the human population. Candidiasis is often observed in immunocompromised individuals such as HIV-positive patients. Candidiasis also may occur in the blood and in the genital tract. *Escherichia coli* is a Gram-negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms.

Pseudomonas aeruginosa is a Gram-negative, aerobic, rod-shaped bacterium with unipolar motility. It is a common bacterium that can cause disease in animals, including humans. It uses a wide range of organic material for food; in animals, the versatility enables the organism to infect damaged tissues or people with reduced immunity. *Staphylococcus aureus* also known as "golden staph" and Oro staphira is a facultative anaerobic, Gram-positive coccus, and is the most common cause of staph infections. It is frequently a part of the skin flora found in the nose and on skin. About 20% of the human populations are long-term carriers of *S. aureus*. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis.

2. Material and methods

2.1. Preparation of seed extract

The dried seeds were pulverized & extracted by soxhlet extraction by using Ethanol and Ethyl acetate. The extracts were concentrated in vacuum using the rotary evaporator. Aqueous extract was obtained by boiling dried seeds in water, filtered and dried using water bath.

2.2. Antimicrobial screening of extracts

2.2.1. Microorganisms and media

Gram +ve (*S. aureus*), Gram −ve (*P. aeruginosa*, *E. coli*) bacteria as well as fungi (*C. albicans*) were used for the broad spectrum antimicrobial study. The cultures of microorganisms were obtained from IMITECH Microbial Type Culture Collection and Gene Bank, Chandigarh. Brain Heart Infusion Agar M211 (Hi Media Laboratories Limited, Mumbai) and Brain Heart Infusion Broth M210 (Hi Media Laboratories Limited, Mumbai.) It contains Agar, Beef infusion form, Dextrose, Calf brain infusion form, Disodium phosphate, Proteose peptone and Sodium chloride.

2.2.1.1. Brain heart infusion agar. 52 gm of brain heart infusion agar M211 was suspended in 1000 mL distilled water. It was boiled to dissolve the medium completely. It was then sterilized by autoclaving using 15 lb pressure at 121 °C for 30 min.

2.2.1.2. Brainheart infusion broth. 37 gm of brain heart infusion broth M210 was suspended in 1000 mL distilled water. It is boiled to dissolve the medium completely. It is then sterilized by autoclaving using 15 lb pressure at 121 °C for 30 min.

2.2.1.3. Standardization of culture. The culture was standardized by using McFarland standard.

Procedure:

Test organism was grown on plates of brain heart infusion agar for 48 h. Inoculum suspension was prepared by picking up 5 colonies of at least 1 mm diameter and suspending the material in 5 mL sterile 85% w/v sodium chloride solution to match that of Mc Farland turbidity standard.

2.2.2. Determination of minimum inhibitory concentration (MIC) for antibacterial activity

The MIC values against bacterial strains were performed using Broth Dilution technique. 0.1 g of dried evaporated extract was dissolved in 100 mL of solvent giving final concentration of 1 mg/mL. The microbial activity was carried out in an

Table 1 Minimum inhibitory concentration (MIC) of aqueous, ethanol and ethyl acetate extracts of *C. intybus* seeds.

Extracts	*S.aureus*	*C. albicans*	*P. aeruginosa*	*E. coli*
Aqueous	70	80	100	100
Ethanol	–	90	–	80
Ethyl acetate	80	90	100	–

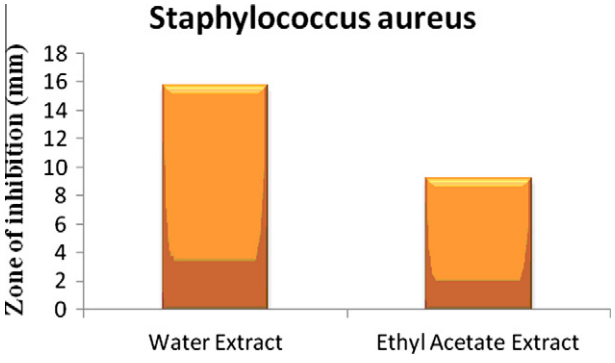

Figure 1 Antimicrobial screening of the extract against *Staphylococcus aureus*.

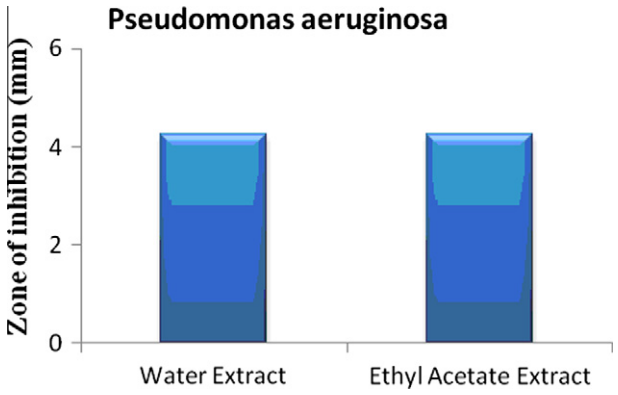

Figure 2 Antimicrobial screening of the extract against *Pseudomonas aeruginosa*.

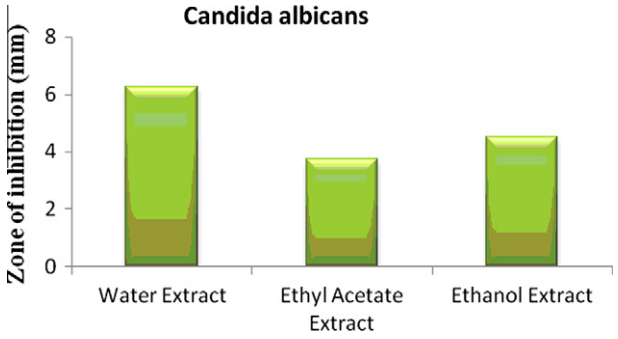

Figure 3 Antimicrobial screening of the extract against *Candida albicans*.

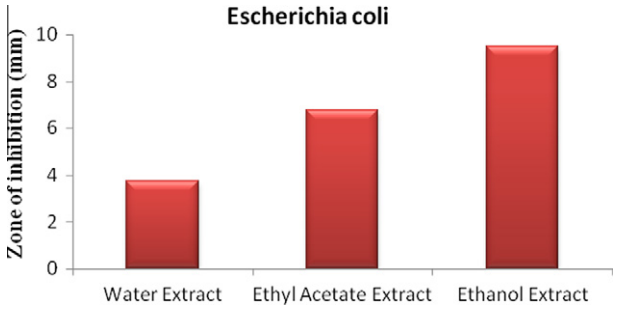

Figure 4 Antimicrobial screening of the extract against *Escherichia coli*.

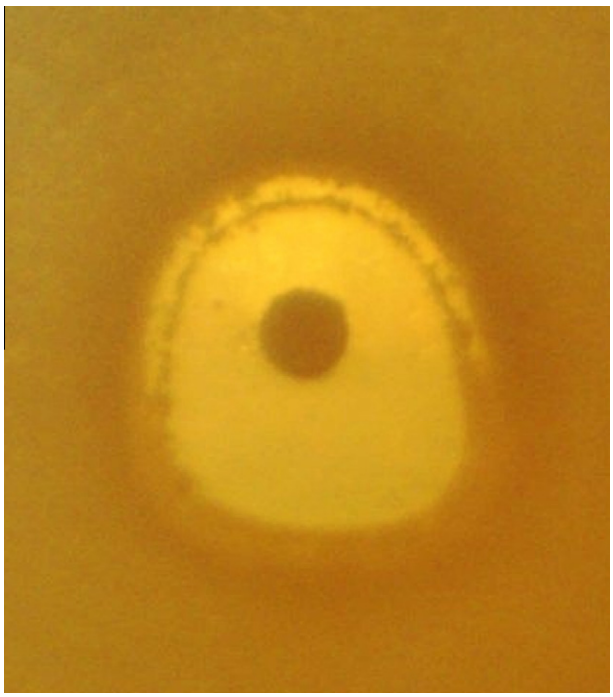

Figure 5 Zone of inhibition of aqueous extract against *Staphylococcus aureus*.

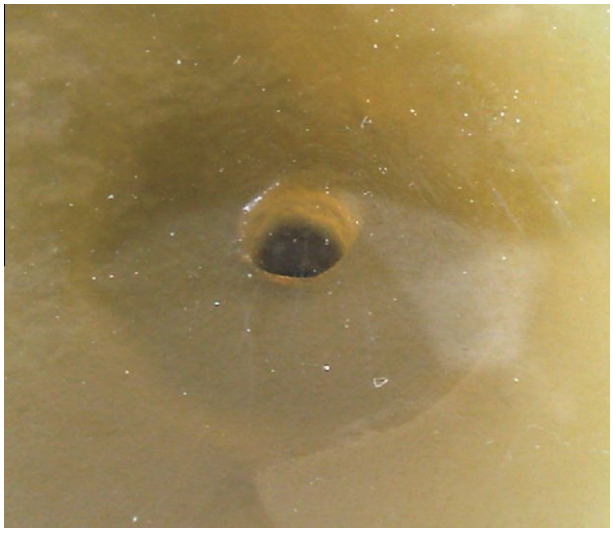

Figure 6 Zone of inhibition of ethyl acetate extract against *Staphylococcus aureus*.

aseptic area. Brain Heart Infusion broth was prepared. The medium was poured in the tubes which were then sterilized by autoclave using 15 lb pressure at 121 °C for 15 min. Using sterile pipettes exact amount of extract was added in concentration of 0.01–0.10 mg/mL to obtain a final volume of 10 mL. MIC was performed for all three extracts against four microorganisms.

The tubes were then inoculated with 0.05 mL of the standardized culture. The tubes were incubated at temperature 30 °C for 48 h. The tubes were observed for growth of microorganism by observing the turbidity produced. The test procedure was repeated three times to check the reproducibility of the results. The lowest concentration that inhibits the growth is the Minimum inhibitory concentration (MIC).

2.2.3. Agar well diffusion assay

Antimicrobial activity was assessed by agar well diffusion assay method as described elsewhere (Hou et al., 2007) with a slight modification. 0.5 mL overnight culture of test microbes and 15 mL of nutrient agar were evenly mixed and poured into sterile Petri plates aseptically and allowed to solidify at room temperature. The holes of 7 mm were bored aseptically using

Figure 7 Zone of inhibition of Ethanol extract against *Escherichia coli*.

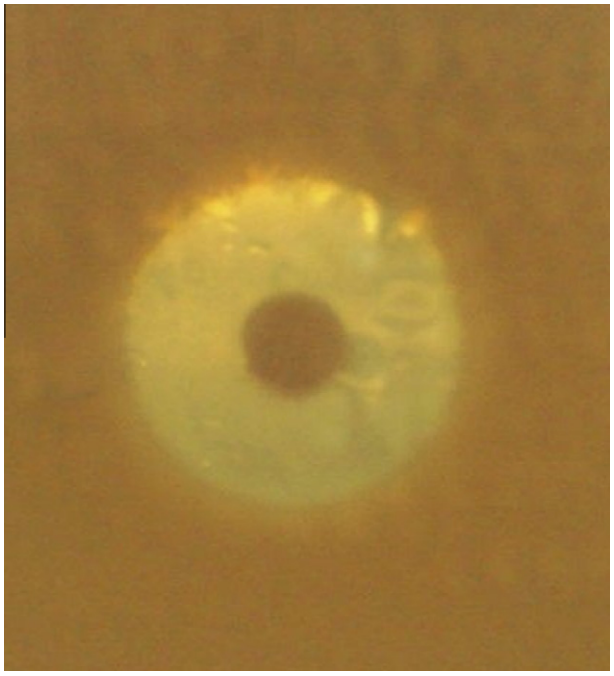

Figure 8 Zone of inhibition standard (Streptomycin).

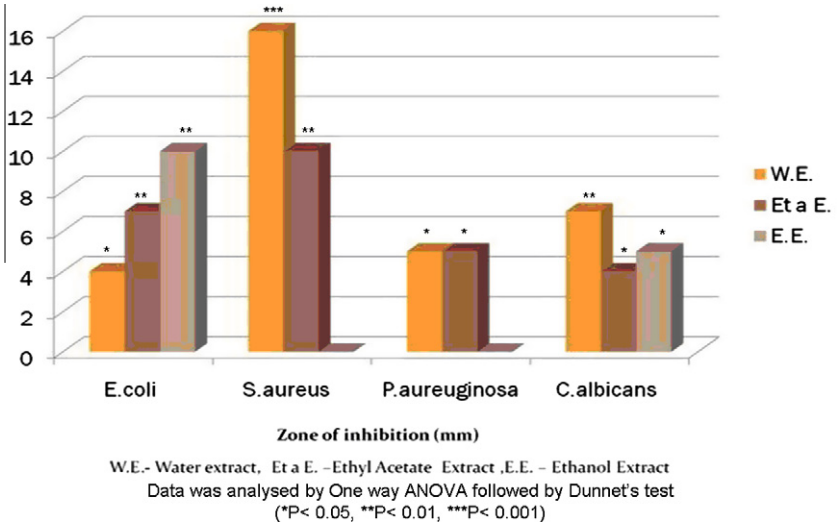

Figure 9 Comparison of activities.

sterile cork borer. The holes were filled completely with extracts in concentration of 3 mg/mL and kept in incubator at 37 °C for 48 h. The average diameters of the zone of inhibition were measured. All the tests were carried out in duplicates to ensure the accuracy and reliability of the results. Streptomycin was used as a standard in concentration of 1 mg/ mL.

3. Results and discussion

3.1. Standardization of culture

The optical densities of cultures were matched with McFarland standard and cultures were successfully standardized.

3.2. Minimum inhibitory concentration determination (MIC)

The results of MIC showed that all three extract were active against all four strains of microorganisms. All the extracts examined showed nearly the same MIC values. The MIC of the extracts of *C.* intybus ranged from 10 to 100 μg/mL. Among all extracts, aqueous extracts showed significant MIC. For aqueous extracts, the MIC values were found to be 70 μg/mL for *S. aureus*, 80 μg/mL for *C.* albicans and 100 μg/mL for *P.* aeruginosa and *E. Coli*. The ethanol extract was found to be active against *E. Coli* with MIC of 80 μg/mL and *C.* albicans with 90 μg/mL. *S.* aureus was found to be sensitive against ethyl acetate extract with MIC of 80 μg/ml,

P. aeruginosa and *C*. albicans of 100 μg/mL and 90 μg/ml for *E. Coli* (Table 1).

3.3. Antimicrobial screening

The antimicrobial activity of the *C*. intybus seed extracts was assessed by agar well diffusion assay method by measuring the zone of inhibition of three different extracts i.e., aqueous, ethanol and ethyl acetate extract. Various crude extracts of *C*. intybus seed showed significant antimicrobial activity against tested microorganisms which are presented in Figs. 1–8). Streptomycin was taken as standard.

The infections caused by *S*. aureus are among the most difficult to treat with standard clinical antimicrobial agents. The growth of *S*. aureus was inhibited by all the extracts but aqueous extract was found to be most significant when compared to other extracts (Fig 1).

Growth of *P*. aeruginosa was potentially inhibited by ethyl acetate extract (Fig 2). Ethanol extract was found to have potential antimicrobial activity against *C. albicans*. (Fig. 3). Ethanol extract showed significant activity against *E.* coli (Fig. 4). The comparative data were depicted in graph 1.

4. Conclusion

In conclusion, all the *C*. intybus seed extracts showed significant antimicrobial activity against tested microorganism, but *S*. aureus was found to be most sensitive and had the widest zone of inhibition. The culture was standardized successfully. The MIC of the extracts of *C*. intybus ranged from 50 to 100 μg/ mL. It was observed that aqueous extract showed maximum activity against *S*. aureus while did not show any significant activity against *C. albicans*. Ethyl acetate extract was found to be significant against *P*. aeruginosa and *S. aureus*. The results obtained from screening contributes to the literature that, chicory contains higher concentration of water soluble compounds (inulin, flavonoids, etc.) and may be responsible for exhibiting higher antimicrobial activity which was not seen in other extracts. The results obtained from the study confirmed that the therapeutic potency of the plant scientifically supports the effectiveness of the medicinal plant locally as well as traditionally to treat infectious disease. The results also form a good basis for selection of candidate plant species for further pharmacological and toxicity studies.

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