Antibacterial activity of *Eachinacia angustfolia*

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ABSTRACT

Antibacterial activity of petroleum ether, methanol and aqueous extracts of *Eachinacia angustfiloa* was investigated. The extracts were tested against both Gram-positive, Gram-negative and fungi organisms *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Staphylococcus epidermidis*, *Aspergillus niger* and *Candida albicans* at different doses of 0.5 mg/ml, 1.5 mg/ml and 1.5 mg/ml using cup plate and minimum inhibitory concentration (MIC) method. The *Eachinacia angustfiloa* extract exhibited broad-spectrum antibacterial activity against the tested organisms. The concentration of the extract that inhibits growth of the organism at a 530 nm MIC was found to be 400, 500, 600 and 900 µg/ml and MBC value was found to be 700, 900 and 1000 µg/ml, respectively.

Keywords: *Eachinacia angustfiloa*, cup plate, MIC, MBC.

INTRODUCTION

Echinacea angustifolia (EEA) is also known as Black Sampson. Echinacea plants are herbaceous perennials reaching 10–60 cm in height. The stem ascends either from a vertical taproot of Compositae family, that is known to have great medicinal importance widely used for the prevention and treatment of acute respiratory infections. It is also known for immune stimulation, anti-cancer and wound healing activity.[1] The plant also reported immunomodulator,[2] anti-inflammatory,[3] anti-stress,[4] anti-cancer,[5] anti-oxidant[6] and wound healing activity.[7] This study is designed to evaluate the antibacterial activity of *Echinacea angustifolia*.

MATERIALS AND METHOD

The ethanolic extract of *Echinacea angustifolia* (ECAG/JA 0071), was a gift sample obtained from Madhur Pharma, Bangalore Hundred grams of dried ethanolic extract of *Echinacea angustifolia* was successively fractioned with various solvents such as petroleum ether (60–80°C), methanol and water having different polarity in separating funnels. After drying, the different extracts were used for antibacterial screening.

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Microorganisms

The microorganisms employed in the current study were procured from the Deccan Medical College, Hydrabad (AP) and Osmania University, Hydrabad (AP) India.

Media

Nutrient broth, nutrient agar, malt extract broth and sabouraud dextrose agar, all products of Himedia Laboratories Mumbai (India) were used in this study.

Antimicrobial agents

Ampicillin, and fluconazole, (1% w/v) were used. The test solutions of the extract were prepared in DMSO at a concentration of 0.5–1.5 mg/ml. Fluconazole was used as standard and was dissolved in sterilized water to get a concentration of 100 µg/0.1 ml The test solutions of the extract were prepared in DMSO at a concentration of 0.5–1.5 mg/ml. Ampicillin was used as standard and was dissolved in sterilized water to get a concentration of 100 µg/0.1 ml DMSO (0.1 ml) was used as solvent control.

Anti-bacterial screening by cup plate method

A sterile borer was used to prepare cups of 10 mm diameter in the agar media spread with the microorganisms.

0.1 ml of inoculums (of 10^4 to 10^6 CFU/ml population prepared from standardized culture, adjusted with peptone water) was spread on an agar plate by spread plate technique. Accurately measured (0.1 ml) solution of each sample and standard were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2–8°C for a period of two hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 h. The presence of definite zones of inhibition around the cup indicated antibacterial activity.[8,9,10]

Determination of minimum inhibitory concentration

Two ml of nutrient broth for bacteria and saboraud dextrose broth for fungi was sterilized by autoclaving at 121°C for 15 minutes in different test tubes. 0.1 ml of required suspension (1×10^6 cfu/ml) of microorganism was added. One ml of different dilutions of extracts ranging from 100–1000 µg was added to the inoculated medium. Two ml of sterile nutrient broth inoculated with 0.1 ml of organism was taken as positive control and 2 ml of uninoculated tube of medium is incubated to serve as negative growth control. All the test tubes were incubated at 37 ± 1°C for 24 hr. The lowest concentration of the extract that inhibits growth of the organism, as detected by lack of visual turbidity (matching the negative growth control), is designated as minimum inhibitory concentration and reported as µg of substance per ml.[11,12,13]

RESULTS AND DISCUSSION

The results of antimicrobial activity test of *Echinacea angustifolia* extracts by different solvents with increasing polarity such as water, methanol, petroleum ether are shown in Tables 1, 2 and 3. The different extracts of this solvent vary in their antibacterial activity against the tested bacteria. The aqueous extraction of *Echinacea angustifolia* gave less antibacterial activities than all other organic solvents extraction. The most active organic extract was the methanol and petroleum ether extract. A methanol extraction from *Echinacea angustifolia* produced an inhibition zone of almost 7–18 mm. Minimum Inhibitory Concentration (MIC) value was found to be 900 µg/ml and Minimum bactericidal concentration MBC value was found to be 1000 µg/ml. The aqueous extract displayed no

Table 1. Antibacterial activity of *Echinacea angustifolia* petroleum ether extract.

Organisms	Zone of inhibition (mm)				
	EEA 0.5 mg/ml	EEA 1 mg/ml	EEA 1.5 mg/ml	Ampicillin 100 µg/ml	Fluconazole 100 µg/ml
Staphylococcus aureus (2079)	8	12	18	21	–
Bacillus subtilis (NCIM-2708)	7	13	18	24	–
Staphylococcus epidermidis (2478)	9	11	15	22	–
Escherichia coli (2685)	8	14	16	21	–
Candida albicans (MTCC1344)	7	14	16	–	21
Aspergillus niger (MTCC184)	7	12	13	–	20

Table 1a. Minimum inhibitory concentration, minimum bactericidal concentration, minimum fungicidal concentration of EEA.

Organisms	Concentration in g/ml of EEA		
	MIC	MBC	MFC
Staphylococcus aureus (2079)	500	700	–
Bacillus subtilis (NCIM-2708)	400	600	–
Staphylococcus epidermidis (2478)	400	500	–
Escherichia coli (2685)	500	700	–
Candida albicans (MTCC1344)	800	–	1000
Aspergillus niger (MTCC184)	900	–	1000

Table 2. Antibacterial activity of *Echinacea angustifolia* methanol extract.

Organisms	Zone of inhibition (mm)				
	EEA 0.5 mg/ml	EEA 1 mg/ml	EEA 1.5 mg/ml	Ampicillin 100 µg/ml	Fluconazole 100 µg/ml
Staphylococcus aureus (2079)	6	11	16	21	–
Bacillus subtilis (NCIM-2708)	5	10	13	21	–
Staphylococcus epidermidis (2478)	6	11	14	20	–
Escherichia coli (2685)	8	11	16	22	–
Candida albicans (MTCC1344)	7	13	16	–	21
Aspergillus niger (MTCC184)	4	11	14	–	20

Table 2a. Minimum inhibitory concentration, minimum bactericidal concentration, minimum fungicidal concentration of EREAS.

Organisms	Concentration in g/ml of EEA		
	MIC	MBC	MFC
Staphylococcus aureus (2079)	400	500	–
Bacillus subtilis (NCIM-2708)	300	500	–
Staphylococcus epidermidis (2478)	400	500	–
Escherichia coli (2685)	500	600	–
Candida albicans (MTCC1344)	600	–	900
Aspergillus niger (MTCC184)	700	–	800

Table 3. Antibacterial activity of *Echinacea angustifolia* aqueous extract.

Organisms	Zone of inhibition (mm)				
	EEA 0.5 mg/ml	EEA 1 mg/ml	EEA 1.5 mg/ml	Ampicillin 100 µg/ml	Fluconazole 100 µg/ml
Staphylococcus aureus (2079)	3	6	10	21	–
Bacillus subtilis (NCIM-2708)	4	6	9	24	–
Staphylococcus epidermidis (2478)	3	7	10	22	–
Escherichia coli (2685)	3	6	8	21	–
Candida albicans (MTCC1344)	2	8	11	–	20
Aspergillus niger (MTCC184)	2	6	10	–	18

Table 3a. Minimum inhibitory concentration, minimum bactericidal concentration, minimum fungicidal concentration of EREAS.

Organisms	Concentration in g/ml of EEA		
	MIC	MBC	MFC
Staphylococcus aureus (2079)	300	400	–
Bacillus subtilis (NCIM-2708)	300	500	–
Staphylococcus epidermidis (2478)	200	500	–
Escherichia coli (2685)	200	700	–
Candida albicans (MTCC1344)	400	–	800
Aspergillus niger (MTCC184)	300	–	700

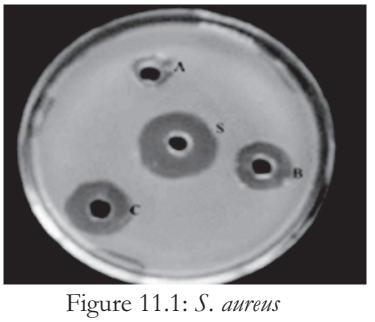

Figure 11.1: *S. aureus*

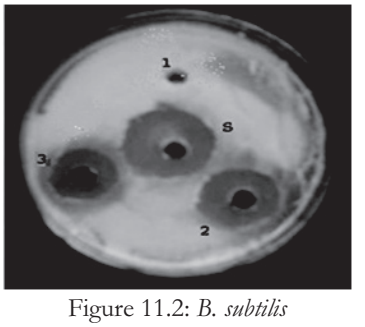

Figure 11.2: *B. subtilis*

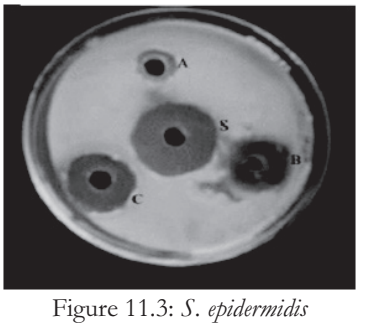

Figure 11.3: *S. epidermidis*

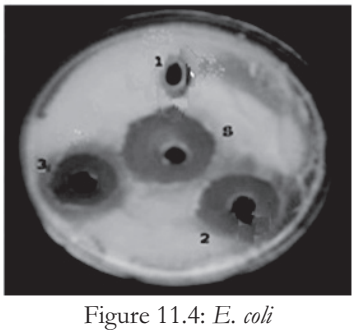

Figure 11.4: *E. coli*

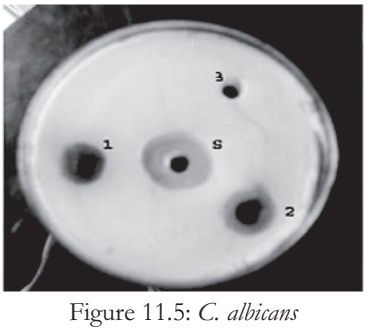

Figure 11.5: *C. albicans*

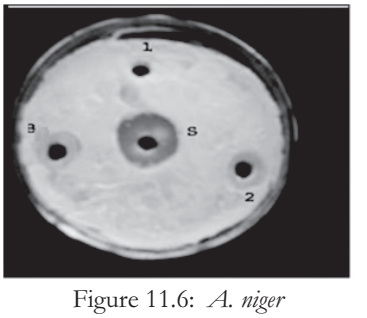

Figure 11.6: *A. niger*

Figure 1. Zone of inhibition of EEA against micro-organisms where **1**: 0.5 mg/ml **2**: 1 mg/ml **3**: 1.5 mg/ml.

antibacterial activity, while the extraction with the petroleum ether gave zone of inhibition equal to 6–15 mm, MIC value was found to be 600 μg/ml and MBC value was found to be 900 μg/ml, respectively. The antifungal activity of the fluconazole and petroleum ether extract was found to be nearly similar since fluconazole is the drug of choice for *Candida albicans*.

The triterpenes while steroids in petroium ether extracts found by Salkowaski test and Libermans test. MIC value for the extract of *Echinacea angustifolia* was 1 mg/ml, 1.5 mg/ml for various viz. *S. aureus, E. coli*, *Staphylococcus epidermidis* and *Candida albicans* activity is due to cichoric acid, echinacoside steroids, terpenoids, flavonoids and Alkaloids, isotussilagine, tussilagine, Alkylamides (alkamides), echinacein was reported.[14] The current work has shown that *Echinacea angustifolia* is a potential source of antimicrobial agents and its activity against various bacteria and fungus may be sufficient to perform further studies for isolation and identification for active principles.

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