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IN-VITRO ANTIMICROBIAL ACTIVITY OF ETHYL ACETATE EXTRACT OF *CENTAUREA BEHEN* LINN

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ABSTRACT

This work aims to evaluate the antimicrobial potential of ethyl acetate extracts of *Centaurea Behen* Linn (*C. Behen*) on some microorganisms. Agar well diffusion method has been used to determine the antimicrobial activities of plant extracts against Gram-positive bacteria, Gram-negative bacteria (*Streptococcus mutans, Staphylococcus aureus, Salmonella bongori* and *Escherichia coli*), and two fungus (*Candida albicans* and *Aspergillus niger*). The extracts exhibited both antibacterial and antifungal activities against tested microorganisms using standard ofloxacin, ciprofloxacin and fluconazole (10-30µg/ml). The antimicrobial activity was determined by measuring the diameter of the zone of inhibition in term of millimeter (mm). To analyze the antimicrobial activity, ethyl acetate extract of *C. Behen* was tested by well diffusion method against six selected strain and which shows significant inhibitory action against all the tested strain. The antimicrobial activity of ethyl acetate extract of root against all microorganisms was concentration dependent but less than standard drug. It is concluded that the antimicrobial activity showed by the plant is due to the presence of phytochemicals. For future studies, phytochemcials responsible for these activities can be isolated and modified for pharmacological purpose.

Keywords: Infectious diseases, *Centaurea Behen* Linn, Gram-positive bacteria, Gram-negative bacteria, Antimicrobial activity.

1. INTRODUCTION

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases [1]. However, emergence and dissemination of multidrug resistant (MDR) strain in pathogenic bacteria have become a significant public health threat as there are fewer, or even sometimes no effective antimicrobial agents available for the infection caused by pathogenic bacteria [2, 3]. Thus, in the light of the evidence of the rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy [4, 5]. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of these problematic bacterial infections [6]. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs [7]. Many plants have been used because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant [8, 9]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found *in vitro* to have antimicrobial properties [10, 11]. A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases as urinary tract infections, gastrointestinal disorders, respiratory disease, and cutaneous infections. *C. Behen* is commonly called as White Behen, Safaid Behmen, Behman abyaz and White Rhapontic belongs to the family Asteraceae/Compositae. It is native to India, Pakistan [12], Israel, Europe, North Africa and China [13]. It is also found in Tehran, Iraq and Turkey [14], which is used as remedial agents in various diseases. *C. behen* has been used to treat weakness of brain, heart and liver, palpitation, hepatitis, melancholia, sexual debility, neurasthenia, spermatorrhoea, fatigue and for diseases of the stomach and intestines [15]. It is also used in jaundice and is a heart tonic [16]. The roots of *C. behen* are used for killing the lice and making the hair good smelling [17]. Roots act as nervine and anabolic tonic, strengthen central nervous system and used in

affections of kidney [12]. Aerial parts of *C. behen* afforded several sesqueterpene lactones, the guaianolides cyanraopicrin, augerin B, desacylcynaropicrin, grosshemin and traces of a ketone which is closely related to solstitialin A [18]. The oil of *C. behen* comprises of five monoterpenes (7.8%), eleven sesquiterpene hydrocarbons (85.9%). Caryooyllane (24.5%), β-selinene (13.9%) and valencene (11.7%) are the major components, followed by δ-cadinene (8.7%), epi-α-muurolene (7.6%), α- humulene (6.5%) and α-copane (4.0%) [19]. The main constituents of the essential oil of aerial parts of *C. behen* are β-caryophyllene (40.3%), β-sesqiphellandrene (18.4%), and caryophene oxide (9.9%) [20]. From the roots of *C. behen*, a crystalline unsaturated lactone behenin having molecular formula C24H48O3 has been obtained [21]. The roots also contain taraxasterol and its acetate, myristate, inulin and a glucoside which on hydrolysis yields centaurea sterol A [13]. Seeds contain 23 % of a yellow semi-drying oil containing palmitic (7.2%), stearic (0.8%), oleic (11.9%) and linoleic (8.1 %) acids. Seeds also contain the enzymes diastase, lipase and protease. Xylose and uronic acids are present in the seed oil. Lipid extract of the plant contains lupeol, stigmasterol and straight-chain hydrocarbons [13]. Considering the vast potentiality of plants as sources for antimicrobial drugs, this study aimed to investigate *in vitro* antibacterial and antifungal activity of ethyl acetate extracts of *C. Behen* against the most common microbial pathogens including MDR bacteria.

2. MATERIAL AND METHOD

2.1. Plant material

Roots of *C. behen,* free of diseases, were collected from local region in separate sterile bags from Bhopal, Madhya Pradesh in the month of October, 2020. Plant material (root part) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was ground using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

2.2. Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvent used in this study were of analytical grade.

2.3. Bacterial strain

The pathogenic microbes used in the current study *Streptococcus mutans* (MTTCC-890), *Bacillus subtilis* (MTCC-441), *Salmonella bongori* (MTCC-3858), *Staphylococcus aureus* (MTCC-3160), *Klebsiella pneumonia* (MTCC-432), *Escherichia coli* (MTCC-40), *Aspergillus niger* (MTCC- 281), *Candida albicans* (MTCC-183) and *Aspergillus flavus* (MTCC-277) were obtained from Microbial Culture collection, National Centre Forcell Science, Pune, Maharashtra, India.

2.4. Defatting of plant material

Shade dried powder (136 gram) of root of *C. behen* was extracted with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

2.5. Successive extraction with different solvents by maceration method

Plant material were extracted in four solvents of different polarity viz water, methanol, ethyl acetate and chloroform. Powdered plant materials were extracted by maceration method. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6×2 cm) and stored in a refrigerator (4°C), till used for analysis [22].

2.6. Maintenance of bacterial culture

The bacterial strains were grown on nutrient agar media and inoculated at 37°C temperature for 24 hours and fungal strains were grown on SDA medium. Then organisms were maintained on nutrient agar slant at 4°C temperature and the purity of the organisms was checked at regular intervals by plating.

2.7. Antimicrobial sensitivity

The antimicrobial sensitivity test was employed on to the all the bacteria used under present study with ethyl acetate extract of *C. Behen*. 6 mm diameter wells with a stock of 100 mg/ml of each extract were used in this experiment, which were subsequently dried under aseptic circumstances. A nutrient agar plate is seeded with particular bacteria with the help of spread plate

technique prior and left for 5 minutes. The drug impregnated filter paper discs were placed in the center of pre-inoculated culture plates then incubated for 24 hours at 37°C. After incubation, plates were observed to see the sensitivity of extracts towards test bacteriums at particular concentration in the form of zone of inhibition.

2.8. Well diffusion method

The agar well diffusion method was adopted to determine the antibacterial activity of the ethyl acetate extract prepared from *C. behen* [23]. Firstly, microbial inoculum was swabbed over the surface of culture media. Broth cultures (undiluted) should never be used as inoculums. Then, four wells of 6 mm in diameter and about 2 cm apart were punched in the culture media containing petri plates with pre-sterilized tips. The plant extracts/standard antibiotics of different concentrations were placed on the holes. Three different concentrations of selected plant extracts (25, 50 and 100 mg/ml) were used in this test. Ciprofloxacin, ofloxacin and fluconazole were used as standard. Ofloxacin (10µg/ml, 20µg/ml, 30µg/ml) was used for *Streptococcus mutans*, Ciprofloxacin (10µg/ml, 20µg/ml, 30µg/ml) was used for *Staphylococcus aureus, Salmonella bongori* and *Escherichia coli,* and fluconazole (10µg/ml, 20µg/ml, 30µg/ml) for *Candida albicans* and *Aspergillus niger*. Subsequently, petri plates were incubated at 37°C for 24-48 hrs and after incubation; they were removed and observed to see clear zones of inhibition around the well. The diameter of zones of inhibition formed around the well was measured in millimeter and their average determined.

3. RESULTS AND DISCUSSION

After the antimicrobial sensitivity test, out of 9 microbes, we used only 4 gram positive, gram negative bacteria and 2 fungal pathogenic microbes for antimicrobial activity of plant (Table 1). The antibacterial and antifungal activities were determined using the well diffusion method. This method is highly effective for rapidly growing microorganisms, and the activities of the test extracts are expressed by measuring the diameter of the zone of inhibition. The antimicrobial activity of ethyl acetate roots extract of *C. Behen* showed bioactivity by inhibiting growth of microbial species selected for the test as shown in table 2 and 3. The zone of inhibition shown by the extracts was comparable to the standard drug. It is effective against *Streptococcus mutans, Staphylococcus aureus, Salmonella bongori*, *Escherichia coli, Aspergillus niger* and *Candida albicans* in concentration dependent manner.

Table 1: Results of sensitivity of ethyl acetate extract of *C. behen*

Microbes Codes	Microbes	Sensitivity of root extract
Bact-1	*Streptococcus mutans*	Yes
Bact-2	*Bacillus subtilis*	No
Bact-3	*Staphylococcus aureus*	Yes
Bact-4	*Salmonella bongori*	Yes
Bact-5	*Klebsiella pneumoniae*	No
Bact-6	*Escherichia coli*	Yes
Fungus-1	*Aspergillus niger*	Yes
Fungus-2	*Candida albicans*	Yes
Fungus-3	*Aspergillus flavus*	No

Table 2: Antimicrobial activity of standard drug against selected microbes

Name of drug	Microbes	Zone of inhibition		
		30 µg/ml	20 µg/ml	10 µg/ml
Ofloxacin	*Streptococcus mutans*	17±0.19	15±0.13	12±0.15
Ciprofloxacin	*Staphylococcus aureus*	22±2.16	18±2.62	17±1.69
	Salmonella bongori	25±0.5	23±0.86	17±0.15
	Escherichia coli	28±0.5	21±0.57	16±0.86
Fluconazole	*Candida albicans*	14±0.5	10±0.5	8±0.86
	Aspergillus niger	14±0.5	10±0.5	8±0.74

Table 3: Antimicrobial activity of ethyl acetate extract of *C. behen* against selected microbes

Name of microbes	Zone of inhibition (mm) Ethyl acetate extract		
	100mg/ml	50 mg/ml	25mg/ml
Streptococcus mutans	19±0.47	14±0.94	10±0.47
Staphylococcus aureus	18±0.47	13±0.47	10±0.47
Salmonella bongori	18±0.00	12±0.47	9±0.47
Escherichia coli	22±0.00	15±0.47	9±0.47
Candida albicans	18±0.47	14±0.47	8±0.47
Aspergillus niger	12±0.47	11±0.81	8±0.47

4. CONCLUSION

Extracts of *C. behen* in this study demonstrated a broad-spectrum of antimicrobial activity against selected microbial species. The antimicrobial activity of the plant extract, possibly due to the identified phytoconstituents, further confirms its use as a health remedy in folklore medicine. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial infections. Identification of these phyto-constituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view of formulating novel chemotherapeutic agents should be the future direction for investigation. Instead of cold percolation method, soxhlet extraction, subfraction, semipure compound, or a pure compound isolated from these plants might exhibit better antimicrobial activity. Further investigations are necessary to evaluate antimycobacterial, antiviral and antiparasitic activity. Moreover, other parts of the plants need to be studied to evaluate the studied plant extracts as a potential antimicrobial agent.

Conflict of interest

None declared

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