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In vitro Antibacterial and Antifungal Activity of *Heliotropium europeaum*

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Abstract:

In this study, we evaluated antibacterial and antifungal analysis of different extract and fractions of Heliotropium europeaum such as methanolic extract, n-hexane fraction, aqueous fraction and butanol fractions. For extraction and fractionation, different solvents were used such as methanol, n-hexane, aqueous and butanol. Agar

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well diffusion method was used for antibacterial and antifungal bioassay. The different strains which were used for analysis are gram negative bacteria E. coli, Salmonella typhi and gram positive bacteria Staphylococcus aureus while the fungi which were used for antifungal activity, are Aspergillus flavus, Aspergillus parasiticus, and Aspergillus ochraceus. Antibacterial activity of methanolic extract of Heliotropium europeam revealed substantial activity in which zone of inhibition ranged from 8mm to 12mm while other fractions also revealed antibacterial activity for instance n-hexane fraction, zone of inhibition from 8mm to 14mm, aqueous fraction, from 9mm to 11mm and the zone of inhibition for butanol is from 8mm to 11mm. Substantial antifungal activity shown by methanolic extract in which zone of inhibition ranged from 22mm to 32mm while other fractions also shown antifungal activity such as n-hexane fraction in which zone of inhibition ranged from 21mm and 31mm, aqueous fraction from 18mm to 22mm and butanol fraction exhibited activity in which zone of inhibition ranged from 21mm to 23mm.

Key words: *Heliotropium europeaum*; Extraction; Fractionation; Antibacterial; Antifungal activity; Agar well diffusion method

INTRODUCTION

From the traditional time, plants are the most supply of treatment and today they are hub of healthful supply not solely in developing countries, however conjointly in developed Countries wherever fashionable medicines are preponderantly used [1]. Medicinal plants are rich wellsprings of safe and powerful medications [2] and are used all through the history of humans either within the form of plant extracts or natural compounds towards numerous infectious diseases [3]. Medicinal plants are regularly used for treating diseases of the gastrointestinal tract and skin in countries having poor socio-economic [4]. Pakistan has different climate and is rather wealthy in medicinal herbs, although scattered over a

significant discipline [5]. Boraginaceae, family contains 100 genera and 1800 species and they are distributed by way of temperate regions however disbursed extra sufficiently in the Middle East. [6].

Heliotropium is a genus of flowering plants within the borage family, Boraginaceous. *Heliotropium europaeum* (Borginaceac), basic heliotrope, is a late spring developing yearly plant. The plant *Heliotropium europaeum* is a major plant from the medicinal point of view. This ethno medicinal plant is an erect or semi-prostrate expanded yearly growing 10 to 50 cm tall and produces an all-around created taproot [8, 9]. It is local to the Mediterranean and in Middle Eastern regions. Numerous types of *Heliotropium* other than *H.europaeum* are found in Iran and Iraq [10]. The *Heliotropium* is famous for its toxicity [11]. Seeds are scattered by water and creatures [12] and by individuals in trade as both a contaminant and wanderer [13, 14]. Boraginacea family plants are well known for the production of Pyrrolizidine Alkaloids and these pure PAs and PAs extracts inhibit the growth of many bacterial species such *as Bacillus Subtilis, E.coli, Staphylococcus and Bacillus* etc. and some Fungal species [15, 16]. Phytochemicals investigation of unrefined and parts of the plants uncovered the nearness of alkaloids, saponin, tannins, steroids, terpenoids, flavonoids, glycosides and phenols [17].

This medicinal and toxic plant is innately present in Mediterranean region countries such as Spain, France, Turkey, Italy, Greece, Monaco, Bosnia, Croatia, Albania and Middle Eastern region countries such as Syria, Saudia Arabia, Iran, Iraq, Egypt and accidently introduced in the region of Australia where this therapeutic plant is a significant weed [18, 19]**.** This plant *H.Europaeum* is weed economically because it contains toxic element to sheep, horses, poultry and pigs [20]. Many chemical components were separated from restorative plants of the Boraginaceae groups and family plants. These incorporate

Pyrrolizidine alkaloids, naphthoquinone, flavonoids, trepenoids, triterpenoids and phenols. These parts display antiviral, antitumor, antimicrobial, injury mending, prophylactic and calming [21]. In this study, we, the researchers in the Institute of Biochemistry, University of Balochistan, Quettan, Pakistan, extracted and fractionated *Heliotropium europaeum* and evaluated antibacterial and antifungal activities.

MATERIAL AND METHODS

Plant Material

Heliotropium europeam were collected from Dera Bhugti, Balochistan, Pakistan and was identified by Prof. Dr. Rasool Bakhsh Tareen, Taxonomist, Department of Botany, University of Balochistan, Quetta, Pakistan.

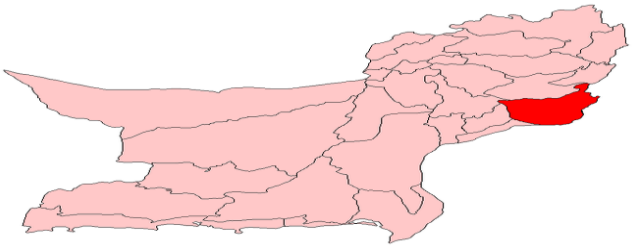

Fig. Dera Bhugti, Balochistan, Pakistan

Extraction and fractionation of *Heliotropium europeam*

This research of extraction and fractionation was completed in the Institute of Biochemistry, University of Balochistan, Quetta, Pakistan. 12.169 kg plant was soaked into extraction containers having methanol [22]. These containers containing soaked plant set aside for the duration of six days. Throughout six days container was shaken two times in 24 hours. After six days, the solvent such as methanol containing compounds extracted from the plant was filtered with the help of suction filtration. The filtered methanol containing plant extract was

with the help of rotary evaporator vaporized. Semisolid crude methanolic extract was removed and was 287.4gm [23, 24].

Formation of Fractions

The main extract due to methanol was separated for into 2 portions. 1 portion (3gm) has been screened for antibacterial and antifungal activities whereas 2 portions (284.4 gm) relocated in the separatory funnel for the formation of different fractions with the help of solvents such as water, n-hexane, and butanol. In a separatory funnel with 284.4gm extract two solvents such as water and n-hexane have been added. With thorough shaking two layers have been created n-hexane layer and water layer. Both layers have been alienated, though; water layer has been three times extracted with n-hexane. n-hexane was with the help of rotary evaporator vaporized. Semisolid n-hexane fraction was removed and was 20gm while water layer was with the help of rotary evaporator vaporized. Semisolid water fraction was removed and was 121gm and further fractionated with butanol solvent.

Formation of Butanol Fraction

In a separatory funnel with water extract two solvents such as water and butanol have been added. With thorough shaking two layers have been created water layer and butanol layer. Both layers have been alienated, though; water layer has been three times extracted with butanol. Butanol was with the help of rotary evaporator vaporized. Semisolid butanol fraction was removed and was 26gm and screened for antibacterial and antifungal activities.

Test microorganisms used

The microorganisms consist of gram negative bacteria E. coli, Salmonella typhi and gram positive bacteria Staphylococcus aureus while the fungi which were used for antifungal activity,

are Aspergillus flavus, Aspergillus parasiticus, and Aspergillus ochraceus. The strains were in the labs at CASVAB, University of Balochistan, Quetta, Pakistan.

Preparation of extracts

The crude extracts were dissolved in 30% dimethyl sulphoxide (DMSO) and further diluted to obtain of each extracted sample of 100mg/L concentration was used for the determination of antibacterial and antifungal activity.

Preparation of media and inoculum

Nutrient Agar/broth was used as the media for culturing of strains. A loopful of pure culture was inoculated in 20ml sterile nutrient broth medium in the test tubes aseptically and this process was repeated for all the strains. The tubes were incubated at 37 C^{o} for 24 hrs. Growth was observed in all the test tubes and this was further used in the experiment.

Standard antibiotics: Ciprofloxacin 0.3% w/v

Antibacterial and antifungal activity Agar well diffusion method

About 1ml of the inoculum was poured in the sterilized nutrient agar media. When media attains a temperature of 30-40 C^{o}, mixed well, and 20ml of this media was poured in all the petriplates and allowed to solidify. Then four wells of 6mm were made in each petriplate with the help of a sterile cork borer, 50ul of the plant extract was poured in each well using sterilized micropipettes. For each bacterial and fungal strain, negative controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones, for positive control, standard antibiotic ciprofloxacin (30mcg) was used. The plates were incubated overnight at 37 C^{o}. Microbial growth was determined by

measuring the diameter of zone of inhibition. The entire process was carried out aseptically in the laminar airflow [25, 26].

RESULTS

Antibacterial Activity of *Heliotropium europeam* Fractions

Antibacterial activity of *Heliotropium europeam* extract and fractions was performed against gram negative bacteria E. coli, Salmonella typhi and gram positive bacteria Staphylococcus aureus, the results are listed in table 1. Methanolic extract of *Heliotropium europeam* revealed substantial activity in which zone of inhibition ranged from 8mm to 12mm while other fractions also revealed activity for instance n-hexane fraction, zone of inhibition from 8mm to 14mm, aqueous fraction, from 9mm to 11mm and the zone of inhibition for butanol is from 8mm to 11mm. This result shows that methanolic extract and n-hexane fraction give significant activity against Staphylococcus aureus in which zone of inhibition is 12mm and 14mm. Methanolic extract also shown activity against E.coli and in which zone of inhibition is 12mm.

Antifungal Activity of *Heliotropium europeam* Fractions

Antifungal activity of Heliotropium europeaum extract and fractions was performed against Aspergillus flavus, Aspergillus parasiticus, and Aspergillus ochraceus. Substantial antifungal activity shown by methanolic extract in which zone of inhibition ranged from 22mm to 32mm while other fractions also shown activity such as n-hexane fraction in which zone of inhibition ranged from 21mm and 31mm, aqueous fraction from 18mm to 22mm and butanol fraction exhibited activity in which zone of inhibition ranged from 21mm to 23mm. this is tabulated in table 1.

Table 1: Antibacterial and antifungal activities of extracts and fractions of *Heliotropium europeaum*

S.NO	Bacterial and Fungal Strains	Zone of inhibition (mm)			
		Methanol	n-hexane	Aqueous	Butanol
1	E. Coli	12mm	8mm	9mm	8mm
2	Salmonella typhi	8mm	9mm	10mm	8mm
3	Staphylococcus aureus.	12mm	14mm	11mm	11mm
4	Aspergillus flavus	25mm	31mm	22mm	23mm
5	Aspergillus parasiticus	32mm	28mm	17mm	23mm
6	, Aspergillus ochraceus	22mm	21mm	18mm	21mm

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