Research Article

In vitro Antimicrobial and Insecticidal Activity on Leaves of Methanolic Extract and its Fractions of *Thuspeinanta brahuica*

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ABSTRACT

In the present paper, we investigated the biological studies of *Thuspeinanta brahuica* leaves. For this purpose different biological assay of crude methanolic extract (CME) and its fractions that are chloroform fraction, *n*-hexane fraction, Ethyl acetate fraction, *n*-butanol fraction and aqueous fraction were carried out. The results from the agar diffusion method indicated that Chloroform fraction showed maximum antibacterial activity against *B. Subtilis* with the inhibition zone (28 mm). Whereas CME crude showed strong activity against *B. subtilis* (24mm). On the other hand, CME showed maximum activity against *Candida albicans* and *Candida glaberata* with % inhibition of (72%) and (67%) respectively. Similarly, Chloroform fraction showed good activity against *Candida glaberata* and *Candida albicans* with % inhibition of (61%) and (60%) respectively. A second block of studies focuses on insecticidal activity, CME showed maximum insecticidal % mortality against *Triboliumca staneum* with (87%) mortality and (78%) mortality against *Sitophils oryzea.* Chloroform fraction also showed moderate activity against *Sitophils oryzea* with (65%) mortality. The extract and fractions were also appreciating for further investigations in future.

Keywords: Antimicrobial, insecticidal, *Thuspeinanta brahuica* leaves.

INTRODUCTION

For centuries, the therapeutic properties of various medicinal plants have been used to treat human diseases. It has been estimated that between 60-90% of the populations of developing countries use traditional and botanical medicines almost exclusively and consider them to be a normal part of primary healthcare[1]. Consumers are increasingly interested in complementary and alternative medicines, including herbal medicine, as they perceive these forms of healing as being both safe and effective. This trend in use of alternative and complementary healthcare has prompted scientists to investigate the various biological activities of medicinal plants. In the USA, a number of medicinal plants have been documented as important source of bioactive compounds.[2]

In herbal medicine, crude plant extracts in the form of infusion, decoction, tincture or herbal extract are traditionally used by the population for the treatment of diseases, including infectious diseases. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents.[3] Plant-derived products contain a great diversity of phytochemicals such as phenolic acids, flavonoids, tannins, lignin, and other small compounds[4]. These compounds possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities[5].

The expanding bacterial resistance to antibiotics has become a growing concern worldwide[6]. Intensive care physicians consider antibiotic-resistant bacteria a significant or major problem in the treatment of patients[7]. Increasing bacterial resistance is prompting resurgence in research of the antimicrobial role of herbs against resistant strains[8, 9]. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds[10]. Medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat[11]. Moreover, a rational approach is being developed to use medicinal plants as an insecticide. The insecticidal activity is due to the presence of active molecules in medicinal plants [12, 13].

The plant genus *Thuspeinanta brahuica (Lamiaceae*), *Annual* herb, 5-20 cm, simple or branched from base, has in its upper part, spreading eglandular hairs and numerous short capitate glandular hairs. Balochistan is blessed with diverse flora and fauna due to diverse ecological condition [14, 15], a series of papers on medicinal plants of Pakistan and included some information on Balochistan[16]. *Thuspeinanta brahuica* species are used as a weed for animals. This is also a continuation of our previous work to find bioactive compounds from medicinal plants[17-24]. Only limited work has been done in the medicinal flora of Balochistan province, since this plant (i.e. *Thuspeinanta brahuica)* has not been previously reported as a medicinal plant, therefore, the present work has been carried out to study the *in vitro* antimicrobial and insecticidal activity of *Thuspeinanta brahuica.*

MATERIALS AND METHODS

Plant material

The leaves of *Thuspeinanta brahuica* was collected from District Kalat, Balochistan province, Pakistan.

Extraction and fractionation

Fresh leaves were washed, sliced and dried under shade for 15 days. The leaves extract was prepared in analytical grade methanol (3 kg in 8 L) for 72 hours. Then, the methanol was removed and residue was immersed in methanol for further seven days. Then,, the methanol was decanted and filtered with Whatman filter paper. The filtrate was subsequently concentrated under reduced pressure at 45°C in rotatory evaporator (Stuart RE 300) and dried to constant weight (460 g) in vacuum oven (LINN high therm) at 45°C. This was crude methanolic leaves extract (CME). The CME was than further fractionalized, where 250g of CME was suspended in 250ml of distilled water. This aqueous suspension was further subjected to solvent-solvent extraction for five fractions, namely, *n*-hexane fraction (NHF), chloroform fraction (CHF), Et-acetate fraction (EAF), *n*- butanol fraction (NBF) and aqueous fraction (AQF).

Biological activities

Following biological activities were performed on the extract and its fractions.

Preparation of the tested organisms

A) Preparation of standard bacterial suspensions:

The average number of viable, *Bacillus subtilis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* organisms per ml of the stock suspensions was determined by means of the surface viable counting technique[25]. About (108- 109) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

B) Preparation of standard fungal suspensions

The fungal cultures (*Aspergillus niger, Candida albicans, Aspergillus fumigatus, A. flavus)* were maintained on Saboraud Dextrose Agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in (100 ml) of sterile normal saline and the suspension was maintained for further use.

Antimicrobial activity

Testing for antibacterial activity

The cup-plate agar diffusion method was used[26] to assess the antibacterial activity of the prepared extracts. 0.6 ml of standardized bacterial stock suspensions of 108 -109 colony- forming units per ml was thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates, 4 cups, also 10 mm in diameter, were cut using a sterile cork borer No. 4 and the agar discs were removed. Alternate cups were filled with 0.1ml of each extracts using micropipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 °C for 18 hours. Two replicates were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation the diameters of the growth inhibition zones were measured, averaged and the mean values were tabulated (Table 1).

Testing for anti-fungal activity

The same method as for bacteria was followed. Instead of nutrient agar media, yeast and mould extract agar was used. The inoculated medium was incubated at 25 °C for two days for the *Candida albicans* and *Aspergillus fumigatus* and three days for *Aspergillus niger* and *Aspergillus flavus.*

Insecticidal activity

Crude extract and all fractions were evaluated against different insect's *viz*., *Triboliumca staneum, Callosbruchus analis,* and *Rhyzoperth adominica*. The test sample was prepared by dissolving 200 mg of crude fractions in 3 ml acetone and loaded in a Petri dishes covered with the filter papers. After 24 hours, 10 test insects were placed in each plate and incubated at 27 °C for 24 hours with 50% relative humidity in growth chamber. The results were analyzed as percentage mortality, calculated with reference to the positive and negative controls. Permethrin was used as a standard drug, while Permethrin, acetone and test insects were used as positive and negative controls[27-31].

The percentage mortality was calculated by the formula:

$$\text{Growth regulation (\%)} = \frac{\text{Number of insects alive in test}}{\text{Number of insects alive in control}} \times 100$$

RESULTS AND DISCUSSION

Antibacterial activity

The methanolic extract and different fractions from the roots of *Thuspeinanta brahuica* showed good antibacterial activity against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, and Staphylococcus aureus*. Table 1 shows the zone of inhibition against different species of gram positive and gram negative bacteria. The results from the agar diffusion method indicated that 100% methanolic extract showed good activity against *Bacillus subtilis*, with the inhibition zone (24 mm). Least activity was exhibited against *Escherichia coli* with the smallest inhibition zone (16mm). Chloroform fraction showed strong activity against *B. subtilis* (28mm) and moderate activity against *Staphylococcus aureus* with (25 mm) of zone inhibition. *Escherichia coli* showed (14mm) of zone inhibition,

Salmonella typhi showed (12 mm) of zone inhibition whereas *Pseudomonas aeruginosa* was resistant to chloroform fraction. *n*- Hexane fraction showed good activity against *Bacillus subtilis*, *Salmonella typhi, Staphylococcus aureus* with inhibition zones (22, 16 and 16 mm respectively). No activity was exhibited against *Pseudomonas aeruginosa*. *n*-butanol fraction showed moderate activity against *Bacillus subtilis* with the inhibition zones (16mm) and showed (13mm) of zone inhibition against both *Salmonella typhi* and *Staphylococcus aureus* while less activity against *Escherichia coli* with zone of inhibition of (10mm), no activity was shown against , *Pseudomonas aeruginosa*. *Et*-acetate fraction was potent against *Bacillus subtilis*, with the inhibition zone (12 mm). Least activity was exhibited against *Staphylococcus aureus* with inhibition zone (11mm). In general, the antimicrobial activity of the tested extract and fractions is comparable with the standard drugs, Imipenum. The results indicated that plant extract and fractions showed least and no activity against *Pseudomonas aeruginosa.*

Table 1: Antimicrobial Activity of leaves of *Thuspeinanta brahuica*

Bacterial species	Zone of Inhibition of Std. drug* (mm)	Zone of inhibition (mm)					
		Crude (CME)	*n*-hexane	Chloroform	Et-acetate	*n*-butanol	Aqueous
Bacillus subtilis	36	24	22	28	12	16	10
Escherichia coli	35	16	12	14	-	10	-
Pseudomonas aeruginosa	*32*	-	-	-	-	-	-
Salmonella typhi	*40*	*21*	*16*	*12*	-	*13*	-
Staphylococcus aureus	*43*	*19*	*16*	*25*	*11*	*13*	*10*

*Imipenum (10µg disc)

Table 2: Antifungal activity of leaves of *Thuspeinanta brahuica*

Fungal species	% Inhibition of Std. drug*	% inhibition					
		Crude	*n*-hexane	Chloroform	Et-acetate	*n*-butanol	Aqueous
Aspergillus niger	98.4 - Miconazole	62	-	50	-	36	-
Candida albicans	110.8 - Miconazole	72	48	60	30	38	27
Candida glaberata	*110.28* - Miconazole	*67*	*39*	*61*	-	*43*	*22*
Aspergillus flavus	*20* - Amophotecin B	*12*	-	*06*	*08*	-	-

Percent inhibition activity, 0-39= Low (non-significant); 40-59= moderate; 60-69= Good; above 70= Significant

Table 3: Insecticidal activity of leaves of *Thuspeinanta brahuica*

Name of Insects	% Mortality of Std. drug*	% Mortality					
		Crude	*n*-hexane	Chloroform	Et-acetate	*n*-butanol	Aqueous
Tribolium castaneum	100	87	42	50	-	50	30
Sitophilus oryzea	100	78	50	65	-	40	-
Rhyzopertha dominica	*100*	*52*	*20*	*30*	-	-	-
Callosobrus husanalis	*100*	*63*	-	*30*	-	-	-

*Permethrin

Antifungal activity

The antifungal activity of the methanolic extract and different fractions from leaves of *Thuspeinanta brahuica* possess good antifungal activity against *Asperigillus niger, Candida albicans, Aspergillus flavus,* and *Candida glaberata*. Table 2. Shows % inhibition against different species of fungi compared to the standard drug (Miconazole and Amphotericin B). The result indicated that CME showed maximum activity against *Candida albicans and Candida glaberata* with % inhibition of (72%) and (67%) respectively and showed least % inhibition against *Aspergillus flavus* with (12%) inhibition. Chloroform fraction showed good activity against *Candida albicans* and *Candida glaberata* with % inhibition of (61%) and (60%) respectively and showed least % inhibition against *Aspergillus flavus* with (06 %) inhibition. *n- hexane* fraction showed moderate % inhibition against *Candida albicans* and *Candida glaberata* with (48%) and (39%) inhibition and did not show any activity against *Aspergillus flavus*. Et-acetate, *n*-butanol and aqueous fractions showed low % inhibition range from (22% to 43%).

Insecticidal Activity

Methanolic extract and its fractions from leaves of *Thuspeinanta brahuica* were evaluated for its insecticidal

activity against *Triboliumca staneum, Sitophilus oryzea, Rhyzopertha dominica* and *Callosbruchus analis* Table 3. Shows the % mortality of different species of insects as compared to standard drug (Permethrin). CME showed maximum insecticidal % mortality against *Triboliumca staneum* with (87%) mortality whereas (78%) mortality against *Sitophilus oryzea* and. Least was against *Rhyzopertha dominica* with 52% mortality. Chloroform fraction showed moderate activity against *Sitophilus oryzea* with (65%) mortality and (50%) against *Triboliumca staneum, w*hile other fractions showed less activity with % mortality less than and equal to 50% whereas Et-acetate fraction did not show any insecticidal activity.

CONCLUSION

This study was the first *in vitro* study of Antimicrobial and insecticidal activity of *Thuspeinanta brahuica* leaves that showed tremendous results against different pathogenic organisms. We recommend the future study on different aspects of pharmacological *in vitro* and *in vivo* studies.

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