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In Vitro Antimicrobial and Insecticidal Activities and Their Phytochemical Estimation of Methanolic Extract and Its Fractions of *Perotis hordeiformi* Leaves

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ABSTRACT

The aim of the present investigation deals with biological evaluation *Perotis hordeiformi* leaves. For this purpose different biological assay (agar diffusion method) of methanolic extract (Crude) 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 Crude showed maximum antibacterial activity against *Staphylococcus aureus* with the inhibition zone (31.52 ± 0.06 mm). On the other hand, crude showed maximum activity against *Candida albicans* and *Candida glaberata* with % inhibition of (74.23 ± 0.02) and (67.53 ± 0.11) respectively. Furthermore, crude showed maximum insecticidal % mortality against both *Tribolium castaneum and Sitophilus oryzea* with (80%) and (70%) respectively. Phytochemical estimation of Crude and its fractions showed the presence of Alkaloids, Flavonoids, Phenols, tannins and Diterpenes.

Keywords: *Antimicrobial, Insecticidal, Phytochemical Estimation, Perotis hordeiformi*

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 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].

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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].

Perotis hordeiformis belongs to family *Poaceae* is an Annual or short-lived perennial. Mainly found along seashores. Guangdong, Hebei, Jiangsu, Yunnan [India, Indonesia, Malaysia, Nepal, Myanmar, Pakistan, Sri Lanka, Thailand].

This species is very close to *Perotis indica* and is sometimes

included within it. No single character by itself is reliable for separating the two, but the combination of characters given in the key will usually suffice. Hence, in continuation of our previous work [6-13], the present study is carried out to study the *in vitro* Antimicrobial and insecticidal activities and phytochemical estimation of *Perotis hordeiformis* leaves.

Materials and Methods

Plant material

The leaves of *Perotis hordeiformis* were collected from Soorab, Balochistan province, Pakistan.

Extraction and fractionation

Fresh leaves were washed, sliced and dried under shade for 25 days. The leaves' extract was prepared in analytical grade methanol (4 kg in 10 L) for 72 hours. Then the methanol was removed and residue was immersed in methanol for a further five days. Thereafter, the methanol was decanted and filtered with Whatman filter paper. The filtrate was subsequently concentrated under reduced pressure at 40 °C in rotatory evaporator (Stuart RE 300) and dried to constant weight (500 g) in vacuum oven (LINN high therm) at 40°C. This was crude methanolic leaves extract (CME). The CME was than further fractionalized, where 300 g of CME was suspended in 350 ml of distilled water. This aqueous suspension was further subjected to solvent-solvent extraction for four fractions, namely, *n*-hexane fraction (NHF), chloroform fraction (CHF), acetone fraction (ACE), 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, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* organisms per ml of the stock suspensions was determined by means of the surface viable counting technique [14]. 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 (*Microsporum canis, Candida albicans, Aspergillus flavus, and Candida glaberata)* 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 [15] 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, 10 mm in diameter, were cut using a sterile cork borer No. 4 and the agar discs were removed. Alternate cups were filled with 0.1 ml 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 *Microsporum canis* and *Candida albicans* and three days for *Candida glaberata* and *Aspergillus flavus.*

Insecticidal activity

Crude extract and all fractions were evaluated against different insects viz., *Tribolium castaneum, Sitophilus oryzea, Callosbruchus analis, and Rhyzopertha dominica*. 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 [16-20].

The percentage mortality was calculated by the formula:

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

Phytochemical Screening

Phytochemical screening for major bioactive constituents like alkaloids, phenolics, flavonoids and tannin were determined by using standard phytochemical methods [21, 22].

A. Determination of Total Phenolics

Total phenolic content of methanolic extract of *Peucedanum beluchistanicum* leaves was determined by FolinCiocalteu method. Phenolic content was expressed as Gallic acid equivalents (GAE mg/g dry weight of extract) and the values were presented as mean ± SD of triplicate analysis with slight modifications [23]. 200 µl of sample (1 mg/ml) was added to 100 µl diluted (1:10) Folin Ciocalteu reagent and equilibrated for few minutes. Then 800 µl of 2.5 % aqueous Na_2CO_3 was added and mixture was allowed to stand for 60 minutes at room temperature with

intermittent shaking. The absorbance of the blue color solution was measured at 765 nm on UV visible spectrophotometer (Shimadzu UVPC-1700 (Japan)). Gallic acid (50 mg /%) was used as standard. The absorbance of solution was compared with Gallic acid calibration curve.

B. Determination of Total Flavonoids

Total flavonoid content was determined by aluminum chloride colorimetric method [24]. This method is based on the formation of a complex flavonoid-aluminium, having the absorbance maximum at 430 nm. 0.5 ml of plant extract (1 mg/ml) was mixed with 1.5 ml of methanol, 0.1 ml of 10 % $AlCl_3$, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 15 minutes, the absorbance of the reaction mixture was measured at 430 nm on UV-visible spectrophotometer (Shimadzu UVPC-1700 (Japan)). Total flavonoid contents of leaves sample were expressed as rutin equivalents (RE mg/g dry weight of extract) through the calibration curve with rutin as standard.

C. Alkaloid Estimation

2.5g of the plant powder was extracted using 100ml of 20% acetic acid in ethanol. The solution was covered for almost 4 hours. Filtrate was concentrated to 25ml. Concentrated ammonium chloride was added stepwise for precipitation. The whole solution was kept as such so that precipitate should settle down. Collected precipitate was washed with dilute ammonium hydroxide and finally filtered. Filtrate was discarded and pellet obtained was dried and weighed [25, 26].

D. Tannins Estimation

The tannin content in samples was estimated by the method of Price and Butler [27]. Different aliquots of sample were taken and final volume to 3 ml was adjusted by distilled water. The samples after vortexing were mixed with 1ml of 0.016 M $K_3Fe(CN)$, followed by 1 ml of 0.02 M $FeCl_3$ in 0.10 M HCl. Vortexing was repeated and the tubes were kept as such for 15 min. 5 ml of stabilizer (3:1:1 ratio of water, H_3PO_4 and 1 % gum Arabic) was added followed by revortexing. Absorbance was measured at 700 nm against blank. Standard curve was plotted using various concentrations of 0.001 M Gallic acid.

Results and Discussions

Antibacterial activity

The antibacterial activity of the methanolic extract and different fractions from leaves of *Perotis Hordeiformi* possess 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 (Crude) showed good activity against *Staphylococcus aureus* and *Bacillus subtilis* with the inhibition zones (31.52 ± 0.08 mm) and (24.10 ± 0.03mm) respectively. Moderate activity of Crude was exhibited against both *Pseudomonas aeruginosa* and *Escherichia coli* with the inhibition zones (20.67 ± 0.08) and (16.34 ± 0.02) respectively. No activity was exhibited against *Salmonella typhi*. Chloroform fraction showed strong activity against *Staphylococcus aureus* with (24.53 ± 0.11mm) zone inhibition and moderate activity against both *Bacillus subtilis* and *Pseudomonas aeruginosa* with the inhibition zone (21.36 ± 0.09) and (17.43 ± 0.02) respectively. *n*-butanol fraction showed moderate activity against *Staphylococcus aureus* with (19.48 ± 0.06mm) zone inhibition. Rest of the fractions showed less activity against all the species of bacteria with zone inhibition less than 15mm. The antimicrobial activity of the tested extract and fractions is comparable with the standard drug, Impenum.

Antifungal activity

The antifungal activity of the methanolic extract and different fractions from leaves of *Perotis Hordeiformi* possess good antifungal activity against *Microsporum canis, 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 Crude showed maximum activity against *Candida albicans* and *Candida glaberata* with % inhibition of (74.23 ± 0.01) and (67.53 ± 0.02) respectively and showed least % inhibition against *Aspergillus flavus* with (13.01 ± 0.01) inhibition. Chloroform fraction showed good activity against *Candida albicans* and *Candida glaberata* with % inhibition of (66.32 ± 0.05) and (60.38 ± 0.01) respectively and showed least % inhibition against *Aspergillus flavus* with (10.55 ± 0.10) inhibition.

Table 1: Antibacterial Activity of leaves of *Perotis Hordeiformi*

Bacterial species	Zone of Inhibition of Std. drug* (mm)	Zone of inhibition (mm)					
		Crude	*n*-hexane	Chloroform	Et-acetate	*n*-butanol	Aqueous
Bacillus subtilis	36.06±0.03	24.10±0.03	15.12±0.01	21.36±0.09	13.22±0.07	10.65±0.10	10.17±0.01
Escherichia coli	35.11±0.02	16.34±0.02	-	-	-	-	-
Pseudomonas aeruginosa	32.01±0.09	20.67±0.08	-	17.43±0.02	-	12.82±0.09	-
Salmonella typhi	40.12±0.01	-	-	-	-	-	-
Staphylococcus aureus	43.22±0.08	31.52±0.06	13.61±0.06	24.53±0.11	11.31±0.03	19.48±0.06	10.14±0.01

**Impenum (10 μg disc)*

Table 2: Antifungal activity of leaves of *Perotis Hordeiformi*

Fungal species	% Inhibition of Std. drug*	% Inhibition					
		Crude	*n*-hexane	Chloroform	Et-acetate	*n*-butanol	Aqueous
Microsporum canis	98.04±0.02 Miconazole	48.66±0.04	30.12±0.09	41.07±0.01	25.72±0.06	23.42±0.07	-
Candida albicans	110.08±0.02 Miconazole	74.23±0.10	52.08±0.03	66.32±0.05	45.18±0.10	39.08±0.10	33.78±0.03
Candida glaberata	110.25±0.06 Miconazole	67.53±0.02	47.10±0.03	60.38±0.01	42.22±0.01	30.75±0.06	-
Aspergillus flavus	20.12±0.06 Amphotericin B	13.01±0.01	07.10±0.05	10.55±0.10	06.22±0.04	-	-

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

Table 3: Insecticidal activity of leaves of *Perotis Hordeiformi*

Name of Insects	% Mortality of Std. drug*	% Mortality					
		Crude	*n*-hexane	Chloroform	Et-acetate	*n*-butanol	Aqueous
Tribolium castaneum	100	80	40	60	50	30	20
Sitophilus oryzea	100	70	30	50	20	-	-
Rhyzopertha dominica	100	60	20	40	-	-	-
Callosobrushus analis	100	-	-	-	-	-	-

Table 4: Quantitative estimation of phytoconstituents present in methanol extract and it is fractions of *Perotis Hordeiformi* leaves.

Phytoconstituents	Quantity (mg/g plant extract and it is fractions)
Alkaloids	16.18 ± 0.06
Phenolics	20.55 ± 0.06
Flavonoids	22.82 ± 0.01
Tanins	18.67 ± 0.08
Diterpenes	13.48 ± 0.06

n-hexane fraction showed moderate % inhibition against *Candida glaberata* with (52.08 ± 0.03) inhibition. *Et*-acetate and *n*-butanol aqueous fractions showed moderate and low % inhibition. Aqueous fraction only showed activity against *Candida albicans* with % inhibition (33.78 ± 0.03).

Insecticidal Activity

Methanolic extract and its fractions from leaves of *Perotis Hordeiformi* were evaluated for its insecticidal activity against *Tribolium castaneum, Sitophilus oryzea, Rhyzopertha dominica* and *Callosbruchus analis*. (Table 3) shows the % mortality of different species of insects as compared to standard drug (Permethrin). Crude showed maximum insecticidal % mortality against *Tribolium castaneum* with (80%) mortality whereas (70%) mortality against *Sitophilus oryzea*. No activity was exhibited against *Callosbruchus analis*. Chloroform fraction also showed good activity against *Tribolium castaneum* with (60%) mortality and (50 %) mortality against Sitophilus *oryzea*. *n*-hexane, Et-acetate and *n*-butanol fractions showed low insecticidal activities with % mortality less than 50% mortality. *Callosbruchus analis* was resistant to Crude and its fractions.

Quantitative Analysis of Phytoconstituents

Phytochemical analysis showed that the Methanolic leaves extract of *Perotis Hordeiformi* are a rich source of Flavonoids, phenols which may be responsible for the antimicrobial and insecticidal activity.

Conflict of Interest

We declare that we have no conflict of interest.

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References

1. World Health Organization: **Traditional medicine-growing needs and potential. WHO policy perspectives on medicine, No. 2**. *WHO, Genève* 2002.
2. Balunas MJ, Kinghorn AD: **Drug discovery from medicinal plants**. *Life sciences* 2005, **78**:431-441.
3. Barnes J, Anderson LA, Phillipson JD, Newall CA: *Herbal medicines*: Pharmaceutical Press London; 2007.
4. Cowan MM: **Plant products as antimicrobial agents**. *Clinical microbiology reviews* 1999, **12**:564-582.
5. Bidlack WR, Omaye ST, Meskin MS, Topham DKW: *Phytochemicals*

as bioactive agents: CRC press; 2000.
6. Nabi S, Ahmed N, Khan MJ, Bazai Z, Yasinzai M, Al-Kahraman YMSA: **In vitro Antileishmanial, Antitumor Activities and phytochemical Studies of Methanolic Extract and its Fractions of Juniperus Excelsa Berries**. *World Applied Sciences Journal* 2012, **19**:1495-1500.
7. Khan MJ, Baloch NU, Nabi S, Ahmed N, Bazai Z, Yasinzai M, Al-Kahraman YMSA: **Antileishmanial, Cytotoxic, Antioxidant Activities and Phytochemical Analysis of Rhazya Stricta Decne Leaves Extracts And Its Fractions**. *Asian Journal of Plant Science and Research* 2012, **2**:593-598.
8. Khan AA, Baloch NU, Nabi S, Khan MJ, Jamali MS, Al-Karhaman YMSA: **In Vitro Antimicrobial and Insecticidal Activity Activity of Methanolic Extract and Its Fractions of Berberis Baluchistanica Roots**. *World Journal of Pharmaceutical Research* 2012, **2**:219-226.
9. Kakar AM, Khan AA, Nabi S, Kakar MA, Yasinzai M, Alkahraman Y: **In Vitro Antileishmanial, Cytotoxic Activity and Phytochemical Analysis of Thuspeinanta Brahuica Leaves Extract and Its Fractions** *IJPBAS* 2013, **2**:520-528.
10. Baloch N, Nabi S, Kakar AM, Wajid Z, Al-Kahraman Y: **In vitro antileishmanial, antitumor, cytotoxic activities and phyto-chemical analysis of Citrullus colocynthis fruit extract and its fractions**. *International Journal of Medicinal and Aromatic Plants* 2013, **3**:78-84.
11. Baloch N, Kakar AM, Nabi S, Yasinzai M, Al-Kahraman DYM: **In Vitro Antimicrobial, Insecticidal, cytotoxic activities and their phytochemical analysis of Methanolic Extract and its Fractions of Peucedanum Beluchistanicum leaves**. *International Journal of Pharma and Bio Sciences* **4**:898-905.
12. Al-kahraman Y, Baloch N, Kakar AM, Nabi S: **In Vitro Antimicrobial, Insecticidal, Antitumor, Antioxidant activities and their Phytochemical Estimation of Methanolic Extract and its Fractions of Nepeta praetervisa leaves**. *International Journal of Phytomedicine* 2013, **4**:531-536.
13. Baloch N, Nabi S, Al-Kahraman YMSA: **In vitro Antileishmanial, Cytotoxic, Antioxidant activities and Their Phytochemical Analysis on Methanolic Extract and it is Fractions of Perotis hordeiformis leaves**. *International journal of Pharmacy* **104**:165-170.
14. Miles AA, Misra SS, Irwin JO: **The estimation of the bactericidal power of the blood**. *Journal of Hygiene* 1938, **38**:732-749.
15. Kavanagh F: *Analytical Microbiology. Edited by F. Kavanagh*: Academic Press; 1963.
16. Abbott WS: **A method of computing the effectiveness of an insecticide**. *J. econ. Entomol* 1925, **18**:265-267.
17. Rahman A-u, Choudhary MI, Thomsen WJ: *Bioassay techniques for drug development*: Harwood Academic Publishers The Netherlands; 2001.
18. Collins PJ: **Resistance to grain protectants and fumigants in insect pests of stored products in Australia**. In *Proceedings of the Australian Postharvest Technical Conference*: CSIRO, Canberra, Australia: 1998:55-57.
19. Rashid R, Farah M, Mirza MN: **Biological screening of Salvia cabulica**. *Pak. J. Bot* 2009, **41**:1453-1462.
20. Tabassum R, Naqvi SNH, Azmi MA, Nurulain SM, Khan MF: **Residual effect of a neem fraction, nimolicine and an insect growth regulator, dimilin, against stored grain pest Callosobruchus analis**. In *Proceedings of the Pakistan Congress of Zoology*: 1997:165-170.
21. Andrews JM: **Determination of minimum inhibitory concentrations**. *Journal of Antimicrobial Chemotherapy* 2001, **48**:5-16.
22. Harborne JB: *Phytochemical methods A Guide to modern techniques of plant analysis*: springer; 1998.
23. Trease E, Evans W: *Text book of pharmacognosy* edn 12th. Oxford: Alden Press; 2007.
24. Chang C-C, Yang M-H, Wen H-M, Chern J-C: **Estimation of total flavonoid content in propolis by two complementary colorimetric methods**. *Journal of food and drug analysis* 2002, **10**:178-182.
25. Edeoga HO, Okwu DE, Mbaebie BO: **Phytochemical constituents of some Nigerian medicinal plants**. *African journal of biotechnology* 2005, **4**:685-688.
26. Okwu DE, Josiah C: **Evaluation of the chemical composition of two Nigerian medicinal plants**. 2006.
27. Price ML, Butler LG: **Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain**. *Journal of Agricultural and Food Chemistry* 1977, **25**:1268-1273.

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