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RESEARCH ARTICLE

Cytotoxic, phytotoxic and antimicrobial characterization of *Melilotus parviflora*

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Abstract

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Melilotus parviflora collected at Bannu District, KPK, Pakistan was evaluated for pharmacological properties by using reported procedures. The results showed that *Melilotus parviflora*'s methanolic extract has reasonable lethal effect on Brine Shrimps. The growth of rice stems and roots were inhibited. The growths of bacteria i.e. *Bacillus Septica*, *E. Coli*, and *Bacillus* were retarded by using *Melilotus parviflora*'s methanolic extract. *Asperillus niger* growth is more stunted as compared to *Asperillus flavus* and *Asperillus fumigates* species of fungi. The linear growth measured for *Asperillus niger*, *Asperillus flavus* and *Asperillus fumigates* on slanted *Melilotus parviflora*'s methanolic extract added media were 1.5 cm, 5 cm and 5.5 cm respectively.

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1. Introduction

Natural products from plants are the prime target for drug development [1]. The chemical compounds within plants show their effects on human body through different processes [2]. About 20% of plants having pharmacological properties are used for various treatments i.e., stomach ailments, skin disease, blood purifiers, rashes, burns and other infectious diseases [3]. Medicinal plants are gaining importance in maintaining and promoting human health because of their therapeutic component as well as the higher growth of drug confrontation for available antibiotics. Medicinal plant's extracts are signifying an affluent resource for antimicrobial activity. Plants are producing secondary metabolites which are precursors for development of microbicides, pesticides and as well as many other pharmaceutical drugs [4]. Plant extracts are used as antimicrobial agents because they contain different types of biologically active compounds. Nearly 40% of the population from the developing countries is put to death because of the infection of microorganisms. In addition, it also spoils food materials [5]. To control plant disease synthetic fungicides have been used, however alternatives are being used due to the negative public perceptions regarding the use of synthetic chemicals as a disease control agent. The plant-derived products have been studied in view of the fact that they have a propensity of low mammalian toxicity as well as less environmental effects and broad community approval [6,7]. The plant extracts having bactericidal and bacteriostatic activity are being studied throughout the world [8]. In this study *Melilotus parviflora* a monocarpic, annual or biennial herb belonging to the legume family (Leguminosae) [9], has been evaluated for cytotoxicity, phytotoxicity and antimicrobial activity.

2. Experimental section

2.1. Plant Collection:

The *Melilotusparviflora* plant was collected from District Bannu and was identified by Prof. Abdur Rehman GPGC Bannu. The plant samples were dried under the shadow at a room temperature for 40 days and then grinded it into fine powder.

2.2. Plant Extraction:

To obtain methanolic extract fine powder of *Melilotusparviflora* has been soaked in sufficient amount of methanol followed by gentle shaking and placed at room temperature for 7 days. After treatment the solution has been filtered with Whatman filter paper and the filtrate was further subjected to evaporation using rotary evaporator to obtain crude extract.

2.3. Cytotoxic Bioassay Brine Shrimp lethality test;

Sample preparation:

In this assay, a stock solution of the methanolic extract was prepared by dissolving 5 mg/5 ml in methanol. From the stock solution a serial diluted solutions of concentrations 100 µg/ml, 500 µg/ml, 1000 µg/ml were prepared using the dilution formula i.e. $M_1V_1 = M_2V_2$. Saline solution was prepared by dissolving 2.8 gram of sea salt in 100 ml of distilled water with the help of magnetic stirring.

Experimental procedure:

In this bioassay, 500 µl of each diluted solutions (100 µg/ml, 500 µg/ml, 1000 µg/ml) were taken in labeled test tubes containing 5 ml of sea salt and eight larvae were transferred with the help of a dropper in each test tube. Control was prepared by transferring eight larvae into a labeled test tube containing only 5 ml of sea salts. The test tubes were packed tightly with cotton and incubated at 28 °C for 24 h. After incubation, live larvae were counted and calculated by using Abbott's formula.

$$\% \text{ death} = (\text{sample control}) \times 100$$

2.4. Phytotoxicity Bioassay;

A stock solution (5 mg/5 ml) of crude methanolic extract was prepared in methanol. From this stock solution three diluted solutions were prepared having concentration of 250 µg/ml, 500 µg/ml, 1000 µg/ml using dilution formula. Each diluted solution of methanolic extract (5 ml) was poured on filter paper placed in respective labeled petri dish. The petri dish containing methanolic extract impregnated filter paper was kept open to evaporate the methanol. Then 5 ml of D/water was added to all petri dishes. The petri dish without impregnation of methanolic extract was considered control for this activity. The seeds of maize were kept in D/water for one hour and from these, 5 seeds were placed on each filter paper placed in labeled petri dishes. The petri dishes were incubated for known period of time at 28 °C. The length of both roots and stems were measured after 3rd and 7th days for the germinated seeds. Similar procedure was used to evaluate the phytotoxicity of *Melilotusparviflora* on rice seed. All the experiments were done in duplicate.

2.5. Antibacterial activity:

The antibacterial activity of *Melilotusparviflora*'s methanolic extract was carried out by the protocols reported in the literature [10].

The antibacterial activity was tested against three strains of bacteria which were *Bacillus subtilis*, *Bacillus septica* and *Escherichia coli*. The bacterial strains were maintained on the nutrient agar media at 4 °C. The nutrient agar medium was prepared by suspending nutrient agar 2g in 100 ml of distilled water and was autoclaved. It was allowed to cool up to 45 °C under laminar flow. The media was seeded with the prepared inoculums to have 10⁶ CFU per ml. A sufficient amount of the inoculated nutrient agar media was poured in the labeled petri plates and allowed to solidify at room temperature. Commercial antibiotic and DMSO were used as positive and negative control. Five wells per plate were dug with cork borer (8mm) and labeled. Using the micropipette, 100 µl of each methanolic extract solution (1 mg/ml, 5 mg/ml, and 10 mg/ml) was poured in the respective wells and incubated at 37 °C for 24 h. The diameters of zone of inhibition around the labeled wells were measured.

2.6. Antifungal activity:

The antifungal activity of the plant extract was screened through the agar tube dilution method. The fungal strains i.e. *Asperillus niger*, *Asperillus flavus*, *Asperillus fumigates* were used in this bioassay.

The stock solution of crude methanolic extract and positive control was prepared by making concentration of 1 mg/mL of each methanolic extract and terbinafine (antifungal) in DMSO separately. The stock solution of both crude methanolic extract and positive control was further diluted with DMSO up to 200 µg/mL and 67 µL of these was further used for experiment. 67 µL of pure DMSO was taken from bottle

and was used as negative control. Sabouraud Dextrose Agar (Merck) media was used for the growth of three fungal species i.e. *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus*.

The media for fungal growth was prepared by dissolving 6.5 gm. of Sabouraud Dextrose Agar (Merck) in 100 mL of distilled water followed by autoclaved at 121 °C for 20 minutes. 4 mL of SDA media solution was poured in the labeled test tubes. Then 67 µL (200µg/mL) of crude methanolic extract, positive control and negative control was poured in the media solution contained in the test tubes labeled for crude methanolic extract, positive control and negative control respectively. After this, all the test tubes were placed in slanting position under laminar flow to solidify the medium at room temperature. After solidification, 10 spores of 7 days old culture of fungal strains were inoculated in the labeled test tubes of crude methanolic extract, positive control and negative control carefully. Two test tubes of each crude methanolic extract, positive control and negative control were specified for each strain of fungus in order to get the result in duplicate. After inoculation, the test tubes were sealed air tightly and incubated at 28 °C for 7 days. The fungal growth inhibition was measured for crude methanolic extract and positive control in respect of negative control and the % inhibition growth was calculated by using the following equation.

% inhibition growth = $(100 - \text{Linear growth in the test tube (mm)} / \text{linear growth in control}) \times 100$

3.Results and Discussions

3.1. Cytotoxic activity:

The cytotoxic effect of meliltois parvifloramethanolic extract was determined by using brine shrimps lethality test as shown in the Figure 1. It is clear that the cytotoxicity of methanolic extract increases with increase in their concentrations. The order of cytotoxicity for various concentrations were 1000 µg/ml > 500 µg/ml > 100 µg/ml. In case of control no such cytotoxic effect was observed. Similarly, other researchers also reported the use of plant's methanolic extract for cytotoxic effect [11]. It was reported that methanolic fraction of herbs showed 100 % cytotoxicity at its high dose. Brine shrimps lethal activity of meliltois parviflora justify its potentiality for cancer and tumor treatment.

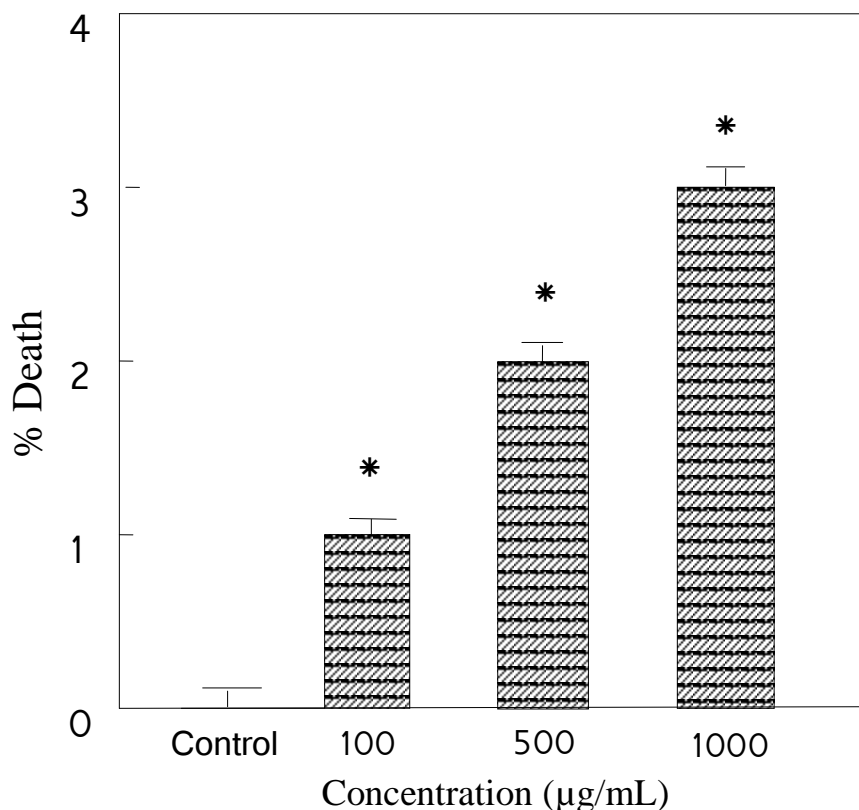


Figure 1 Cytotoxic effect of meliltois parviflora methanolic extract on brine shrimps cell.

3.2. Phytotoxic activity:

The Meliltois parviflora methanolic extract showed good herbicidal activities as shown in Figure 2&3. The phytotoxicity of Meliltois parviflora methanolic extract is in relationship with their concentrations. For

example the highest concentration (1000 $\mu\text{g/ml}$) of methanolic extract inhibited the growth of rice root up to 1.2mm and 3.2mm after 3rd & 7th day respectively. Similarly, the growth inhibition of rice stem was also observed which were 5mm and 7.5 mm after 3rd & 7th day respectively. The other lower concentrations also inhibit the growth of rice stems and roots. The results showed, justify the use of *Melilotus parviflora* as herbicidal agent. Similar findings of growth inhibition were reported as observed in other studies [12]. Fureported their findings which are in accordance to our obtained results. The growth inhibition property of *Melilotus parviflora* is due to the presence of bioactive polyphenolic compounds [13].

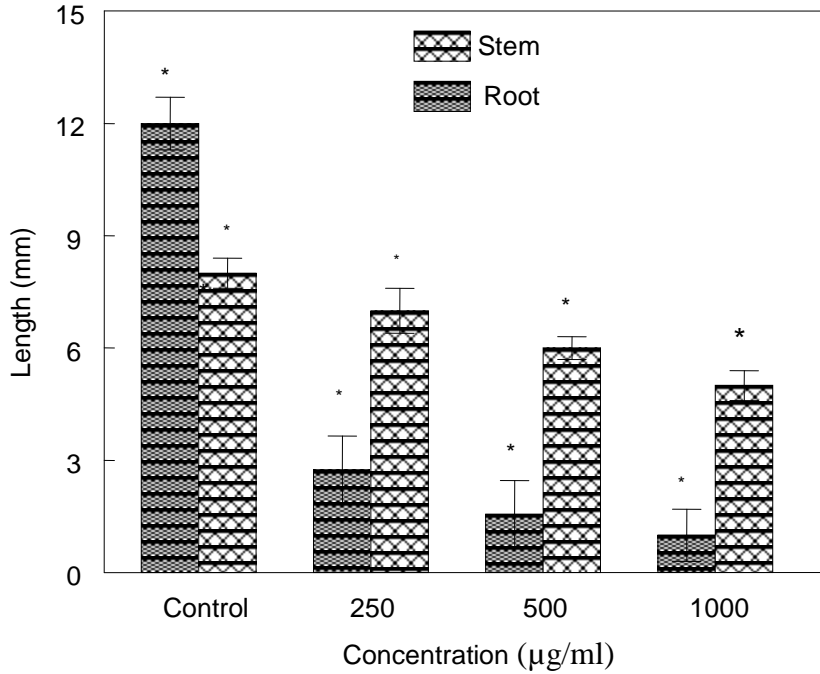


Figure 2. Inhibition activity of *Melilotus parviflora* methanolic extract for rice after three days

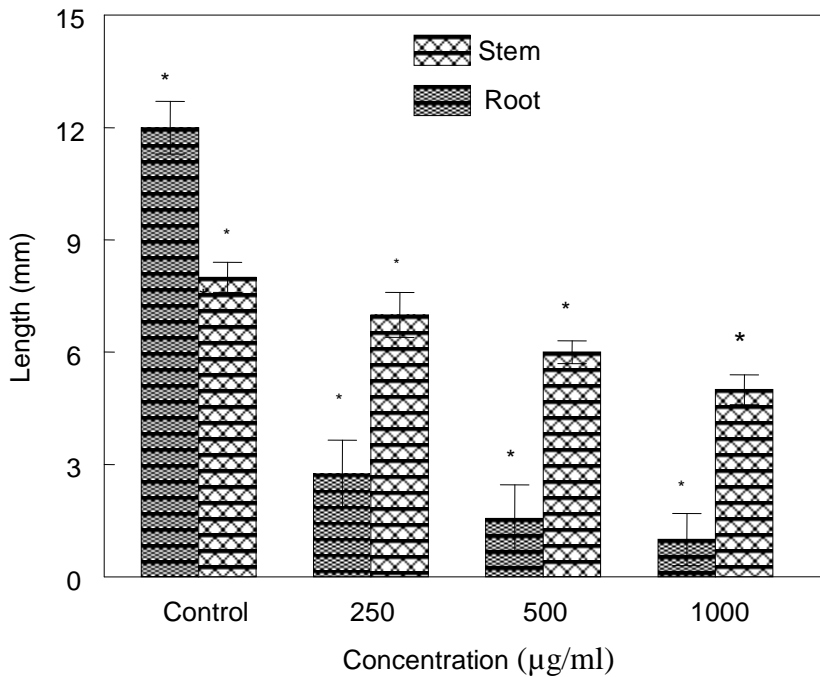


Figure 3. Inhibition activity of Melilotus parviflora methanolic extract for rice after seven days**4.5 Antibacterial assay:**

The results for antimicrobial activity of methanolic extract have been tabulated in table 1. The methanolic extract has the rational antimicrobial activity for the studied three species of bacteria i.e. Bacillus Septica, E.Coli, and Bacillus Subtilisin. The antimicrobial activity was noted to increase with increase in concentrations of Melilotus parvifloramethanolic extract. For example as the concentration of methanolic extract increases from 1mg/mL to 10 mg/mL the zone of inhibition for Bacillus Septica was measured to 10 to 18 mm respectively. In comparison to other species of bacteria, high antimicrobial activity was observed by Bacillus Septica bacterium. The commercial antibiotics (positive control) purchase from local market has more antibacterial activity as compare to methanolic extract of Melilotus parviflora. DMSO was used as negative control and has no such effect on the growth of studied species of bacteria. The result obtained from the present work closely related to the work of Aboaba et al [14], studied the antimicrobial potential of South African plant i.e. Alobarberae. The Dalia asiatica's leaf and stem have been reported for antibacterial activity against gram positive as well as gram negative bacteria [15] this has strongly supported our work. The results of our present study support the traditional usage of the studied plants for control of bacterial infectious diseases.

Table 1 Antibacterial activity of Melilotus parvifloramethanolic extract

Bacterial Name	DMSO	Antibiotic	Sample
Bacillus septica	0 mm	30 mm	1 mg/mL = 10 mm
			5 mg/mL = 16 mm
			10 mg/mL = 18 mm
E.Coli	0 mm	31 mm	1 mg/mL = 5 mm
			5 mg/mL = 10 mm
			10 mg/mL = 15 mm
BacillusSubtilus	0 mm	35 mm	1 mg/mL = 8 mm
			5 mg/mL = 11 mm
			10 mg/mL = 16 mm

4.6. Antifungal activity:

The result of the antifungal assay showed that the methanolic extract of Melilotus parviflora possessed the antifungal activity against Asperillus niger, Asperillus flavus and Asperillus fumigates as shown in table 2. The growth of Asperillus nigeris more stunted as compared to other species of fungi. The growth measured for Asperillus niger, Asperillus flavus and Asperillus fumigates are 1.5 cm, 5cm and 5.5 cm respectively. All the three species are observed to have no growth on terbinafine (positive control) added media while for negative control (DMSO added media) growth was observed. The antifungal activity of various plant extracts were correlated to the presence of phenolic and polyphenolic compounds like saponins, catechin and tannins etc [16-20].

Table 2 Antifungal activity of Melilotus parvifloramethanolic extract

S. No	A, Niger growth on DMSO	A. Niger growth on TB	A. Niger Growth on methanoic extract	A.Flavius growth on DMSO	A.Flavius growth on TB	A. Flavius growth on methanoic extract	A.Fumegatus growth on DMSO	A.Fumegatus growth on TB	Fumegatus growth on methanoic extract
Average growth	9 cm	0.0 cm	1.5cm	10 cm	0.0 cm	5 cm	9.5 cm	0.0 cm	5.5cm

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