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Determination of antifungal and phytotoxic activities of three different fractions of *Lonicera quinquelocularis* (Translucent Honeysuckle) plant.

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ABSTRACT

Fungi are the cause of many human and plants diseases and these are the main cause of reduction in crops yields. Synthetic antifungal drugs have several drawbacks which limited their scope and achievements. Antifungal agents from natural sources such as plants have proved to be most advantageous. The present study was designed to evaluate the antifungal and phototoxic activities of three different fractions i.e. ethanol, chloroform and ethyl acetate extracted from *Lonicera quinquelocularis*. The results were compared with terbinafine (positive control) and DMSO (negative control). The chloroform fraction extract of *Lonicera quinquelocularis* show relatively high activity 35.5% against *Aspergillus niger* and low activity 25% against *Aspergillus flavus*, Ethanolic fraction of *Lonicera quinquelocularis* showed 25% against *Aspergillus niger* and 50% against *Aspergillus flavus* and ethyl acetate crude extract of *Lonicera quinquelocularis* show 41.2% against *Aspergillus niger* and 22.5% against *Aspergillus flavus*. The result of phytotoxic revealed that the different fraction of *Lonicera quinquelocularis* significantly inhibited shoot growth of wheat compared to control after 3rd and 7th days. The data obtained from this experimental work revealed that all the three fractions isolated from *Lonicera quinquelocularis* possess antifungal and phytotoxic properties.

Keywords: *Lonicera quinquelocularis*, phytotoxicity, antifungal, chloroform

1. Introduction

There is urgent need to discover new antimicrobial compounds with diverse chemical structures and novel and new mechanisms of action, because there has been an upsetting increase in the incidence of new and re-emerging infectious diseases. Another big anxiety is the development of resistance to the antibiotics in current clinical use. Higher plants produce hundreds to thousands of diverse chemical compounds having different biological activities [1]. Antimicrobial compounds which are produced by plants are active against plant and human pathogenic microorganisms [2]. There are several reports in the literature concerning the antimicrobial activity of plant crude extracts, and the bioassay-guided fractionation of them to yield active principles [3]. The secondary metabolites of plant provide protection against infections and are a big source of plant survival [4]. These secondary metabolites behave like allelochemicals [5]. The plants' extracts can act as herbicides and the plants have great agricultural applications [6]. The natural mechanism in which living beings affect the growth and the reproduction of neighboring organism by the releasing of allelochemicals is known as allelopathy. Allelochemicals are the secondary metabolites it may have positive or harmful effects on the neighboring organism. Alleochemicals are very essential for defense against herbivory [7]. Allelochemicals are present in all parts of plants like leaves, stem, root and flower and it have broad spectrum inhibitory effects [8]. Synthetic herbicides may cause problem to human beings and to the environment. Therefore, it is necessary to explore non-toxic and environmental friendly compounds [9]. The continuous use of artificial herbicides not only damages the agriculture products, but also causes severe biological and environmental problems [10]. It is thus necessary to study and describe the phytotoxic activity of plants and their compounds. Bioactive terpenoids play an important role in the defense system of many organisms and have efficient use in agriculture and pharmaceutical fields [11].

and a very large number of extremely phytotoxic compound (allelochemicals) are the derivative of terpenoid [12].

2. Materials and Methods

2.1 Extraction procedure and preparation of crude extract.

Plant of *Lonicera quinquelocularis* was collected from the main Mandan area, Bannu, Khyber Pakhtunkhwa, Pakistan in the month of July 2010. The plant was identified by Taxonomist Prof: HASHAM KHAN: Department of Botany, Government Post Graduate College, Bannu. The plant materials were washed by the distilled water and were shade dried at room temperature for two weeks, chopped and grinded mechanically of mesh size 1 mm. 3 kg powder of *Lonicera quinquelocularis* was extracted in 3 liter of 70% methanol by random shaking. After a week, the extract was filtered by using Whatman filter paper No.1. After filtration, the filtrate was further concentrated by using rotary vacuum evaporator at 38 ^{0}C in order to get the methanolic crude extract of the plant. The combined ethanolic extract was evaporated under reduced pressure to obtain a thick greenish gummy material. The methanolic extract was further partitioned with chloroform (20 g), ethyl acetate (13 g) respectively. The methanolic crude extract was stored at 4 ^{0}C in the refrigerator for further phytochemical studies and in vitro investigation.

2.2 Phytotoxic Assay procedure

For performance of this experiment, first of all, filter paper was set in the autoclaved Petri plates. The experiment was done in duplicate. 5 ml from 100 µg/ml solution was poured by micropipette on the filter paper of each petri plates very carefully. The same process was repeated for 1000 µg/ml Petri plates already labeled. But for the control, the petri plates were not treated by the sample solution. All of the treated Petri plates were placed in the oven at 40 ^{0}C for complete evaporation of methanol from the filter papers. Then 5ml of distilled water were poured in all the treated Petri plates and also in the three control Petri plates. Then 8 wheat seeds, first seed were washed by distilled water and then they were placed in equal distance by scientific way. All the Petri plates were incubated in growth room for 3 days. After 3 days, shoot and root inhibition was noted by ruler with respect to control and was averaged and again after 7 days the inhibition was noted by same way and took average mean. Similarly the fresh and dry weight of all the treated and control fractions were recorded by using electronic digital balance and was averaged. Then the difference between the fresh and dried of the treated fractions with respect to the control was calculated.

2.3 Antifungal assay

To check the antifungal activity of cruds extracts derived from *Lonicera quinquelocularis.* Protocol of Duraipandiyan and ignacimuthe (2009) was used for this assay.

2.3.1 Requirements

Crude extracts derived from *Lonicera quinquelocularis*, DMSO, terbinafine, distilled water, micropipette, autoclaved tips, flasks, beakers, ruler, test tubes, incubator and autoclave, SDA, electronic digital balance, laminar flow and microorganisms were used.

Aspergillus niger (0198*)*
Aspergillus flavus (0064)

2.3.2 Preparation of samples

Stock solutions for each fraction of *Lonicera quinquelocularis* were prepared by dissolving 5mg extract of the respective fraction in 5ml DMSO. From theses stock solutions, further 1ml solutions of 200µg/ml were prepared for each of the three fractions in DMSO.

Similarly, the stock solution, of 5µg/ml of turbinafine (positive control/antifungal agent) was prepared in the DMSO. From this stock solution, further solution of required concentration (200µg/ml) was prepared in DMSO. Similarly; 1ml DMSO was taken and used as a negative control.

2.3.3 Media for antifungal assay

Sabouraud dextrose agar (SDA) contains peptones. It was created by Raymond Sabouraud, and then named after; Raymond Sabouraud in 1892. It is widely used to cultivate dermatophytes and other types of fungi. Later this was adjusted by Emmons, its pH level was adjusted such that it was brought closer to the neutral range to support the growth of other subcultures of fungi. SDA (MERCK) was used to grow fungus for inoculums preparation.

2.4. Assay procedure

For the preparation of media for fungus growth, 3.2 g of SDA was dissolved in 50 ml distilled water in flask and was autoclaved at 121 ^{0}C for 20 min. 4 ml media solution was poured in all the autoclaved test tubes marked up to 10 cm in the Laminar flow for fungal strains then 100 µl extract Solution from the required concentration (200 µg/ml) was put in all the 8 test tubes, 100 micro liter of terbinafine solution that is positive control of the required concentration (200 µg/ml) was put in all the four test tubes (2 for each) of 2 fungal strains. After this, all the test tubes were adjusted in the slanting position in the Laminar flow in order to solidify media in the test tubes at room temperature. After solidification, 10 to 12 spores from 7 day old culture of each fungus strain were placed in the incubator at 28 ^{0}C for 7 days. After 7 days measured their growth and calculated in percentage inhibition with the reference to negative control by using the formula

Percentage (%) inhibition growth= (dc-dt) ×100

Where c is used for negative control growth and 't' is used for sample growth in test tube.

3. Results

1 kg powder of *Lonicera quinquelocularis* was dissolved in ethanol and after the filtration and drying, ethanolic extract was obtained. This extract was further fractionated with chloroform, and ethyl acetate. The three different fractions i.e. ethanolic, chloroform and ethyl acetate of the plant *Lonicera quinquelocularis* were then used for phytotoxic and antifungal activities. The results showed that all the three fractions of the plant of *Lonicera quinquelocularis* have significant antifungal abilities. 67 µl (200 µg/ml) of crude extract of *Lonicera quinquelocularis*, 67 µL DMSO (99.9%) and terbinafine 67 µl, were used for screening of antifungal activity. The chloroform crude fraction of *Lonicera quinquelocularis* show relatively high activity 35.5% against *Aspergillus niger* and low activity that is 25% against *Aspergillus flavus* (Table 1), Ethanolic crude fraction of *Lonicera quinquelocularis* showed 25% against *Aspergillus niger* and 50% against *Aspergillus flavus* (Table 2) and Ethyl acetate crude extract of *Lonicera*

quinquelocularis show 41.2% against *Aspergillus niger* and 22.5% against *Aspergillus flavus* (Table 3). The terbinafine, a positive control indicated 100% against *Aspergillus flavus* and *Aspergillus niger*. Similarly, DMSO, A negative control indicated 0% against the *Aspergillus niger* and *Aspergillus flavus.*

The results for phytotoxic assay of plant extract were recorded against wheat seeds growth under controlled conditions. The current study was performed to investigate the allelopathic potential of the extract. The phytotoxic study was performed in plate study. Two different concentrations (100 µg/ml and 1000 µg/ml) are used for the phytotoxic efficacy of three different fractions i.e. ethanolic, chloroform and ethyl acetate of the plant *Lonicera quinquelocularis.* The result showed that the three different fractions i.e. ethanolic, chloroform and ethyl acetate of *Lonicera quinquelocularis* significantly inhibited shoot growth of wheat compared to control after 3rd and 7th days (fig 1-3) and (fig 4-6). After complete treatment, fresh and dry weight of all replicates are calculated which exposed that extensively controlled the Fresh as well as dry weight

Table.1: Antifungal activities of terbinafine, DMSO and of chloroform extract of *Lonicera quinquelocularis* against Aspergillus flavus and Aspergillus niger fungus strains. Data is the mean value of three different experiments performed in duplicates.

Fungus Specy	Chloroform fraction of *Lonicera quinquelocularis*	DMSO	Terbinafine	% Inhibition of sample
Aspergillus flavus	6 cm	8 cm	0	25
Aspergillus niger	5 cm	8 cm	0	37.5

Table.2: Antifungal activities of terbinafine, DMSO and of ethanolic fraction of *Lonicera quinquelocularis* against *Aspergillus flavus* and *Aspergillus niger* fungus strains. Data is the mean value of three different experiments performed in duplicates

FS	Ethanolic fraction of *Lonicera quinquelocularis*	DMSO	TERBINAFINE	% Inhibition of sample
Aspergillus flavus	6 cm	8 cm	0	25
Aspergillus niger	4 cm	8 cm	0	50

Table.3: Antifungal activities of terbinafine, DMSO and ethyl acetate fraction of *Lonicera quinquelocularis* against *Aspergillus flavus* and *Aspergillus niger* fungus strains. Data is the mean value of three different experiments performed in duplicates

Fungus Speciey	Ethyl acetate fraction of *Lonicera quinquelocularis*	DMSO	Terbinafine	% Inhibition of sample
Aspergillus flavus	6.2 cm	8cm	0	22.5
Aspergillus niger	4.7cm	8 cm	0	41.2

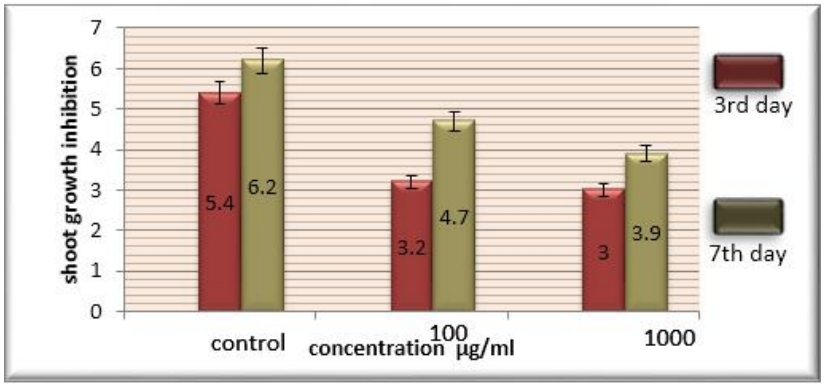

Fig 1: Phytotoxic effects of chloroform fraction of *Lonicera quinquelocularis* on the growth of wheat shoot. Results represents mean values of three different experiments run in duplicates.

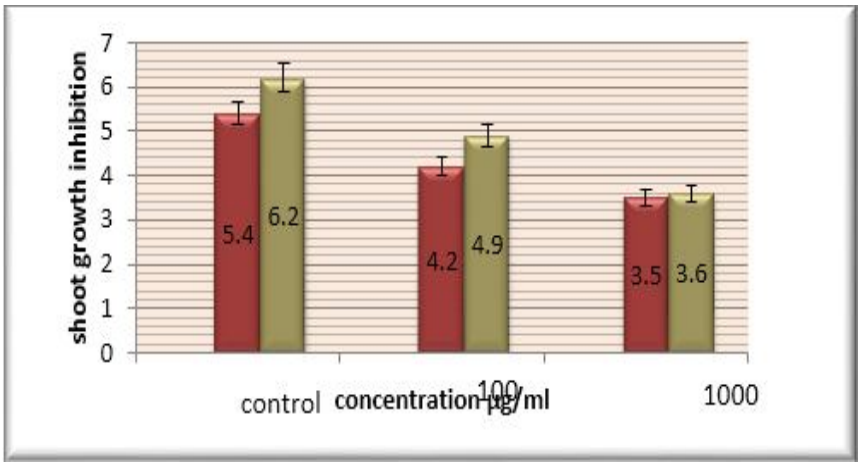

Fig 2: Phytotoxic effects of ethyle acetate fraction of *Lonicera quinquelocularis* on the growth of wheat shoots. Results represents mean values of three different experiments run in duplicates.

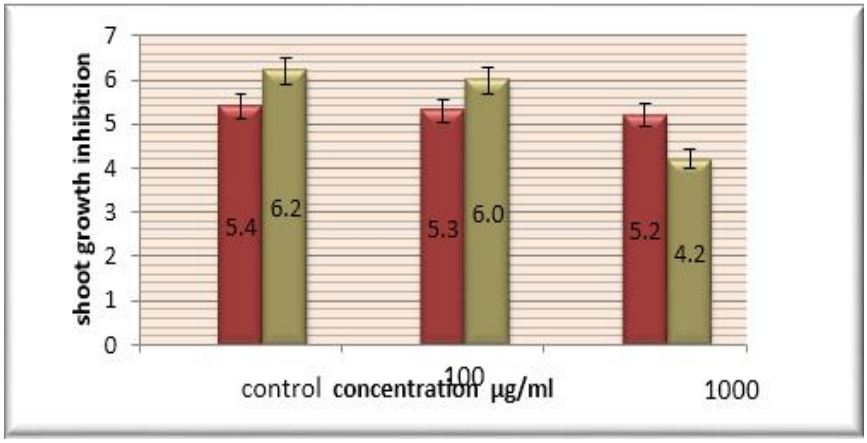

Fig 3: Phytotoxic effects of ethanolic fraction of *Lonicera quinquelocularis* on growth of wheat shoots. Results represents mean values of three different experiments run in duplicates.

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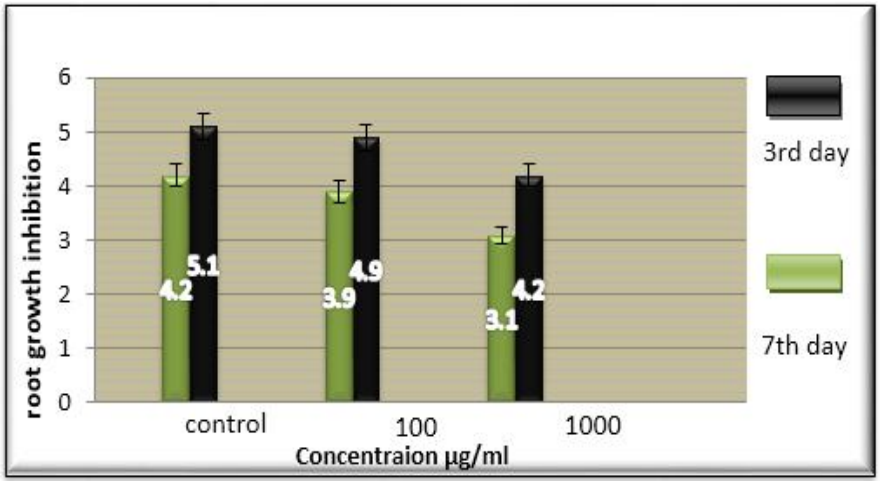

Fig 4: Phytotoxic effects of chloroform fraction of *Lonicera quinquelocularis* on the growth of wheat roots. Results represents mean values of three different experiments run in duplicates.

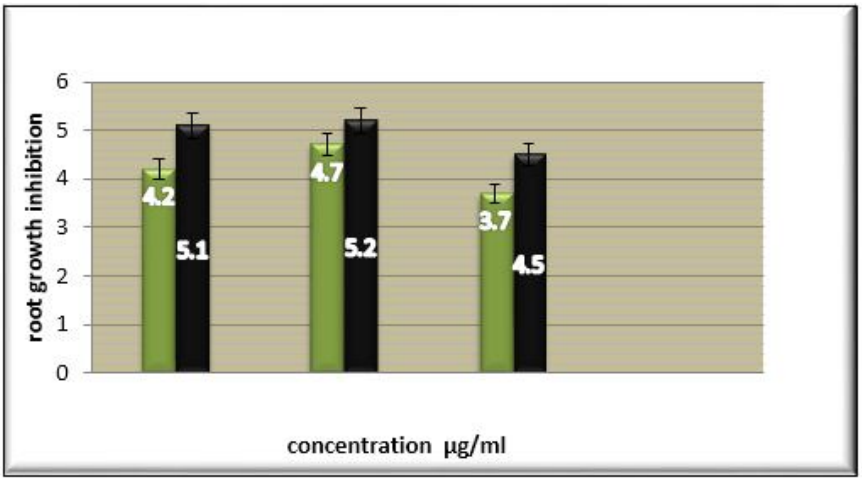

Fig 5: Phytotoxic effects of ethyl acetate fraction of *Lonicera quinquelocularis* on the growth of wheat roots. Results represents mean values of three different experiments run in duplicates.

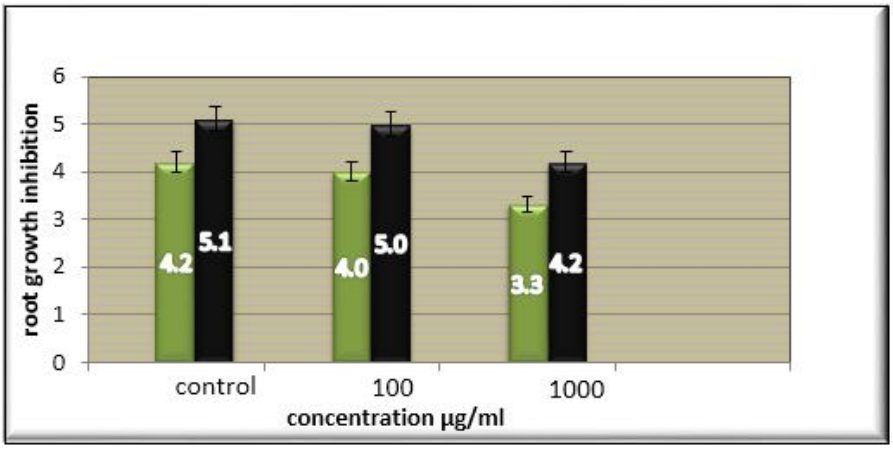

Fig 6: Phytotoxic effects of ethanolic fraction of *Lonicera quinquelocularis* on the growth of wheat roots. Results represents mean values of three different experiments run in duplicates.

4. Discussion

Medicinal plants had showed a rich source of antimicrobial agents [13]. Many of the plant materials used in traditional medicine are easily available in rural areas at comparatively cheaper than modern medicine [14]. Generally plants produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products are the principal source of pharmaceutical agents used in traditional medicine [15]. In the present study, *Lonicera quinquelocularis* plant showed antifungal property. The three different fractions i.e. ethanolic, chloroform and ethyl acetate of *Lonicera quinquelocularis* showed good antifungal property (Table 1-3). The ethanolic crude extract has showed high antifungal property than chloroformic and ethyl acetate fraction of *Lonicera quinquelocularis* (Table 1-3). Our results show similarity with the finding of Mann *et al*. (2008) and Satish *et al.* (2008) [13, 14].

The ethanolic, ethyl acetate and chloroform fractions of *Lonicera quinquelocularis* plant were subjected tophytotoxic bio assay. From the results it was concluded that all fraction had phytotoxic effects (Figure 1-3). It may due to the various compounds present in each fraction that caused phytotoxicity. Duke *et al* (2004) result has similarity with our result.

5. Conclusion

The present findings have clearly demonstrated that the plant has excellent potential towards the phytotoxic activity. The growth inhibition of the plant against Aspergillus *flavus* and *Aspergillus niger* fungus strains identified that the plant can be used as antifungal agent for the treatment of different fungal diseases. So the further research and studies are needed to purify the active constituents of the plants that are responsible for its phytotoxic and antifungal activity to get a valuable herbicidal and antifungal agent.

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