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Phytotoxic, antioxidant and antifungal activity of crude methanolic extract of *Equisetum debile*

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Abstract

Natural Products, especially plants, have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient time and an impressive number of modern drugs have been developed from them. They are good and cheap sources of many useful bioactive chemical compounds which can be utilized as anitimirobial , anticancer and anti oxidant agents. In case of *Equisetum debile* , it have been used for the treatment of different ailments like infections, rheumatism and inflammation etc. The present study indicate that *Equisetum debile* methanolic extract (100 & 1000 µg/ml) significantly inhibit the growth of shoots (hypocotyls) and roots (radicals) of rice when compared to control after three and seven days treatment. Furthermore, the *Equisetum debile* methanolic extract (50 to 1000 µg/ml) exhibit antioxidant properties and scavenge the free radical in dose dependent manner when compared with standard antioxidant (ascorbic acid). *Equisetum debile* methanolic extract also have antifungal property that inhibit 42.26% and 53.84% growth of *Aspergillus flavous* and *Aspergillus niger* respectively, while using the extract 200 µg/ml.

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Introduction

Plants always have great importance in many cultures. Human beings are user of plants for their basic requirements like feeding, clothing, sheltering, hunting and nursing. As source of medicines, plants have formed the basis for sophisticated traditional systems and continue providing mankind with new remedies. In recent years, the interest in folk medicine has highly increased. This discipline is gaining the scientific basis for its appropriate application within official medicine.

In 1937 Pro: Hans Molisch defined the term "Allelopathy". as the effects of one plant on another by releasing chemicals to the environment. "Allelopathy" is actually the expression of harmful, stimulatory enhanced and useful effects of one plant on the other plant and specific chemicals are formatted and released into the environment. This property of plants is very important in biological control of weeds. Many medicinal plants and their purified ingredients have revealed as useful therapeutic potentials. Kokate *et al.,* (2004) defined antioxidants as they such type of compounds which prevent or decelerate the oxidation process. Some of the best examples of free redicals which cause oxidation are superoxide anion (O), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO) and reactive radicals (OH). Some of the nitrogen derived free radicals or substances include nitric oxide (NO), peroxy nitrite anion (ONOO), nitrogen dioxide (NO_2) and dinitrogen trioxide (N_2O_3). These free redicals circulate inside the body and tend to react with biomolecules such as DNA, amino acids etc which are present inside the body and leads to the abnormal conditions like cancer, ischemia, aging, adult respiratory distress syndromes and rheumatoid arthritis etc (Kokate *et al.,* 2004). Antioxidants have been identified in numerous agricultural and food products like cereals, vegetables, oil seeds and fruits (Adom *et al*, 2003; Naczk and Shahidi, 2006; Netzel *et al*, 2007).

The knowledge of herbal remedies, developed through trial and error over the centuries, is being used as guide to lead the chemists towards different classes of compounds. It is a fact that the 25% of all medical prescriptions are based on substances derived from plants or plant-derived synthetic analogues (Sara *et al*., 2009). Indigenous medicine is now recognized world wide as a healthcare resource. The World Health Organization (WHO) has pointed out that traditional medicine is an important contributor toward health goals. Scientists are interested in investigating medicinal plants which are commonly used by public and derived from folklore or anecdotal information (Helton, 1996; Mail *et al*., 1989). Dubick 1986 reported that the medical use of herbs is deeply rooted in human history and folklore, and incorporated into the historical medicine of virtually all human cultures. He describe the history of Gineseng and Garlic as two famous plants widely used –till now- in traditional medicine and proved to have many active constituents (Dubick, 1986).

Materials and methods

Plant collection

Equisetum debile plant was collected during summer, in the month of July 2011, from Kakki District Bannu Khyber Pakhtunkhwa Pakistan. It was identified by Professor Abdur-Rehman, Chairman Department of Botany, Government Post Graduate College Bannu. The materials (Leaves) of the plant were properly washed by means of distilled water and shaded dried at room temperature for about two weeks, chopped and mechanically grinded about of mesh size 1 mm.

Preparation of plant extract

100g powder of *Equisetum debaile* was dissolved in 300 ml of 70% diluted methanol. After few days, the extract was filtered with the help of Whatman filter paper No. 1. The filtrate was further concentrated after the filtration by using rotary vacuum evaporator at 42 C^0 for the purpose to obtain methanolic crude extract of the plant. After getting the methanolic crude extract, it was stored at 4 ^{0}C in refrigerator for further phytochemical studies and in vitro investigation.

Phytotoxicity bioassay

This experiment was conducted according to the modified protocol of McLaughlin (1988). First of all filter papers were set up in the autoclaved Petri plates for this experiment. The experiment was conducted in duplicate Petri plates and sprayed each filter paper in the Petri plate with the help of micro pipette very carefully with 100µg/ml and 1000µg/ml solution and the Perti plates were labeled. But in case of the control Petri plate, it was not treated with the sample solution. After that all the treated Petri plates were kept at 40 ºC in the oven in order to evaporate methanol completely from the filter papers (as methanol is toxic). After the completion of evaporation process, 5 ml distilled water was poured in each one of all the treated Petri plates as well as to the control too. 8 rice seeds were first washed with distilled water and then were placed in each Petri plate at equal distance according to the scientific method. After the completion of this process, all the Petri plates were kept in incubator in growth room for three days. After passing 3 days, hypocotyls/shoot and radical/root inhibition was measured with the help of ruler with respect to the control one and was averaged. Again after 7 days, the length of hypocotyls/shoot and radical/root was measure and the average mean was taken.

Antioxidant assay

DPPH radical scavenging activity

The DPPH (1, 2-dyphenyl-2-picrylhydrazyl) assay was conducted according to the procedure of Gyamfi *et al.,* (1999) with some modification.

The experiment was conducted in duplicate test tubes. 100 µl of the plant extract each one with the concentration of (50, 100, 150, 200, 250 and 1000 µg/ml) was taken in separate test tubes and to these test tubes, 900 µl DPPH solution was added to each of the concentration. The same process was conducted with ascorbic acid concentration which was used as reference. 1 ml (1000 µl) was directly taken from the DPPH solution in a separate test tube.

All these treated test tubes were labeled separately as extract, ascorbic acid and DPPH and were shaken well and kept in incubator in dark for about 30 minutes at 25 ºC. By means of spectrophotometer, the absorbance was taken and calculated as 320 nm.

Antifungal assay

In order to check the antifungal activity of methanolic crude extract of *kalanchoe pinnata,* the protocol of Duraipandiyan and Ignacimuthu (2009) was followed.

6.5 g SDA media was dissolved in 100 ml distilled/ autoclaved water in flask for fungus growth and autoclaved at 121 ºC for 15 minutes. 4 ml of this media was poured in the all the autoclaved test tubes and marked up to 10 cm in the Laminar flow cabinet for two fungal strains. From the required concentration (200 µg/ ml) of the solution, 67 µl of extract solution was put in all the 4 test tubes, which were specified and duplicate for the two fungal strains by micro pipette.

In the same way, the terbinofine solution of 67µl (for positive control) of the required concentration (200 µg/ ml) was put in all the two test tubes (one for each) of the two fungal strains. Likewise, 67 µl DMSO (negative control) was poured in another set of two test tubes (one for each) of the two fungal strains. After the completion of this whole process, all the test tubes were place in the laminar flow in slanting position for solidifying the media in the test tubes at room temperature. After the solidification process, 15 spores from 7 days old culture were placed of each fungus strain in all the test tubes (Extract + Control) and were specified very carefully for each strain. All the test tubes were packed air tightly and were placed in incubator at 36 C^0 for 7 days.

Results

Phytotoxic activity of Equisetum debile

Petri plate study

Two different concentrations (100 µg/ml and 1000 µg/ml) were used for the phytotoxic efficacy of *Equisetum debile* methanolic extract (EDME). The result revealed that crud extract significantly inhibited the shoot (hypocotyls) growth of rice compare to control after three and seven days (Fig 4.1

& 4.2). The data also indicated that EDME are significantly inhibited roots growth as compared to non treated water (control) group as shown in Fig 4.3 & 4.4

In vitro Bioassays

DPPH free radical scavenging assay

DPPH (1, 1-diphenyl 1-2-picryl-hydrazyl) a free radical has the ability to oxidized a range of compounds, take electron from it. Therefore, it is widely used for estimation of *in vitro* antioxidant scavenging activities of medicinal plants. The Fig.4.1 shows the % scavenging activity of *Equisetum debile* methanolic extract (PAME) for free radicals of DPPH.

Table 1. Stain used.

Fungal strains	*Aspergillus flavus*	*Aspergillus niger*
DMSO (67 µl)	0	0
Terbinafine 67 µl	63.91	80
Extract 67 µl	42.26	53.84

In this study we used various concentration of *Equisetum debile* methanolic extract Significant scavenging activity was observed by various concentration of *Equisetum debile* methanolic extract with increasing concentration (50 µg/ml < 100 µg/ml < 150 µg/ml < 200 µg/ml < 250 µg/ml < 1000 µg/ml). Ascorbic acid was used as a reference compound and Similar result was presented by various concentration of Ascorbic acid (50 µg/ml < 100 µg/ml < 150 µg/ml < 200 µg/ml < 250 µg/ml and 1000µg/ml).

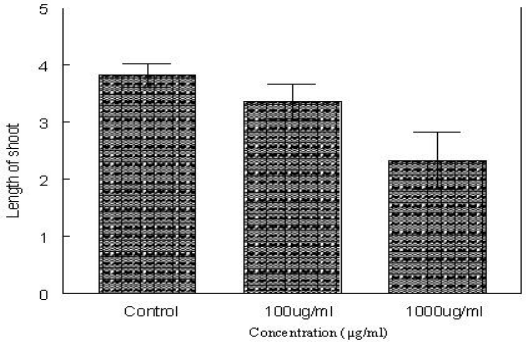

Fig. 1. Shoot growth of rice in the presence and absence of different concentration of *Equisetum debile* after 3rd day treatment.

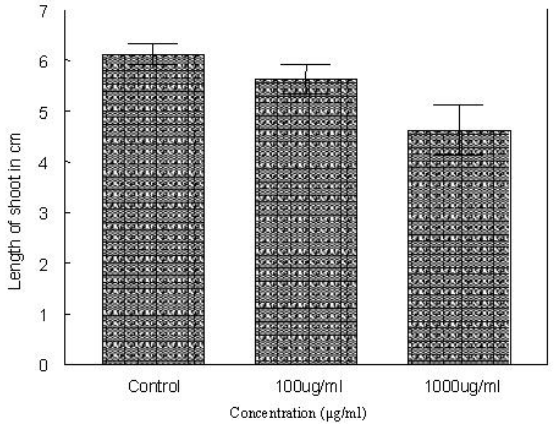

Fig. 2. Shoot growth of rice in the presence and absence of different concentration of *Equisetum debile* after 7rd day treatment.

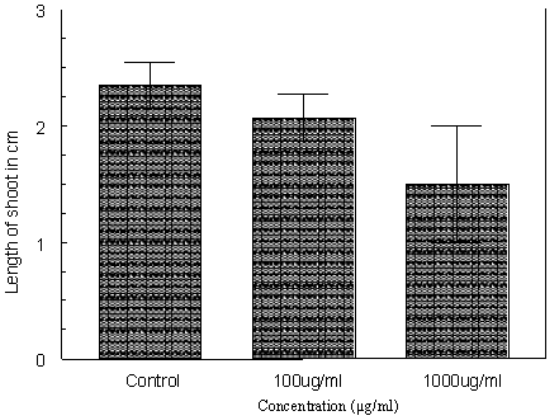

Fig. 3. Root growth of rice in the presence and absence of different concentration of *Equisetum debile* after 3rd day treatment.

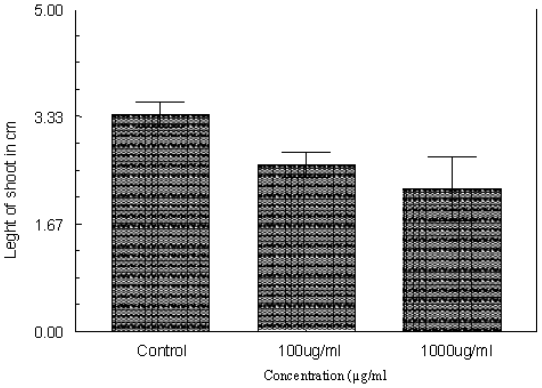

Fig. 4. Root growth of rice in the presence and absence of different concentration of *Equisetum debile* after 7rd day treatment.

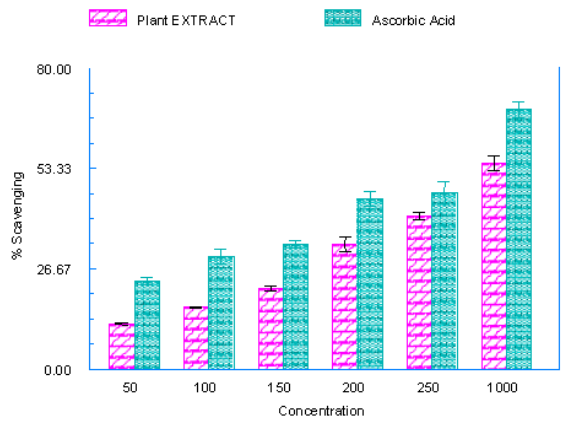

Fig. 5. DPPH free radical scavenging of *Equisetum debile* methanolic extract (EDME) and ascorbic acid

Antifungal activity of Equisetum debile methanolic extract (EDME)

Antifungal activity in term of % Inhibition

67 µl (200 µg/ml) of *Equisetum debile* methanolic extracts (EDME), 67 µl DMSO (99.9%) and terbinafine 67 µl (200 µg/ml) were used for screening of antibacterial activity. The *Equisetum debile* methanolic extracts (EDME) indicated low activity (11.11%) against, *Aspergillus niger,* while highest activity was shown against *Aspergillus flavus* (89.58%), The terbinafine, a positive control indicated low activity (87.75%) against *Aspergillus flavus* however comparatively high activity was found against *Aspergillus niger*. Similarly, DMSO, a negative/normal control indicated zero percent (0%) activity/inhibition against all the two used fungal strains.

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