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Antifungal activity in the methanolic, aqueous and hexane extracts of *Calligonum polygonoides*

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Abstract

Calligonum polygonoides is locally called as Phog which belongs to the Polygonaceae family. It is traditionally used as an antifungal. The methanolic extract, hexane extract, ethyl acetate extract and aqueous extract were screened against Candida albicans and Aspergillus niger in seven concentrations, that is, 1.8, 2.9, 6.5, 12.6, 25, 50 and 75 µg/mL/disc. Calligonum polygonoides showed significant activity against Candida albicans as the observed minimum inhibitory concentration (MIC) is 6.5 µg/mL for methanolic extract, 9.8 µg/mL for ethyl acetate extract, whereas aqueous and hexane extracts showed no activity. Calligonum polygonoides did not show any significant activity against Aspergillus niger.

Keywords

antifungal activity, *Aspergillus niger*, *Calligonum polygonoides*

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Introduction

Calligonum polygonoides is locally called as Phog which belongs to the Polygonaceae family.[1] Essential oil is extracted from buds and roots of Calligonum polygonoides. In total, 27 compounds were analysed qualitatively and quantitatively, accounting for 68.42% and 82.12% total contents of the essential oils of buds and roots, respectively. It contains a complex mixture of terpenoids, hydrocarbons, phenolic compounds, acid derivatives and ketones. The main components of the essential oil were ethyl homovanillate (11.79%) in buds and drimenol (29.42%) in roots. Calligonolides A(1) and B(2) are two new butenolides and a new steroidal ester has been isolated from the whole plant of Calligonum polygonoides, together with four known compounds: tetracosane-4-olide, sitosterol and its glucoside, and ursolic acid.[2] Cancer is a life-threatening disease, which is associated with a number of potentially lethal pathogens including bacteria and fungi. In a study, the increased risk of paediatric cancer was compared with biofilm formation by pathogenic bacteria and Candida albicans. The cancer patients are more prone to other infections (particularly fungal infections) due to their decreased immunity and the excessive use of anticancer treatments. So, cancer patients are on greater risks not only due to cancer but also due to the associated diseases.[3]

Materials and methods

General experiment

The commercial grade solvents/chemicals (i.e. methanol, ethyl acetate and petroleum ether) were used for the soaking of plants and further fractionation. For the antifungal screening on solid

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media, Yeast Peptone Dextrose Agar (YPDA) media was used. The analytical balance is of Ohaus Company with accuracy up to 0.1 mg. Rotary evaporator, heating bath, recirculating chiller and vacuum pump are of Buchi Company. Laminar flow cabinet was of Camsco micro-biosafety cabinet. Incubator was of Memmert. Hotplate was of WiseStir MSH-2A. The electric autoclave was of No. 25X, made in the United States. The microscope used was of No. 890032, made in China. Wisd Laboratory Instruments and the pipette used are of Labnet BioPette Plus. The glassware used was made of Pyrex.

Collection of plant material

Calligonum polygonoides, commonly called as Phog, belongs to the family Polygonaceae. The whole plant with roots was collected from Baghdad-ul-Jadeed and identified by our taxonomist Mr M. Warris. The fresh weight of the plant was 15 kg. The root of Calligonum polygonoides was chopped and was shade dried for 25 days to get 9.900 kg of dried plant material (66% water content). The root of Calligonum polygonoides was powdered and soaked in 11 L methanol for 1 week and then it was filtered. The process was repeated twice and total solvent used was 32 L. The solvent was evaporated off under high vacuum on a rotary evaporator. The dried methanolic extract (63.28 g) was used for further fractionation. Dried methanolic extracts of 15 g were dissolved in 150 mL of distilled water. The extraction with petroleum ether (hexane) did not give hexane layer. Successive extraction of aqueous extracts was also done with ethyl acetate layer (450 mL × 3) to get ethyl acetate extracts of 6.91 g. The methanolic extract, hexane extract, ethyl acetate extract and aqueous extract were screened against Candida albicans and Aspergillus niger in seven concentrations, that is, 1.8, 2.9, 6.5, 12.6, 25, 50 and 75 μg/mL/disc.

Antifungal assay

Media preparation

For the antifungal screening on solid media, YPDA media was prepared. Take 10 g of yeast extract and 20 g of peptone, agar and glucose. These were dissolved in the 1-L distilled water and autoclaved. After autoclave media was poured into autoclaved Petri plates, it was allowed to solidify at room temperature, and then streaked with Candida albicans and Aspergillus niger. The streaking loop was then sterilized by making it red hot on the flame, and this is done to avoid contamination of other cultures. Disc diffusion method was used by Ahmadi et al.[4] A separate dose was added to each disc to check their antifungal activities. After this, the culture was incubated at 28°C that facilitates the yeast growth in Candida albicans.

Culture preparation

In order to prepare the culture, fresh YPDA media was prepared and then inoculated with Candida albicans and Aspergillus niger. A small amount of culture was transferred to 2–3 mL of distilled water or normal saline in a screw-capped tube with few glass beads (1 mm in diameter) and vortexed for 5–10 min to make a homogenous suspension of fungal culture. The culture was incubated at 28°C and 37°C with constant shaking for overnight culturing to favour the yeast and hyphal growth of Candida albicans, respectively. The cultures of Candida albicans and Aspergillus niger were taken from the pathology lab of Quaid-e-Azam Medical College, Bahawalpur. The voucher number of Candida albicans and Aspergillus niger was 2915 and 2, respectively.

Antifungal screening

In this system, we added the Candida albicans and Aspergillus niger in the YPDA media (1 L)-prepared Petri dishes. Sterile filter discs were placed at the centre of the Petri dish and doses of different concentrations were added on the disc (1.8, 2.9, 6.5, 12.6, 25, 50 and 75 μg/mL). The Petri dishes were placed in the incubator for 48 h at 28°C. Results were recorded by measuring the zone of inhibition in mm.

Determination of minimum inhibitory concentration

Different extracts showing a zone of inhibition of 15 mm or more were considered significant and selected for the determination of their minimum inhibitory concentration (MIC) by the disc diffusion method.[5]

Antifungal activity of Calligonum polygonoides *Linn*

The results showing the in-vitro antifungal activity of methanolic, aqueous, hexane and ethyl acetate root extracts of Calligonum polygonoides are used for the antifungal activity. The Calligonum polygonoides shows significant results against Candida albicans. This data show that Calligonum polygonoides has some phytochemicals that have activity against fungus, especially against Candida albicans. The MIC for methanolic root extracts of Calligonum polygonoides was found to be 6.5 µg/mL, and with the increase in concentration, there was an increase in inhibitory effect that was measured in terms of zone of inhibition (Table 1).

The ethyl acetate extract of *Calligonum polygonoides* gives significant results against *Candida albicans*. The MIC 9.8 µg/mL of *Calligonum polygonoides* with the increase in ethyl acetate extract of *Calligonum polygonoides* shows an increase in inhibitory value (see Table 1).

At all the concentration of methanolic and ethyl acetate extracts of *Calligonum polygonoides* was effective only on the *Candida albicans*. It was also observed that the hexane and aqueous extracts did not give any results against *Candida albicans* (Table 1). At different doses of methanolic, aqueous, hexane and ethyl acetate extracts of *Calligonum polygonoides* was used it does not give any inhibitory concentration against *Aspergillus niger*.

These results were also compared with the standard drug (Fluconazole). The root extract of *Calligonum polygonoides* shows significant results against *Candida albicans*. The microscopic examination was done by preparing the slides of each concentration followed by microscopic examination under 40X of positive control. *Candida albicans* cells are diploid, but after drug treatment, the cells are dead and when observed under microscope, the cells are deshaped (Figure 1(a) and (b)).

Discussion

The *Calligonum polygonoides*, locally called as Phog, belongs to the Polygonaceae family. The plant with root was collected from the desert area of Baghdad-ul-Jadeed campus. The plant was identified by the taxonomist Mr M. Warris. The total fresh weight of the *Calligonum polygonoides* root was 15 kg. The root was chopped and shade dried for 25 days to get 9.900 kg of dried plant material with 66% water content. The methanolic, ethyl acetate and aqueous root extracts of *Calligonum polygonoides* tested against the two fungi, that is, *Candida albicans* and *Aspergillus niger*, showed a dose-dependent antifungal activity. The higher significant differences were obtained in the activity of root extracts at higher doses when compared to Fluconazole on *Candida albicans*. However, on *Aspergillus niger*, the root extracts of *Calligonum polygonoides* did not exhibit antifungal activity. The susceptibility of the plant extract could be attributed to the presence of some phytochemicals for the *Candida albicans*. The effect of methanolic extracts of *Calligonum polygonoides* gave the MIC 6.5 µg/mL with 1.4 mm inhibition zone. With the increase in dose concentration, the inhibition zone also increases. The maximum dose of 75 µg/mL of crude methanolic extracts of *Calligonum polygonoides* gave 39 mm inhibition zone, 50 µg/mL dose showed 17. 34 mm inhibition zone, 25 µg/mL dose showed 7 mm inhibition zone, 12.6 µg/mL dose gave 3.17 mm inhibition zone and 6.5 µg/mL dose showed 1.4 mm inhibition zone. The methanolic extract doses of 2.9 and 1.8 µg/mL of *Calligonum polygonoides* showed no zone of inhibition. The ethyl acetate extract of *Calligonum polygonoides* is also applied against *Candida albicans* which showed that the MIC 9.8 µg/mL against *Candida albicans* gave 2.84 mm inhibition zone. The maximum dose in the ethyl acetate extract of *Calligonum polygonoides* was 12.6 µg/mL which gave 4.74 mm inhibition zone, and doses 6.5, 2.9 and 1.8 µg/mL did not show any zone of inhibition against *Candida albicans. Calligonum polygonoides* showed activity only against the *Candida albicans*, and variable doses did not give significant results against *Aspergillus niger*. The lowest doses showed the highest MIC values against the *Candida albicans*. The results of the study showed that the methanolic root extract of *Candida albicans* was significantly higher than the aqueous root extract of *Calligonum polygonoides*. Antimicrobial or antifungal activity of medicinal plant is due to the presence of phenolic or saponins compounds[6,7] which might be present in this plant. The results provide the justification for the use of *Calligonum polygonoides* in folk medicines to treat various infectious diseases and suggest that methanolic extract possesses some bioactive compounds with antifungal activities.

Table 1. Antifungal activity of various extract of Calligonum Polygonoides on Candida albicans and Aspergillus niger.

Dose (µg/mL)	Replicate	Methanolic extract			Ethyl acetate extracts			Aqueous & hexane extracts
		Zone of inhibition (*Candida albicans)* (mm)	Mean zone of inhibition (mm)	Zone of inhibition (*Aspergillus niger)* (mm)	Zone of inhibition (*Candida albicans)* (mm)	Mean zone of inhibition (mm)	Zone of inhibition (*Aspergillus niger)* (mm)	Mean zone of inhibition (*Candida albicans* and *Aspergillus niger)* (mm)
75	01 02 03	39 40 36	39	Nil	Nil	Nil	Nil	Nil
50	01 02 03	16 19 17	17.34	Nil	Nil	Nil	Nil	Nil
25	01 02 03	8 6 7	07	Nil	Nil	Nil	Nil	Nil
12.6	01 02 03	3.5 4 2	3.17	Nil	03 5.2 06	4.74	Nil	Nil
9.8	1 2 3	Nil	Nil	Nil	02 03 3.5	2.4	Nil	Nil
6.5	01 02 03	1 1.7 1.5	1.4	Nil	Nil	Nil	Nil	Nil
2.9	01 02 03	Nil	Nil	Nil	Nil	Nil	Nil	Nil
1.8	01 02 03	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Positive control Fluconazole, 100	01 02 03	33 35 36	34	34	Nil	Nil	Nil	Nil
Positive control Fluconazole, 13	Nil	Nil	Nil	Nil	12 10 09	10.3	Nil	Nil

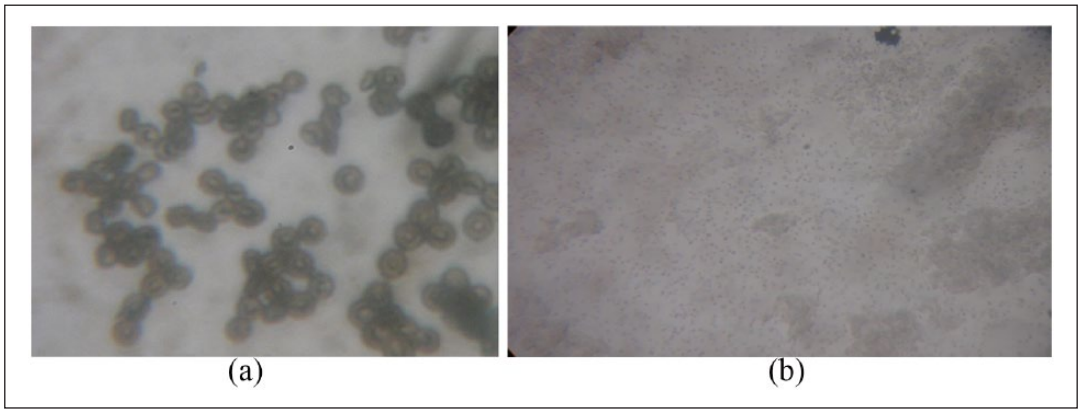

Figure 1. (a) Cells of *Candida albicans* before drug treatment under 40X and (b) after drug treatment under 40X.

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