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IN VITRO ANTIFUNGAL ACTIVITY AND TOTAL PHENOLIC CONTENT OF FERMENTATION EXTRACT OF ENDOPHYTIC FUNGI ISOLATED FROM *FICUS ELASTICA DECORA*

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ABSTRACT: *The aim of this study was to identify the endophytic fungi isolated from Ficus elastica decora and to investigate their potential antifungal activity and their total phenolic content of their fermentation extract. Two fungal species of endophytic fungi were successfully isolated from leaves including Aspergillus parasiticus and penicillium verrocusom. The fungal extracts were assessed for antifungal activity against Candida albicans where penicillium verrocusom extract showed larger inhibition zone than Aspergillus parasiticus one while Aspergillus parasiricus extract had higher total phenolic content than Penicilllium verrocusum one.*

Key words: *Endophytes- Ficus elastica decora-antifungal-total phenolic content.*

INTRODUCTION

Endophytes are microorganisms that are present in living tissue of various plants (root, fruit, stem, seed, leaf etc.) establishing mutual relationship without apparently any symptom of diseases, Sandhu *et al*. (2014). The endophytic fungi play important physiological and ecological roles in their host life. Recent investigations have been intensified by the potentialities of endophytic fungal strains in production of bioactive metabolites like taxol, pestaloside, torreyanic acid and enzymes, i.e: Xylanase**,** Isoflavonoids**,** Asparaginase, Theantana *et al*. (2007). Medicinal plants are known to harbor endophytic fungi that are believed to be associated with the production of pharmaceutical products, Zhang *et al*. (2006). Therefore, it is important to explore endophytic mycoflora in the medicinal plants. The present study was carried out to isolate, identify and test antifungal activity and total phenolic content of extracts of endophytic fungi which were isolated from *Ficus elastic decora which* belongs to the family Moraceae commonly known as the rubber bush, rubber tree, rubber plant, or Indian rubber bush, native to northeast India and southern Indonesia Kiem PV *et al*. (2012).

MATERIALS AND METHODS

Plant Material

In the present study fungal species were isolated from leaves of *Ficus elastica decora* which was collected from different sites of Sadat city (Egypt). Healthy and mature plant was carefully chosen for sampling. The plant parts were brought to the laboratory in sterilized bags and processed within a few hours after sampling.

Isolation of Endophytic Fungi

Isolation of endophytic fungi from plant parts was done according to the method described by Petrini (1986) with modification. First the plant material was rinsed in tap water to remove the dust and debris then cut into small pieces by a sterilized blade under aseptic conditions. Each sample was surface sterilized by 70% ethanol for 1 minute

and after that the samples were rinsed in sterile distilled water for 1 minute and then allowed to surface dry on filter paper. After proper drying 4 pieces of plant parts were inoculated in potato dextrose agar (PDA) plate supplemented with antibiotic (chloramphenicol) and incubated at 28 ±1°C for 5 to 7 days. Pure colonies were transferred on PDA slant. The fungal strains in the pure culture were preserved on PDA slant at 4 to 5°C with proper labeling and were sub-cultured from time to time.

Morphological Identification of Endophytic Fungi

The fungi were identified on the basis of morphological characteristics . The colonies appearing on petri plates were sub-cultured into the tube containing potato dextrose agar medium for identification. Fungi were again cultured from slant to petri plates containing potato dextrose agar medium without antibiotic (chloramphenicol) for 7 days. Morphological identification was done according to the standard taxonomic key included colony diameter, texture, color and the dimensions and morphology of hyphae and conidia.

Production of Secondary Metabolites

100 ml of Potato Dextrose Broth was prepared in 250 ml flasks and autoclaved at 15 lbs psi for 20 min. The medium was inoculated with fungal discs of solid culture and incubated at 28±1°C in the shaker incubator at 120 rpm. After 10 days of incubation the crude culture broth was collected.

Extraction:

The whole broth was then extracted with equal volumes of ethyl acetate on shaker for 24 hours then the ethyl acetate layer was separated after centrifugation, evaporated using rotatory evaporator and the resulting residue is dissolved in dimethyl sulfoxide (DMSO).

Microbial susceptibility testing:

Tested organisms: *Candida albicans* :

Stock culture of the tested organism was obtained from the microbiological lab at Faculty of Medicine in Mansoura University.

Filter paper disc assay*:*

The antifungal activity of the fungal extracts were estimated by filter paper disc method (Murray *et al*., 1998) using inoculums containing 10^6 fungal cells / ml to spread on nutrient agar plates.

The sterilised filter paper discs (Whatman no.1, 6mm in diameter) were saturated with the tested extracts. The discs were placed on the surface of agar plates seeded with the test organism. The plates were incubated at 30^{o}C. Diameters of inhibition zone (mm) were measured after 24-36 hours , Sardari *et al*., (1998).

Total phenolic compounds content*:*

The total phenolics content of fungal extracts was measured using the modified Folin Ciocalteu colorimetric assay developed by Wolfe *et al*., (2003).

A known volume (1 ml) of plant extract or standard solutions of gallic acid was added 10% 5 ml of Folin Ciocaltu reagent. After 3 minutes, 4 ml of sodium carbonate (75 g/L) was added and the mixture allowed to stand for 30 minutes at 40°C to the exclusion of light. The absorbance was measured at 765nm.

RESULTS

Isolation and identification of endophytic fungi:

In the present study, fungal strains were isolated from the leaves of *Ficus elastica decora*. A total of 2 fungi were isolated which belong to Ascmycetes. Identification of these fungal strains was done on the basis of their cultural and microscopic properties. These fungi were successfully identified as *Aspergillus parasiticus and penicillium verrocusom.*

Screening of endophytic fungi for antifungal activity:

Screening of endophytic fungi to determine the antifungal activity was done against *Candida albicans* . *Penicillium verrocusom* provided zone of inhibition equals (9mm) *and Aspergillus parasiticus*(8mm).

Total phenolic content:

In present study, 2 fungal extracts were investigated for total phenolic contents by using the modified Folin Ciocalteu colorimetric assay. The total phenolics content of *penicillium verrocusom was (3.765mg/ml)and Aspergillus parasiticus* was (6.135 mg/ml).

Discussion:

The antifungal activity of endophytic fungal extracts was proven in other studies as Colletotric acid, a metabolite of *Colletotrichum gloeosporioides*, an endophytic fungus from the plant *Artemisia mongolica*, displayed antimicrobial activity against bacteria as well as against fungus, *Helminthosporium sativum*, Zou *et al*. (2000). In another study *Colletotrichum* sp. isolated from *Artemisia annua*, produces bioactive metabolites that showed varied antibacterial and antifungal activity, Hong *et al*. (2000). For *Penicillium citrinum*; *Wen et al. (2014)* have proven the production of a novel antifungal protein by *Penicillium citrinum W1*, which was isolated from a Southwest Indian Ocean sediment sample, purified and characterized. The culture supernatant of *P. citrinum W1* inhibited the mycelial growth of some plant pathogenic fungi.

Phenols and terpenes are the main chemical constituents responsible forreducing lipid peroxidation and hence act as primary and secondary antioxidants Gulcin,(2006). In this study, extracts having high phenolic content also showed good antioxidant activity. Previous studies conclude that there is a linear correlation between total phenolic content and antioxidant potential of any sample, Sultana *et al*. (2007).

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النشاط المضاد للفطريات والمحتوي الفينولي لمستخلص التخمر للفطريات المعزولة من نبات الفيكس ديكورا معمليا

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الملخص العربي

يهدف هذا البحث الي عزل الفطريات التي تنمو داخليا في نبات الفيكس ديكورا وهذه الفطريات تعتبر غير ضارة بالنبات نفسه ,تم عمل تخمر للفطريات المعزولة لمدة تصل الي عشرة أيام تحت درجة حرارة تصل الي 28 درجة مئوية ثم عمل استخلاص لنواتج التخمر باستخدام اسيتات الايثيل لمدة يوم واحد واختبار المستخلص الناتج كمضاد للفطريات وكذلك معرفة محتوي المستخلص من المركبات الفينولية وقد تم استخلاص فطرين هما الاسبرجيلس باراسيتيكس والبنيسيلليوم فيروكوسم وقد وجد ان لكليهما تأثير مضاد للفطريات وقد قيست منطقة منع النمو علي الكانديدا البيكانس وكانت 8مم و9مم بالترتيب ,كما وجد أن محتوي المركبات الفينولية في مستخلص الفطرين 6,135 و3,765 مجم/ملل بالترتيب.