ANTIOXIDANT AND ANTICANDIDAL ACTIVITY STUDIES ON PHYLLANTHIN COMPOUND FROM PHYLLANTHUS NIRURI

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ABSTRACT

Objective: The present study was to investigate the phyllanthin present in Phyllanthus niruri which belongs to the family, Euphorbiaceae. The vital aim was to evaluate the antioxidant and to determine the anticanical efficacies against Candida albicans.

Methods: Phyllanthin, is one of the active lignan which was isolated from Phyllanthus niruri by silica gel column chromatography employing gradient elution with hexane–ethyl acetate solvent mixture. The eluted samples were further identified and estimated using HPTLC. Free radical scavenging activity of phyllanthin was examined using DPPH assay. Anticanical activity was studied by analysing the growth inhibition and Minimum Fungidal Concentration (MFC) rate.

Results: The retention time of phyllanthin is 23.4 and its total run time was 45 min. Phyllanthin exhibited very high antioxidative property by its low IC50=7.5 μmol/mL. The isolated phyllanthin alters the growth of C. albicans and showed significant results in vitro. Among the various concentrations of phyllanthin, 250 μL/L concentration revealed the high rate of inhibition.

Conclusion: The phyllanthin compound from the medicinal plant, Phyllanthus niruri confirmed as an effective antifungal agent to prevent the candidiasis and the secondary infections caused in various diseases.

Keywords: Phyllanthus niruri, Phyllanthin, Antioxidant, Anticanical activity, Candida albicans.

INTRODUCTION

Phyllanthus is a large genus of shrubs, trees and rare herbs of the family Euphorbiaceae, comprising more than 600 species. Pharmacological properties of Phyllanthus species has become a focal point due to their broad therapeutic use in folk medicine [1]. Phyllanthus species were traditionally used to cure cough, jaundice, gonorrhea, dysentery, diabetes, skin ulcers, headache, stomach -ache, eye wash, sore through, dysentery and dressing of wounds [2-3]. Some of the ailments prepared from these plants are related to cure microbial causing diseases with oxidative stresses [4].

Among the Phyllanthus species, Phyllanthus niruri is a small erect, annual herb growing up to 30–40 cm in height and indigenous to the tropical areas, including South East Asia, Southern India and China [5]. P. niruri is an important plant in Indian Ayurvedic system of medicine which is being used for curing the problems in stomach, genitourinary system, liver, kidney and spleen. It has some bitter, astringent, stomachic, diuretic, febrifuge and antiseptic properties. The whole plant is used in gonorrhoea, menorrhagia and other genital infections. It is useful in gastropathy, diarrhoea, dysentery, intermittent fevers, ophthalmopathy, scabies and wounds [6].

The major active lignan compounds of the Phyllanthus species are phyllanthin and hypophyllanthin which have been shown to be anti-hepatotoxic activity [7]. The therapeutic properties of medicinal plants are conditioned by the presence of active substances which are biologically active in relation to the causative agents of various diseases.

Candida albicans is the most common fungal microorganism in healthy individuals, as well as the most common fungal pathogen causing lethal infections [8-9]. Candida is considered as an opportunistic pathogen because it can harmlessly colonize the human digestive tract, mouth, skin, and genitourinary tract [10-11]. The side effects of most systemic antifungal drugs are comparable and include headache, gastrointestinal symptoms, hepatitis, kidney toxicity, and lupus-like syndromes, among others [12-14]. Although most cases of candida infection are treated with some type of antifungal agent, the formulation of the medication will largely depend on the location and clinical presentation of the infection [15-16]. In immune-compromised individuals, C. albicans can proliferate unimpeded, invade the bloodstream and the tissues, and causes a variety of infections including pneumonia, septicaemia, endocarditis and systemic candidiasis. The present study mainly concentrates on the estimation of antioxidant and anticanical activity of phyllanthin from P. niruri.

MATERIALS AND METHODS

Collection of plant materials

The plant specimen, Phyllanthus niruri for the proposed study was collected from the tropical areas of Kanyakumari District (Tamil Nadu), India and these species were identified on the basis of morphological characteristics and compared with the voucher specimens recorded in the Central Herbarium of Botanical Survey of India.

Preparation of extracts

The fresh leaves of Phyllanthus niruri were shade dried and pulverized. The powdered leaves were then stored in an air light containers. The sample was crushed with powder, using a manual grinding machine, so as to enhance effective constant solvent with sites on the plant material.

The powder was extracted with ethanol in soxhlet apparatus for 5 days [17-18]. The extracts were pooled upon for the removal of the solvents by evaporation at 40 °C reduced temperature and vacuum dried. The crude methanol extract was subjected for chromatography studies.

Phyllanthin extraction

The residue (10 g) obtained was mixed with silica gel (18 g) to form an admixture and chromatographed over silica gel column (500 mm length × 20 mm diameter, 63–200 μm particle size, 70–230 mesh size). Gradient elution was done in the following sequence, hexane: ethyl acetate (98:2 v/v), hexane: ethyl acetate (95:5 v/v), hexane: ethyl acetate (90:10 v/v) and hexane: ethyl acetate (80:20 v/v). The samples were spotted with a 100 μL microsyringe on a pre-coated...
silica gel aluminium plates 60 F 254 (20 cm × 10 cm) with 250 μm thickness.

After air drying, the TLC plates were scanned with a TLC scanner-III in absorbance mode at λmax. freshly prepared 10% sulphuric acid reagent was used to spray above the plates. To develop spots, the plate was heated at 110 °C for 10 min after drying. The scanning and quantification of the spots were performed at 200 nm. The sample was dissolved in 0.05% DMSO solution and further identified by HPTLC. The HPTLC analysis was done using the method described by Murugaiyah and Chan, (2007) [19-20].

**DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay**

The antioxidant of phyllanthin was measured in vitro using DPPH, a stable free radical [21]. The reaction mixture contain 0.1 mL of 1 mM DPPH, 0.8 mL of 99 % ethanol and 0.1 mL of phyllanthin (2.5–25 μmol/mL). The solution was mixed rapidly and allowed to stand for 30 min in dark. The scavenging activity was measured by noting the decrease in absorbance at 517 nm as compared to DPPH control. The antioxidative properties of the tested isolates were expressed as IC₅₀, which is defined as the concentration required for inhibition of DPPH radical by 50%.

**In vitro anticandidal assay**

**Inoculum preparation**

*Candida albicans* inoculum was prepared in 0.85% saline corresponding to No. 0.5 McFarland turbidity standard [22].

**Growth Inhibition test**

The antifungal assay was carried out in petri dishes containing Potato Dextrose Agar (PDA). When the temperature of the medium (PDA) reached about 40 °C, specific initial concentrations (0 and 250 μL/L) of 50% stocks of phyllanthin (diluted in ethanol 96% and sterilized using 0.2 μm filters) were added to PDA and mixed thoroughly. The rate of mycelial growth inhibition was measured after placing an active mycelial plug of fungi on petri dishes containing PDA with specific initial concentrations and incubated at 28±1 °C. The observations were recorded every 12 hours after first 10 hours for acclimation period to complete growth of control treatments. The rates of mycelia growth inhibition (GI %) was calculated by the following formula:

\[
GI \% = \frac{dc - dt}{dc} \times 100
\]

where, \(dc\) is mean colony diameter of control sets and \(dt\) is mean colony diameter of treatment sets [23].

**MFC (Minimum Fungicidal Concentration) determination**

For the determination of MFC, fresh Potato dextrose agar (PDA) agar plates were inoculated with one loopful of culture taken from each of the inoculums. MFC assay plates were incubated 27 °C for 3 to 7 days. The lowest concentration of the extract with no fungal growth on the solid medium after the incubation was regard as the MFC values for this extract [24].

**RESULTS AND DISCUSSION**

The results of the present study revealed the availability of highly bioactive phyllanthin in *P. niruri*. The leaves of *P. niruri* were dried and extracted in solvent ethanol which has the ability to separate maximum lignans from the sample. The extracts were subjected for column chromatography. After the extensive process, the complete separation was achieved with hexane: ethyl acetate (80:20 v/v). The retention time of phyllanthin was 23.4 and the total run time taken for the extraction was 45 min. The extracted phyllanthin (Fig. 1) was compared with that of the standard phyllanthin. From the whole dried leaves, the phyllanthin yield was 0.85% (w/w). The HPTLC method for the quantitative estimation of phyllanthin was validated with regard to their specificity, precision, accuracy and linearity. In one of the earlier report, Murali et al. (2001) [25] Somananabandhu et al. (1993) [26] have unambiguously characterized phyllanthin and hypophyllanthin by IR, 1H NMR, 13C NMR and mass spectroscopy.

Fig.1: HPTLC Chromatogram of *Phyllanthus niruri* for the recovery of Phyllanthin

The DPPH radical scavenging activity of phyllanthin is shown in Fig. 2. It was observed that the DPPH free radical scavenging activity of phyllanthin reaches a maximum at a concentration of 30 μmol/mL. This result indicates that the phyllanthin exhibited very high antioxidative property by its low IC₅₀ = 7.5 μmol/mL. The DPPH assay is based on the reduction of stable radical DPPH to yellow coloured diphenyl picryl hydrazine.

Thus, the ability of the tested products to quench this radical is a measure of its antioxidative ability. Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical induced oxidative stress. Moderate radical scavenging activity of crude extract was reported in the case of Phyllanthus species as well as phyllanthin [27-28].
Our study involves the in vitro evaluation of phyllanthin extracts against candida species. The antifungal assay indicated that all the Candida albicans were highly susceptible to the different concentrations of the extract and their susceptibilities were concentration dependent.

The phyllanthin compounds extracted was highly concentrated. Hence, the efficiency examination was carried out with different concentrations such as 50, 100, 150, 200 and 250 µL/L. With an increase in the concentration of the extract, there was increase in the inhibition zones. Among these concentrations, 250 µL/L of phyllanthin sample has 98.43 % inhibition rate followed by 200 µL/L (91.46 %), 150 µL/L (85.27 %), 100 µL/L (78.48 %) and 50 µL/L (61.33 %) respectively (Fig: 3).

The present study showed the high efficiency of antifungal activity against Candida albicans. Apart from efficacy, safety of this natural medicine extract phyllanthin is of paramount importance as there is not much evidence about other Phyllanthus species extracts that are used in traditional medicine.

The extract phyllanthin obtained from the selected medicinal plant, Phyllanthus niruri has high quantity of antioxidants and antifungal activity. Hence, it can be used in the preparation of novel natural therapeutic drugs against Candida associated infections. These results corroborate the importance of ethnomedical surveys in the selection of plants with bioactive compounds with high antioxidants.

CONCLUSION

In conclusion, the present study represents a worthy as well as expressive contribution to the characterization of phyllanthin from P. niruri which has a power to treat infections caused by C. albicans. Candida causing diseases are highly infectious and have a high mortality rate all over the world.

Due to the emergence of the resistant strains against commonly used antifungal agents. Thus phyllanthin extracts can exhibits a potential attention to develop natural antifungal agents in pharmaceutical research.

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REFERENCES


