POTENTIAL INHIBITORY EFFECTS OF GLORIOSA SUPERBA ON HUMAN PATHOGENIC FUNGI

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ABSTRACT

The different solvent extracts viz., acetone, dichloromethane, chloroform and methanol extracts of Gloriosa superba L. (Liliaceae) were screened for antifungal activity, by disc diffusion method against human pathogenic yeast and mould. The test organisms included were Candida albicans and Aspergillus flavus. The methanol extract of tubers recorded high significant antifungal activity against all the tested fungi. The least activity was found in the chloroform extracts.

Keywords: Gloriosa superba, disc diffusion method, antifungal activity, Candida albicans and Aspergillus flavus.

INTRODUCTION

Man always been surrounded by countless microorganisms. The disease producing microbes are playing a very important role in human life. Pathogenic microorganisms are always trying to develop resistance to the various antimicrobial agents used for their control. Infectious diseases accounts for high proportion of health problems in the developing countries like India. There are alarming reports of opportunistic fungal infection[1]. There is an increasing awareness amongst clinicians and microbiologists pertaining to importance of infection caused by opportunistic fungi[2]. Therefore, the chemotherapy of infectious diseases has proved to be a continuous struggle. Scientists are always in search of new antimicrobial agents to control the ever increasing menace of the microbes. Thus it is of paramount importance for the microbiologists to develop new resistant strains.

Many of the medicinal plants were screened for various biological and pharmacological activities including antifungal, antibacterial, insecticidal activities[3,4,5]. Researchers have shown that all different parts of the plants which include; stem, root, leaves, flowers, seeds etc possess antimicrobial property[6]. Medicinal plants are gifts of
nature to cure limitless number of diseases among human beings. Medicinal plants represent a rich source of antimicrobial agents. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine. The use of plants of plant material as fungicide is of great importance which needs more attention.

Candiasis is an increasingly important disease that has a world wide distribution due to the fact that it’s is a frequent opportunistic pathogen in AIDS patients which is caused by the human pathogenic fungus Candida albicans. It is common in the gastrointestinal and urogenital tracts of human and is also the cause of Candiasis in women.

The genus Aspergillus is a saprophytic mould living in different habitats like water, soil, organic matter, etc. However, more than 60 species of Aspergillus have been recognized as human pathogen. Important diseases caused by them are invasive aspergillosis, bronchopulmonary aspergillosis, pulmonary aspergilloma and different forms of allergies. Of the several Aspergillus species, the widespread and dangerous pathogens are Aspergillus fumigatus, A. flavus and A. niger. Aspergillus fumigatus and Aspergillus flavus are known to produce a carcinogenic and a histo-toxic secondary metabolite called ‘Aflatoxin’. These fungi, when infect nuts and other food grains cause contamination through its toxin and create serious problems in human beings and animals. Aspergillosis is caused due to the inhalation of Aspergillus flavus spores. Among different species of fungi, Aspergillus flavus is associated with heavy loss of grains, fruits, vegetables and other plant products rendering them unfit for human consumption of producing mycotoxins and affecting their nutritive value.

Studies on the use of plant extracts for the control of diseases have shown the importance of natural chemicals (Phytochemicals) as possible sources of non-phytotoxic and easily biodegradable alternative fungicides and antibiotics. Against this background, the endangered medicinal plant Gloriosa superba was selected to evaluate its potential inhibitory effect against Candida albicans and Aspergillus flavus.

Gloriosa superba, L. (Family: Liliaceae) commonly called as Glory lily is a handsome, perennial branched herbaceous, tendril climber with underground cylindrical white tuberous rhizome; common in forest of Orissa and throughout India and

www.pharmasm.com IC Value – 4.01 2019
in Andaman Islands. Root, made into paste with mustard oil, is applied on body for curing periodic fever [22]. The sap from the leaf tip is used as a smoothening agent for pimples and skin eruptions [23]. In case of induced abortion, rhizome/tuber of Gloriosa superba is ground and mixed with ghee and used orally [24]. Seeds and tubers are mainly used to treat gout and rheumatism [25]. To our knowledge, there are only few reports on antifungal activity of G. superba. Therefore an attempt was made to study the antifungal potential of G. superba against human pathogenic fungi.

MATERIALS AND METHODS

Source of plant material

The test plant, Gloriosa superba leaves and tubers were collected in November, 2008 from the fields of Attur village, Salem district, Tamilnadu, India. The plant was identified by Dr. V. Balasubramaniam, Associate Professor in Botany, Kongunadu Arts and Science College, Coimbatore and a voucher specimen was submitted in the Herbarium of Kongunadu Arts and Science College, Coimbatore, Tamilnadu.

TEST ORGANISMS AND CULTURE MEDIA:

The test fungal pathogens, Candida albicans and Aspergillus flavus used for the antifungal activity were obtained from K.G. Hospitals, Coimbatore, Tamilnadu, India. The fungal isolates were cultured on Potato Dextrose Agar medium (Potato tubers-200g, Dextrose-20g, Agar agar-20g, Distilled water-1000ml at pH-5.6) and potato dextrose broth medium (same contents as for Potato Dextrose Agar medium excluding agar-agar). The cultures were maintained at 37°C in the Microbiology laboratory, Kongunadu Arts and Science College, Coimbatore.

EXTRACTION OF PLANT MATERIAL:

The fresh leaves and tubers of G. superba were shade dried, pulverized, sieved through 40 mesh and retained in 60-mesh size were collected. The powders thus obtained (60gm) were extracted with the 250 ml of different organic solvents such as acetone, dichloromethane, chloroform and methanol in soxhlet apparatus for 10 hours. Then the extracts were concentrated to dryness to yield the crude residues which were used for the further study.
ANTIFUNGAL ACTIVITY:

The extracts were screened for their antifungal activity by disc diffusion method [26]. Sterile potato dextrose plates were prepared and inoculated by spread plate method under aseptic conditions. The filter paper discs of 6mm diameter (Whatman’s No. 1 filter paper) were prepared and sterilized. The plant extracts to be tested were prepared with various concentrations at 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml and were added to each disc of holding capacity of 10 microlitres. The sterile impregnated discs with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Control discs of Kanamycin were prepared and placed on the agar surface. All the plates were incubated at 37°C for 72 hours. After incubation, the size (diameter) of the inhibition zones was measured. The mean zone of inhibition of the three replicated tests (triplicate analysis) of the plant extracts is expressed in millimeters. All the data were statistically analyzed using the SPSS Version 2007 WINSAT software.

RESULTS

Antimicrobial activities of various herbs and spices in plant leaves, flowers, stems, roots, or fruits have been reported by many workers [27]. Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The inhibitory effects of four extracts of leaves and tubers of G. superba against pathogenic species in potato dextrose agar by disc diffusion method are shown in Table 1 and 2. All the extracts tested exhibited different degrees of antifungal activity against Candida albicans and Aspergillus flavus. Among the different solvent extracts, the tuber methanol extract (100%) had interesting activity against Aspergillus flavus (19±0.89 mm diameter) and Candida albicans (18.33±5.78 mm diameter). The least zone of inhibition was noted in chloroform leaf extract against Candida albicans (4.34±2.18 mm diameter).
DISCUSSION

In the present study, the extracts from the tubers and leaves of Gloriosa superba were screened for their activity against Candida albicans and Aspergillus flavus. The results of this study showed that the methanol extracts of tuber were more inhibitory effect on Candida albicans. This is in line with the observations made by Swarnkar and Katewa\textsuperscript{30} who reported that methanol extracts of G. superba had significant inhibitory activity against Candida albicans. The methanolic tuber extract also showed maximum zone of inhibition against Aspergillus flavus which is in close agreement with Hemaishwarya Shanmugham et al., 2009 \textsuperscript{31} who reported high activity of methanol tuber extracts of G. superba against Aspergillus niger.
The least zone of inhibition was seen in chloroform extracts. It is known that different solvents extract possess different compounds, and some active components can only be extracted by polar compounds, while some by less polar and yet some by non-polar compounds. Considering the fact that the chloroform fraction lacks some phytochemical components, it may be clear that there is a form of synergism in the activities of the compounds hence the absence of some reduced activity of the chloroform fraction [32, 33].

The inhibitory effect of the extracts of G. superba is probably due to the presence of phytochemicals (i.e. bioactive ingredients) which are antimicrobial agents that are inhibitory to the growth of these pathogens [34]. The difference in the antifungal properties of the plant extracts is attributable to the parts of the plant used, age of the plant used, freshness of the plant materials, physical factors (temperature, light, water) and contamination by field microbes [35,36,37].
CONCLUSION

From the results obtained, it is evident that *G. superba* possesses potential inhibitory activity against human pathogens. Hence, there is a need to isolate possibly by purification the various phytochemical groups in the extracts. The further isolation of such bioactive components could perhaps clarify the pharmacological properties of *G. superba* and be further exploited for pharmaceutical use.

REFERENCES


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