



Antifungal Activities of Methanolic Extracts of *Podophyllum hexandrum* and *Rheum emodi* Against Human Pathogenic Fungal strains

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ABSTRACT

Podophyllum hexandrum and *Rheum emodi* were investigated for their antimicrobial properties against pure cultures of clinical isolates of *Aspergillus niger* ATCC 1197 and *Candida albicans* ATCC 10231. Disc diffusion methods were used in determining the antifungal activities of the different plant extracts on the test organisms. The minimum inhibitory concentration (MIC) of the extracts on the test organisms were 16.66 mg/ml for *Aspergillus niger* in case of *Podophyllum hexandrum* rhizome extract and 50 mg/ml in case of *Rheum emodi* rhizome extract. In case of *Candida albicans* it was 25 mg/ml for *Podophyllum hexandrum* rhizome extract and 16.66 mg /ml in case of *Rheum emodi* rhizome extract.

Keywords: Antifungal activity, Phytochemical screening, Plant Extract.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and since the beginning of man. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity¹⁻³. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components gave the plant as a whole a safety and efficiency, much superior to that of its isolated and pure active components⁴.

Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects⁴. There is therefore the need to search for plants of medicinal value. The plants used in the present study are *Podophyllum hexandrum* and *Rheum emodi*. *Podophyllum hexandrum* belongs to family Berberidaceae and in Kashmir valley it is locally known as Banwangun. The rhizome powder is used as a poultice to treat warts and tumorous growth on the skin. The traditional medicinal uses of *Podophyllum hexandrum* is in the treatment of colds, constipation, septic wounds, burning sensation, erysipelas, mental disorders, plague, allergic and inflammatory conditions of the skin, cancer of brain, bladder and lung, venereal warts, monocytoid leukemia, Hodgkins disease and non- Hodgkins lymphoma⁵.

Rheum emodi (Polygonaceae) is a leafy perennial herb distributed in altitudes ranging from 2800 to 3800m in the temperate and subtropical regions of Himalayas from Kashmir to Sikkim in India. *Rheum emodi*, pambchallan (kashmiri) has been traditionally used to treat pathological ailments like fevers, ulcers, bacterial

infections, fungal infections, jaundice and liver disorders.⁶⁻⁹

MATERIALS AND METHODS

Plant Material

The rhizomes of both the plants *Podophyllum hexandrum* and *Rheum emodi* were identified and collected from Gulmarg area of J & K. The plant parts were shade dried for some days and ground into powder with the help of an electric grinder and latter stored in air tight bottles for further use. 200 gms of each powdered plant materials was filled in the thistle funnels of two separate Soxhlet extractors and extracted with 250 ml 99% methanol (MERCK) up to 48 hours.

Test Organisms Used

The test microorganisms used were *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 1197. These microorganisms were obtained from Center for Microbiology and Biotechnology, Research and Training Institute Bhopal.

Phytochemical Screening

Standard methods^{10,11} were used for preliminary phytochemical screening of methanolic extracts, to know the nature of phyto- constituents present in it.

Antifungal Assay

Extracts of *Podophyllum hexandrum* and *Rheum emodi* prepared with methanol (MERCK) were used to test their antifungal activity. Antifungal activity was demonstrated using a modification of the method originally developed by Bauer et al which is widely used for the antimicrobial susceptibility testing¹². Liquid nutrient potato dextrose agar media and the petri plates were sterilized by autoclaving at 120°C for 30 minutes. Under septic conditions in the laminar airflow chamber, about 20 ml of

the agar medium was dispensed into each petriplate to yield a uniform depth of 4mm. After solidification of the media, the fungal strains were swabbed on the surface of the plates.

Whatmann no.1 filter paper was cut into small discs of diameter 0.4cm and autoclaved. The discs were dipped into the different plant extracts of each four concentrations namely 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml. The dipped discs were placed on the appropriate swabbed petriplates such as that each petriplate have the four concentrations of each plant extract. Amphotericin B was used as the standard drug (Figure no. 3). It was then kept in incubator maintaining the temperature at about 25°C for 48 hours and then the zones of inhibition were measured in mm.

Minimum Inhibitory Concentration (MIC)

It is the minimum concentration of active soluble principle in the extract, which inhibits the growth of the organism. The potent inhibitory extracts providing larger zones of inhibition were selected for MIC studies. Decreasing concentrations of these extracts were prepared and their inhibitory efficacy was observed. Lowest concentration of the extract, below which no inhibitory zone was observed, was considered as MIC. Disc diffusion method was used for detecting MIC. Gradually decreasing concentrations of extracts were prepared. Discs of 0.4mm were impregnated in various extract concentrations and transferred to petriplates

containing autoclaved sterilized Sabouraud's dextrose agar medium for antifungal activity.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of extracts of *Podophyllum hexandrum* and *Rheum emodi* showed the presence of bioactive components like Tannins, glycosides, flavonoids, terpenes, saponins (table. 1). The results of the antimicrobial assay of the methanolic extracts of both the plant extracts indicated that the plants exhibited antimicrobial activity against the tested microorganisms at four different concentrations of 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml. The potent sensitivity of the extract was obtained against the two microorganisms tested and the zone of inhibition was recorded and presented below in the tabulation drawn (table. 2 and 3) and figure 1 and 2.

Table 1.

Tests	<i>Podophyllum hexandrum</i>	<i>Rheum emodi</i>
Alkaloids	– –	+ +
Tannins	+ +	+ +
Glycosides	+ +	+ +
Flavonoids	+ +	+ +
Terpenes	+ +	+ +
Saponins	+ +	+ +
Carbohydrates	– –	– –

++ = Present -- = Absent

Table 2: Zones of Inhibition in mm at four different concentrations of plant extract *Podophyllum hexandrum*.

Test organism	25mg/ml	50mg/ml	75mg/ml	100mg/ml	Amphotericin B. ** 1 mg/ml
<i>Candida albicans</i>	12±0.5	11±0.1	14±1.5	15±1.5	28±2.2
<i>Aspergillus niger</i>	13±0.1	10±0.0	-	-	25±0.5

Table 3: Zones of Inhibition in mm at four different concentrations of plant extract *Rheum emodi*

Test organism	25mg/ml	50mg/ml	75mg/ml	100mg/ml	Amphotericin B. ** 1 mg/ml
<i>Candida albicans</i>	11±0.1	10±0.0	12±0.5	11±1.1	28±2.2
<i>Aspergillus niger</i>	10±0.0	10±0.0	10±0.0	13±0.1	25±0.5

** Amphotericin taken as standard; Values are mean ± S.D of three replicates

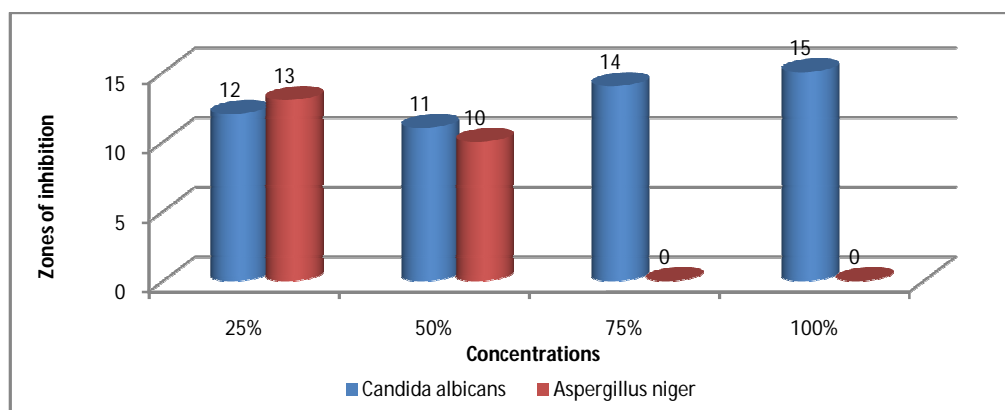


Figure 1: Zones of inhibition (mm) in *Podophyllum hexandrum* extract

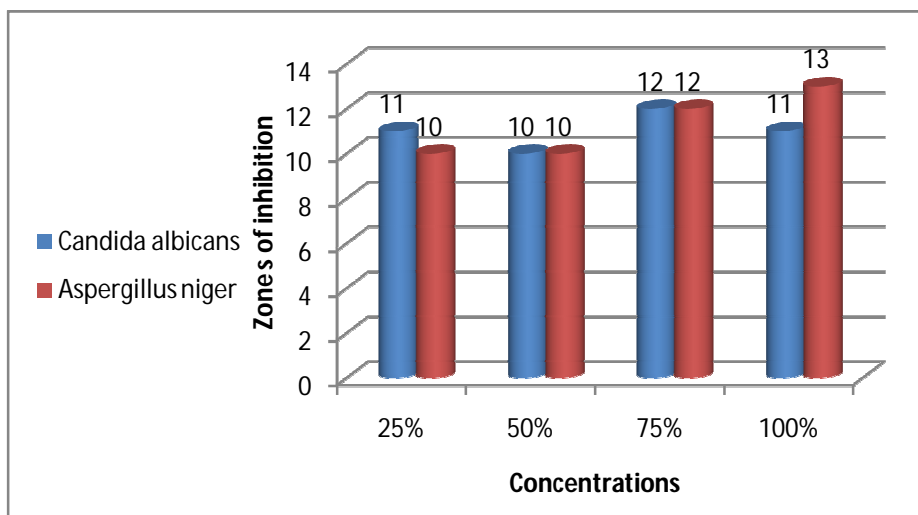


Figure 2: Zones of inhibition (mm) in Rheum emodi extract

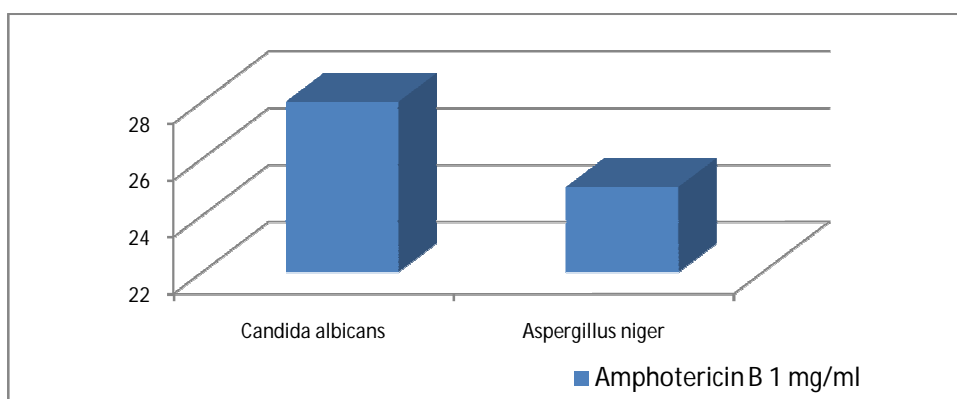


Figure 3: Zones of inhibition (mm) with Amphotericin B 1 mg/ml

In the present era plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization¹³. Although a significant no. of studies have been used to obtain purified plant chemical. Very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bio-active phyto-components present in the plants¹⁴. In the present investigation, the active phyto-components of *Podophyllum hexandrum* and *Rheum emodi* were studied and further the antimicrobial activity of the plant extracts were also tested against two pathogenic microorganisms *Candida albicans* and *Aspergillus niger* at different concentrations of the extract to understand the most effective activity. Also MIC of the two extracts against the selected pathogens were determined. The minimum inhibitory concentration for *Candida albicans* was 100mg/ml in case of *Podophyllum hexandrum* rhizome extract and 33.3mg/ml in case of *Rheum emodi* plant extract (Table 4).

Table 4: MIC of Methanolic extracts of *Podophyllum hexandrum* and *Rheum emodi* on test organisms in mg/ml.

Test organism	<i>Podophyllum hexandrum</i> extract	<i>Rheum emodi</i> extract
<i>Aspergillus niger</i>	16.66	50
<i>Candida albicans</i>	25	16.66

CONCLUSION

From the above studies, it was concluded that the traditional medicinal plants may represent new sources of anti-microbials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. Such studies are of paramount importance in the discovery of new classes of antibiotics that could serve in the maintenance of human health.

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