

Antifungal activity of stem bark of *Kigelia pinnata* Linn.

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ABSTRACT

The effect of petroleum ether, chloroform and methanol extract of stem bark of *Kigelia pinnata* was investigated in different fungus i.e. *Cryptococcus neoformans*, *Candida tropicalis*, *Trychophyton rubrum*, *Microsporum furfure*, *Epidermophyton floccosum* to evaluate the antifungal activity. For this evaluation Sabouraud's glucose broth media was used. All the three extracts exhibited antifungal activity. The chloroform extract showed significant antifungal activity; where as the petroleum ether and methanolic extract showed weak antifungal activity.

Keywords: Antifungal activity, *Kigelia pinnata*, minimal inhibitory concentration.

INTRODUCTION

Kigelia pinnata a plant belonging to family Bignoniaceae colloquially called the sausage tree on account of its large fruits has a variety of uses throughout Africa where it grows as an endemic species in many areas¹. It is cultivated in many parts of India as an ornamental and roadside tree. The phytochemical studies reveal the presence of Quercetin, Kaempferol, β - sitosterol, naphthaquinones, iridoids and flavonoids²⁻⁵. The stem bark and fruit extract showed activity against melanoma and carcinoma cell lines⁶. Extracts of rootbark and stembark exhibited antitrypanosomal activity⁷. Besides it is found to be active as an anti-ulcer and anti-rheumatic activity, it is necessary to explore and establish the analgesic activity of stembark of *K.pinnata*.

The stem bark was collected from Nashik (MS) and authenticated by a botanist from Botany department of K.T.H.M.college, Nashik. The stem bark was dried and powdered. The powder was extracted, successively with petroleum ether, chloroform and methanol using soxhlet extractor. The extracts were evaporated under

vacuum. Extractive values (% w/w) of petroleum ether, chloroform and methanol dry extracts were 0.52, 0.85 and 12.5.

MATERIALS AND METHODS:

Plant material

The fresh woody stem of *Kigelia pinnata* collected from Toranmal hills of Maharashtra, India in June and authenticated by Dr. D. A. Patil, HOD Botany Dept, SSVPS College, Dhule, Maharashtra, India. The woody stem was shed dried, ground and sieved with a 40 mesh sieve.

Preparation of extracts.

About 4 kg of bark powder was subjected to hot extraction using soxhlet extractor, successively with petroleum ether, chloroform and methanol. All the extracts were concentrated under reduce pressure by using rotary flash vacuum evaporator and then dried by using vacuum dryer, giving PEK (0.65%), CEK (0.73%), and MEK (2.50%) respectively.

Test microorganisms

Fungal Strains were obtained from National Chemical Laboratories (NCL), Pune Maharashtra. *Candida neoformans* NCIM 3471, *candida tropicalis* NCIM 545, *Epidermophyton floccosum* NCIM 1099, *Trichoderma rubrum* NCIM 1221 and *Malassezia furfure* NCIM 1197 were used as test organisms.

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Table 1. Minimum Inhibitory concentration ($\mu\text{g/ml}$) of *Kigelia pinnata* stem bark extracts by tube dilution method

Name of Fungus	Petroleum ether extract $\mu\text{g/ml}$	Chloroform extract $\mu\text{g/ml}$	Methanolic extract $\mu\text{g/ml}$	Standard Drug $\mu\text{g/disc}$
<i>C. neoformans</i>	1.25	1.25	2.5	0.625
<i>C. tropicalis</i>	2.5	1.25	2.5	0.312
<i>T. rubrum</i>	0.625	0.625	0.625	0.625
<i>M. furfure</i>	0.625	0.625	1.25	0.312
<i>E. floccosum</i>	1.25	0.625	1.25	0.625

Values are Minimal Inhibitory Concentration ($\mu\text{g/ml}$), and an average of triplicate. Standard Drugs: Ketoconazole Incubation conditions for bacteria—1 day at 37° C. For fungi—7 days at 27° C.

Determination of anti-fungal activity:

Anti-fungal activity of extracts against *Cryptococcus neoformans*, *Candida tropicalis*, *Trychophyton rubrum*, *Microsporum furfure*, *Epidermophyton floccosum* was performed by use of Sabouraud’s glucose broth as media for assay. The inoculated tubes were incubated for 48 hours.

Preparation of Sabouraud’s glucose broth:

Glucose and peptone were dissolved in distilled water with aid of heating. Then the medium was cooled and filtered, pH was adjusted to 5.4 with 10% lactic acid. The media was sterilized by autoclaving at 15-lb/Psi pressure for 15 minutes.

One ml of sterilized media was poured into sterilized test tubes. The stock solution of petroleum ether, chloroform, and methanolic extract having concentration 10 $\mu\text{g/ml}$ were used. The extracts were serially diluted to give a concentration of 5, 2.5, 1.25, 0.625, 0.312 and 0.156 $\mu\text{g/ml}$. In all the tubes 0.1 ml of suspension of bacteria in saline was added and the tubes were incubated at 37°C for 24 hours. The growth in the tube was observed visually for turbidity and inhibition was determined by the absence of growth. MIC was determined by the lowest concentration of sample that prevented the development of turbidity. The procedure was performed for three bacterial species for three extracts. The procedure was repeated to confirm the MIC. Ketoconazole used as standard drug.

Method of preparation of test organism suspension

Test organism maintained on slants of medium containing 300mg of manganese sulphate per liter and transferred to fresh slant once a week. Then the slants incubated at temperature 32°C for 24 hours. Organism was washed by using 3 ml of saline solution from agar slant onto a large agar surface of medium such as Roux bottle containing 250 ml of agar. It was incubated for 24 hour. Using 50 ml saline solution, the growth from the nutrient surface was washed. Then organism stored under refrigeration. Inoculum was adjusted at 530 nm, which give transmission equivalent to 1×10^8 cells/ml.

RESULT AND DISCUSSION:

All the three extracts exhibited antifungal activity. The chloroform extract showed significant antifungal activity; where as the petroleum ether and methanolic extract showed weak antifungal activity.

Thus the chloroform extract was more potent compared to petroleum ether and methanolic extract.

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