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IN-VITRO EVALUATION OF THE ANTIFUNGAL ACTIVITY OF LEAF EXTRACTS OF *MESUA NAGASSARIUM* (BURM.F.) *AND KIGELIA PINNATA*.

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ABSTRACT
In the present study the in vitro antifungal activity of different extracts of leaves of Mesua nagassarium and Kigelia pinnata were evaluated against three pathogenic microorganisms using the Stokes disc diffusion methods. The leaves extracts of M. nagassarium showed highest antifungal activity than K. pinnata. The methanolic extract and its pet-ether and carbon tetrachloride soluble fractions are showed the highest antifungal activity. The carbon tetrachloride soluble fraction of leaf extracts of M. nagassarium showed the maximum inhibition zone of 21.33 mm against Sacharomyces cerevacae and Candida albicans (19.7 mm) and Aspergillus niger (20.3 mm) respectively. The carbon tetrachloride and Chloroform soluble fractions of leaves of K. pinnata showed the mild antifungal activity. The maximum zone of inhibition found at 10 mm against A. niger and 9mm against C. albicans by Chloroform soluble fractions of leaves of K. pinnata. In this study Nystatin (30µg/disc) was used as standard antifungal agent. Carbon tetrachloride soluble fraction of leaf extracts of M. nagassarium can be used in the control of S. cerevacae, A. niger induced diseases as herbal medicines following clinical trials.

Key words: *Mesua nagassarium*, *Kigelia pinnata* , Antifungal, *C. albicans, A. niger*, *S. cerevacae*, Stokes Disc diffusion, herbal medicines.

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INTRODUCTION

Mesua nagassarium (Bengali - nagesar, nageswar) is a medium-sized or fairly large evergreen tree up to 36 m tall. A mixture of pounded kernels and seed oil isolated from this plant is used for poultice as wounds. The seed-oil is used for treating itch and other skin eruptions, dandruff and rheumatism (Orwa et al., 2009).The flowers are known to be useful for the treatment of severe colds, bleeding hemorrhoids, dysentery with mucus, excessive thirst, excessive perspiration, cough and digestion, rheumatism and iron induced lipid peroxidation

(Yadav *et al .,* 2010; Konwarh *et al.,* 2010). Pet ether extracts of leaf of *M. nagassarium* also shows highly antibacterial activity (Sikder *et al., 2011)*. Phenolic extract of seed oil of *M. nagassarium* revealed potent antiasthmatic effect (Bhide, 1977).
Kigelia is a genus of flowering plants in the family Bignoniaceae. The roots, the wood and the leaves have been investigated chemically. They contain naphthoquinones, dihydroisocoumarines, flavonoids and aldehydic iridoids. Among the naphthoquinones kigelinole, isokigelinole, pinnatal and isopinnatal were isolated Akunyili DN, Houghton PJ (1993). Various pharmacological examinations such as antibacterial, antiviral and antioxidant activities have been carried out. The success story of chemotherapy lies in the continuous search for new drugs to counter the challenges posed by resistant strains of microorganism (Khan et al., 2003). There are increasing interest in plants as a source of agent to fight microbial diseases and treatment of several infections (Chariandy et al., 1999; Aburjai et al., 2001). As a part of our ongoing program to investigate the unexplored bioactivity of traditionally used medicinal plant of Bangladesh we studied the antifungal activity of leaf of *M. nagassarium* and *K. pinnata* were evaluated.

MATERIALS AND METHODS

Plant Material

The leaves of *Mesua nagassarium* were collected from Dhaka and *Kigelia pinnata* were collected from Rangpur (Bangladesh) in March 2010. Then oven dried for 24 hours at considerably low temperature (not more than 40^0C) for better grinding. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no. DACB- 35158 and DACB- 35180). The dried leaves were then ground to a coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy, and University of Dhaka. The powdered plant sample (600 gm) was soaked in 2.5 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator at low temperature (40-45 ºC) and reduced pressure. The concentrated methanolic extract (MES) was partitioned by modified Kupchan method (Van Wagenen et al., 1993) and subsequent evaporation of solvents yielded pet-ether (PESF), carbon tetrachloride (CTCSF), Chloroform (CSF) and aqueous (AQSF) soluble fractions which were used for the experimental processes.

Disc diffusion method

In this classical method, antifungal disc diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antifungal (Nystatin) discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media (Barry, 1976). The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antifungal property inhibit fungal growth in the media surrounding the discs and thereby yield a clear, distinct area defined as **zone of inhibition**. The antifungal activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter (Barry, 1976; Bayer *et al.,* 1966.). In the present study the crude extracts as well as fractions were tested for antifungal activity by disc diffusion method. The experiment is carried out more than once and the mean of the readings is required (R. C. Jagessar *et al.,* 2008, Bayer *et al.,* 1966).

Test organisms

The bacterial and fungal strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka and they are listed in the Table 1.

Table 1: Test organisms

Fungi
Aspergillus niger
Candida albicans
Sacharomyces cerevacae

Preparation of discs

Measured amount of each test sample (specified in table 2) was dissolved in specific volume of solvent (Chloroform or methanol) to obtain the desired concentrations in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank Petridis under the laminar hood. Then discs were soaked with solutions of test samples and dried.

Standard Nystatin (30 µg/disc) discs were used as positive control to ensure the activity of standard antifungal against the test organisms as well as for comparison of the response produced by the known antifungal agent with that of produced by the test sample. Blank discs were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

Table 2: sample discs of Leaf of *M. nagassarium*

Plant part	Sample code	Test Sample	Calculated amount (mg)
Leaf of *M. nagassarium*	MESF	Methanol soluble extract of leaf of *M. nagassarium*	4.0
	PESF	Petroleum ether soluble fraction	4.0
	CTCSF	Carbon tetrachloride soluble fraction	4.0
	CSF	Chloroform soluble extract fraction	4.0
	AQSF	Aqueous soluble fraction	4.0

Table 3: sample discs of Leaf of *K. pinnata*

Plant part	Sample code	Test Sample	Calculated amount (mg)
Leaf of Leaf of *K. pinnata*	MESF	Methanol soluble extract of leaf of *K. pinnata*	4.0
	PESF	Petroleum ether soluble fraction	4.0
	CTCSF	Carbon tetrachloride soluble fraction	4.0
	CSF	Chloroform soluble extract fraction	4.0
	AQSF	Aqueous soluble fraction	4.0

Antifungal activity screening

The disc diffusion method (Bauer et al., 1966, Rahman and Rashid, 2008, R. C. Jagessar *et al.,* 2008) was used to test antifungal activity of the extractives against three fungi (Table-1), collected as pure cultures from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka, Bangladesh. Solutions of known concentration (400 µg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with

known amounts of the test substances using micropipette and the residual solvents were completely evaporated. Discs containing the test materials (400 µg/disc according to disc diffusion method (R. C. Jagessar *et al.,* 2008, Bauer et al., 1966, Rahman and Rashid, 2008) were placed on to nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of Nystatin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4 ^{o}C) for 24 hours to allow maximum diffusion of the test materials and Nytastin. The plates were finally incubated at 37 ^{o}C for 24 hours to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antifungal activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The experiment was carried out in triplicate and the mean value was taken.

RESULTS AND DISCUSSION

The crude methanolic extract (MES) of leaf of *M. nagassarium and K.pinnata* and its pet-ether (PESF), carbon tetrachloride (CTCSF), Chloroform (CSF) and aqueous (AQSF) soluble partitionates were subjected to antifungal screening at 400 µg/disc. Among the extractives, MES, PESF and CTCSF exhibited very strong antifungal activity (Table 4).

The carbon tetrachloride soluble fraction (CTCSF) of *M. nagassarium* revealed the highest inhibition against fungal growth having zone of inhibition ranged from 21.33 mm to 20.67 mm. The maximum zone of inhibition produced by CTCSF was found to be 21.33 mm against *S. cerevacae.* This partitionate showed significant antifungal activity having zone of inhibition 20.33mm against *C. albicans* (Table 4). The methanol extract also demonstrated significant inhibition of fungal growth having zone of inhibition ranging from 21.0mm to 19.30 mm. This extract exerted highest inhibitory activity against *S. cerevacae* (having zone of inhibition of 19.30 mm). The Petroleum ether soluble fraction (PESF) of *M. nagassarium* also demonstrated significant inhibition of fungal growth having zone of inhibition ranging from 21.30mm to 19.70 mm and Chloroform soluble fraction showed also mild antifungal activity having zone of inhibition 11.70 mm against *C. albicans,* 10.0 mm against *S. cerevacae.* whereas aquous fraction showed also mild antifungal activity having zone of inhibition 11.30 mm against *C. albicans,* 10.0 mm against *S. cerevacae* (Table 4).

On the other hand the Chloroform (CSF) and carbon tetrachloride soluble fraction (CTCSF) of *K. pinnata* showed the mild inhibition against fungal growth having zone of inhibition ranged from 10.00 mm to 8.0 mm. The maximum zone of inhibition produced by CSF was found to be 10.00 mm against *C. albicans* (Table 5).This partitionate also showed significant antifungal growth having zone of inhibition 20.33mm against *C. albicans.* Carbon tetra chloride soluble fraction zone of inhibition ranged from 8.5 mm to 8.0 mm. (Table 5) and standard antifungal agent nystatin produce zone of inhibitor range of 44.67 mm to 40.00 mm against the test organisms (Table 4, 5).

Table 4: Antifungal activity of test samples of *M. nagassarium*

Test microorganisms	**Diameter of zone of inhibition (mm)**					
	Methanol	**Pet-ether**	**CCl_4**	**$CHCl_3$**	**Aqueous**	**Nystatin**
Candida albicans	20.3±2.51	19.7±1.53	20.33±1.53	11.7±2.88	11.3±2.08	42.83±0.28
Aspergillus niger	19.3±1.52	20.3±0.58	20.67±1.15	9.33±0.57	11.3±1.15	41.66±0.57
Sacharomyces cerevacae	21.0±1.52	19.3±2.08	21.33±1.53	10.0±1.0	10.0±0	43.33±0.28

Values are expressed as mean ± S.D. (n=3)

Table 5: Antifungal activity of test samples of *K. pinnata*

Test microorganisms	Diameter of zone of inhibition (mm)					
	Methanol	Pet-ether	CCl_4	$CHCl_3$	Aqueous	Nystatin
Candida albicans	Nil	Nil	8.26±0.25	9.1±0.15	Nil	42.6±0.57
Aspergillus niger	Nil	Nil	8.56±0.11	10.1±0.28	Nil	41.16±.76
Sacharomyces cerevacae	Nil	Nil	8.1±0.15	8.03±0.05	Nil	43.5±0.5

Values are expressed as mean ± S.D. (n=3)

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