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RESEARCH PAPER

ANTIFUNGAL ACTIVITY OF ACETONIC EXTRACT OF *FLACOURTIA INERMIS* FRUIT AGAINST HUMAN OPPORTUNISTIC PATHOGENS

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Abstract: The fruits of *Flacourtia inermis* are widely used by people of Kerala but its medicinal significance is still unknown. In the present study, acetonic extract of *Flacourtia inermis* fruit was examined for their antifungal activity against opportunistic human pathogens such as *Aspergillus fumigatus*; *Aspergillus flavus*, *Aspergillus niger*, *Mucor ramosissimus* and *Chrysosporium* species. The study showed that the fruit extract possessed highest activity against *Aspergillus fumigatus* with an average inhibition zone of 47 mm. Least susceptibility was shown by *Aspergillus niger* with a mean zone of inhibition of 30 mm, which also is a promising result for a plant extract. *Aspergillus flavus*, *Mucor ramosissimus*, and *Chrysosporium* sp. were also powerfully inhibited by the fruit extract. MIC of the acetonic extract was also detected. The study reveals that the acetonic extract of *Flacourtia inermis* fruit contains powerful agents active against human opportunistic pathogenic fungi.

Keywords: *Flacourtia inermis*, Loika , Lavalolikka, antifungal, opportunistic pathogen,aspergillus sp.,

INTRODUCTION

Modern life style, increased urbanization and disturbances of ecological balance provide ample scope to microorganisms for their rapid pervasion among world population. On considering different microbial infections, fungal infections are found to be increasing in recent years [1]. Latest investigations revealed that most of the saprophytic fungi of the early days have now changed into opportunistic human pathogen especially among patients with immunological disorders [2]. The fungal infections especially internal infections are difficult to cure using the present antifungal drugs. Some internal infections may lead to secondary infections, tissue necrosis or even organ damage. Fungal toxin like 'Aflatoxin' is very dangerous to man and animals. They are powerful carcinogens [3, 4, and 5].In modern medicine, the drugs available to treat mycoses and other fungal diseases are limited. More over, these drugs have many problems including rapid development of resistance by pathogen, interaction problems between drugs, histotoxicity, and so on [6]. Thus, there is an urgent need for the development of more efficient antifungal agents with fewer limitations, less side effects and a broad-spectrum antifungal activity [7].Since the 19th century, plants and their products have been extensively used in medicine for treating various fungal diseases [8, 9]. The reason is that plants and their parts contain several compounds of different chemical composition called secondary metabolites or natural products. These biologically active compounds may serve as a drug for direct use, as a precursor for synthesis of

other drugs, or as a prototype for synthesis of *de novo* drugs [10].*Flacourtia inermis* Roxb. belongs to the family Flacourtiaceae. This tree is common in rural areas and villages of Kerala State in India. In Kerala, its fruit is commonly known as Loika or Lavalolikka. Fruit is reddish purple in colour with the size of a cherry [11]. In South India, these are used for preparing jams, jellies, syrup and chutney. These fruits have not yet been identified in folk or modern medicine because its antimicrobial and other biological properties are still unknown. However, one report shows that its fruit contains tremendous antioxidant compounds [12]. In the present study, a first attempt is made for investigating the antifungal property of *Flacourtia inermis* fruit against different saprophytic fungi.

MATERIALS AND METHODS

Preparation of the crude extract

Fresh fruits of *Flacourtia inermis* were collected from villages of Kerala state. Fruits were brought to the lab in fresh condition and washed first with tap water and then with saline water followed by distilled water, wiped dry and cut into several small pieces, dried in Hot air oven at 60 °C and was powdered in a mixer grinder. 250 grams of the dried powder was serially extracted with 500 ml each of petroleum ether, acetone, methanol and water using a Soxhlet extractor. The extract was concentrated by vacuum evaporation using a rotary evaporator and the concentrate was stocked in a refrigerator at 4 °C and used for antifungal studies. Only the acetonic extract was used for antifungal studies because other extracts were found to be less active during preliminary antifungal studies. From the acetone-Soxhlet extract stock, aliquot was taken and re-dissolved in 1ml acetone to get a final concentration of 50 mg/ml. This was used for the antifungal susceptibility test. For MIC, 50 mg/ml of acetonic extract was taken as the stock sample. From this stock, 40 mg/ml, 30 mg/ml, 20 mg/ml, 10 mg/ml and 0.5 mg/ml were prepared.

Fugal strains

Fungal strains used for the study were opportunistic human pathogens. They were *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Mucor ramosissimus* and *Chrysosporium* species. Strains were sub cultured on Sabouraud's Dextrose Agar (SDA) medium, incubated at 25 °C – 28 °C for 2 – 10 days and were maintained at 4 °C in a refrigerator.

Antifungal susceptibility test

Antifungal susceptibility test was done by Agar Well Diffusion method [13, 14]. After adding the fungal spore's inoculum into the sterile SDA medium aseptically, it was poured to plates and allowed to set. A well of 6mm diameter was made at the centre of each plate by a sterile cork borer. Depth of the well was sufficient to accommodate 1ml extract. Then 1ml of the acetonic extract with a concentration of 50 mg/ml was poured into the well. For MIC determination, 1 ml of acetonic extract with each respective concentration was added separately into each plate. In control experiment, 1ml of acetone alone (without fruit extract) was added. Both test and control plates were incubated at 25 °C - 29 °C for 2-7 days. After the incubation period, antifungal activity was evaluated by measuring the diameter of zone of inhibition in millimeters. Test was repeated three times, mean value was calculated and recorded as the result.

RESULTS AND DISCUSSION

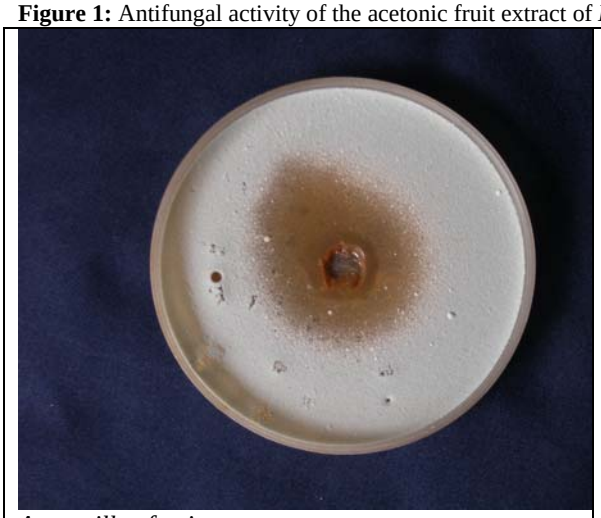
Table 1 and Figure 1 show the antifungal activities of the acetonic extract of fruit of *Flacourtia inermis*. Control experiment did not show inhibitory zone. The solvent, acetone, alone is ineffective to produce antifungal activity. However, acetonic extract showed prominent inhibitory

activity against all the tested strains, which shows that the components of the extract rather than the solvent is responsible for antifungal activity. Maximum susceptibility was given by *Aspergillus fumigatus* with an average inhibition zone of 47 mm against the extract, which is an exciting result from a plant extract. Among the susceptible strains, least susceptibility was shown by *Aspergillus niger*, although its mean zone of inhibition was 30 mm, which is also a promising result when compared to the antifungal activity of other plant extracts. *Aspergillus flavus*, *Mucor ramosissimus*, and *Chrysosporium* sp. were also powerfully inhibited by the fuit extract.

Table 1: Antifungal activity of the acetonic extract of *Flacourtia inermis* fruit against opportunistic pathogenic fungi.

S No.	Fungal Strains tested	Diameter of zone of inhibition in mm			
		Test I	Test II	Test III	Mean value
1	*Aspergillus fumigatus*	45	46	50	47
2	*Aspergillus niger*	30	25	35	30
3	*Aspergillus flavus*	30	32	34	32
4	*Mucor ramosissimus*	42	44	40	42
5	*Chrysosporium* sp.	33	34	35	34

Figure 1: Antifungal activity of the acetonic fruit extract of *Flacourtia inermis* against opportunistic human pathogens



Aspergillus fumigatus: acetone extract

Aspergillus flavus: acetone extract

MIC of the acetonic extract of the *Flacourtia inermis* fruit

MIC represents those lowest concentrations of the acetonic crude extract of the fruit that could effectively inhibit the growth of tested organism. Table 2 and Figure 2 show the MIC of the acetonic extract of fruit of *Flacourtia inermis* against opportunistic pathogenic fungi. 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, bronchoplumonary aspergillosis, pulmonary aspergilloma and different forms of allergies. Respiratory mucosal membrane is the primary target of these pathogens [15]. Infections are more prevalent in persons who have taken multiple antibiotics, steroid drugs, immunosuppressive drugs and patients with AIDS. Hence, they are regarded as opportunistic pathogen [16].of the several *Aspergillus* species, the widespread and dangerous pathogens are *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*. *Aspergillus fumigatus* and *Aspergillus flavus* are known to produce a carcinogenic and a histo-toxic secondary metabolite called 'Aflatoxin'. These fungi, when infects nuts and other food grains cause contamination through its toxin [5, 17, 18] and create serious problems in man and animals.*Aspergillus fumigatus*, in addition to 'aflatoxin',

causes opportunistic lung aspergillosis in patients with previous lung diseases. Its large production of hyphae in the affected lung causes severe damage and malfunctioning of the lung. Sometimes it causes blood clot in lungs, allergies and rhinitis [19]. In MIC studies, this pathogen showed increased susceptibility even at 10 mg/ml concentration of the crude acetonic extract. However, a concentration of 20 mg/ml of the crude extract is required for a better result. In the absence of previous antifungal studies, this work suggests that the acetonic fruit extract of *Flacourtia inermis* can be used as an effective alternative agent against the dangerous pathogen, *Aspergillus fumigatus*. *Aspergillus niger* is another human pathogen but the frequency of its infection is less than that of *Aspergillus fumigatus* and *flavus*. However, its spores can cause lung aspergillosis and otomycosis. It causes stem rot, root rot, boll rot and crown rot diseases in plants, depending on the plant variety [14]. This species also showed effective susceptibility towards the extract at a concentration of 20 mg/ml and is a promising result in the antifungal therapy.*Aspergillus flavus* was found to be a mutagenic and teratogenic agent in man and animals. Its 'Aflatoxin' is recognized as a major cause for certain type of human liver cancer. Its infections include corneal, joint, skin and ear mycosis and lung aspergillosis. Among fungal allergy, it stands in the first place [5, 20]. Analysis of the results showed that a concentration of 20 mg/ml of the crude acetonic extract is sufficient against this pathogen. Here also, increasing the concentration of the sample increases the activity. Hence, this fruit extract is an effective antifungal agent against it.*Mucor* sp. is generally saprophytes, found in soil, plant body and on humus. Among different species, *Mucor ramosissimus* is reported as a pathogen to man and animals. In birds, it causes feather loss and dermatitis [21]. In humans, its spore activates complementary system, which indicates its importance in medical mycology [22]. In the present study, an MIC of 20 mg/ml of the crude extract is found to be effective against it. Therefore, this fruit extract in acetone can be used as an agent against *Mucor* sp.*Chrysosporium* is a keratin dependent saprophytic fungus found in birds, soil, plant materials and dung. For their nutrition, they can digest the keratin of feathers and hairs that fall on land. Occasionally they develop as human pathogen, especially on skin. Increased frequency of infection is shown in patients with granulomatous diseases and bone marrow transplanted patients. This shows their medical significance [19]. When its MIC was determined, it was found that a concentration of 20 mg/ml of the crude acetonic extract is required for satisfactory activity. Therefore, this acetonic extract of *Flacourtia inermis* fruit can be considered as an effective antifungal agent against *Chrysosporium* sp.

Table 2: MIC of the purified compound of *Flacourtia inermis* against opportunistic pathogenic fungi

S. No.	Tested strains	Zone of inhibition in mm				
		40 mg/ml	30 mg/ml	20 mg/ml	10 mg/ml	0.5 mg/ml
1	*Aspergillus fumigatus*	40	32	21	11	0
3	*Aspergillus niger*	25	20	13	8	0
2	*Aspergillus flavus*	24	19	12	7	0
4	*Mucor ramosissimus*	33	27	20	10	0
5	*Chrysosporium* sp.	27	22	14	8	0

Figure 2: MIC of the *Flacourtia inermis* fruit extract against opportunistic pathogenic fungi

Aspergillus fumigatus:- 30 mg/ml of crude acetonic extract

Aspergillus flavus:- 20 mg/ml of the crude acetonic extract

CONCLUSION

The tested fungi are generally saprophytes. However, in man they may cause opportunistic infections especially in immuno- incompetent patients. A mean value between 30 mm to 47 mm of inhibition zone by the acetonic extract at a concentration of 50 mg/ml reveals that the *Flacourtia inermis* fruit is a potential antifungal agent against these opportunistic pathogens. MIC study showed that a concentration of 20 mg/ml of the acetonic crude extract of the *Flacourtia inermis* is sufficient to produce a satisfactory result.For centuries, this fruit is widely used by the people of Kerala. Therefore, it can be assumed that the chemical constituents of its fruits are non-toxic to man. Hence, this fruit can be considered as a better source for developing new antifungal drugs in folk and modern medicine.

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