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Original Research Article

An In-vitro Investigation of the Anti-fungal Efficacy of Carica Papaya Leaf Extract Against Candida Albicans and the Synergistic Effect of the Fluconazole

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Abstract

Aim: To evaluate the anti-fungal activity of Carica papaya leaf extract against candida albicans and its synergy with fluconazole.

Methods: A prospective observational study was conducted in the Department of Pharmacology, Darbhanga medical college and Hospital, Laheriasarai, Darbhanga, Bihar, India from July 2021 to August 2021, 1 mg of plant extract powder was taken and mixed with 1 ml of DMSO obtaining the concentration of 1 mg/ml. This assay determines the studied agent's capability to inhibit the growth of known micro-organism. Minimum Inhibitory concentration is achieved by passing the sample through the method of successive dilution.

Results: The antifungal activity was not demonstrated with aqueous extract of papaya leaf extract with increasing concentrations. In ethanolic extract of plant, demonstrated the antifungal activity against Candida albicans, of which highest concentration showed significant activity with zone diameter of 12.55±0.21 mm. The synergistic activity with Fluconazole and plant extract showed highly significant antifungal activity with zone of inhibition of 13.88±0.78mm when compared with the standard drug (fluconazole) In this test, the plant extract showed moderate antifungal activity with MIC of 300 µg/ml when compared to the

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control. This test also demonstrated the synergistic activity of the antifungal property of the extract with MIC of 130 µg/ml.
Conclusion: The Carica papaya leaf extract has a significant antifungal property and exhibit synergistic effect when used with fluconazole.
Keywords: MIC, fluconazole

Introduction

Carica papaya Linn belonging to family Caricaceae is commonly known as papaya in English, Papita in Hindi and Erandakarkati in Sanskrit. Papaya is a powerhouse of nutrients and is available throughout the year. It is a rich source of threes powerful antioxidant vitamin (C A & E); the minerals(magnesium and potassium;) the B vitamin pantothenic acid and folate and fiber.[1] The plant is native to tropical America and was introduced to India in 16th century. Papaya tree is basically a short lived Indian tree. In the historic times,it was considered as an exotic fruit because of its buttery taste and apperence.[2] The plant is recognised by its weak and usually unbranched soft stem and yielding copious white latex and crowded by a terminal cluster of large and long stalked leaves, is rapidly growing and can grow up to 20m tall.Traditionally leaves have been used for treatment of a wide range of ailments, like in treatment of malaria, dengue, jaundice, immunomodulatory and antiviral activity.[3] Young leaves are rich in flavonoids (kaempferol and myricetin), alkaloids (carpaine,pseudocarpaine, dehydrocarpaine I an d II), phenolic compounds (ferulic acid, caffeic acid,chlorogenic acid), the cynogenetic compounds (benzyl glucosinolate) found in leaves.[4] Both leaf and fruit of the Carica papaya Linn. possess carotenoids namely β- carotene, lycopene,anthraquinones glycoside, as compared to matured leaves and hence possess medicinal properties like anti-inflammatory, hypoglycaemic, anti-fertility, abortifacient, hepatoprotective, wound healing, recently its antihypertensive and antitumor activities have also been established.[3,4] Leaves being an important part of several traditional formulations are undertaken for standardization for various parameters like moisture content, extractive values, ash values, swelling index, etc.[5,6]

Materials and Methods

A prospective observational study was conducted in the Department of Pharmacology, Darbhanga medical college and Hospital, Laheriasarai, Darbhanga, Bihar, India from July 2021 to August 2021, after taking the approval of the protocol review committee and institutional ethics committee.

Preparation of Plant Extract

The fresh leaves of Caricia papaya were procured from the local nursery. The samples were washed, sun dried and made into a powder. The leaves of Caricia papaya were extracted with solvent of aqueous and 70 % ethanol. The containers were kept in the dark for 3-5 hours. Then the solutions were filtered and left in hot air oven at 50^{o}c till the extract got dried. The dried extracts were dissolved in dimethyl sulfoxide, making extracts of different concentrations.

Pathogens

The fungal pathogens (Candida albicans) available with LIFETEK research centre were sub cultured and used in the study.

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Preparation of the Media

Sabouraud dextrose agar was used to culture Candia albicans. Loops full of fungal culture was inoculated in the Sabouraud dextrose agar medium and incubated for 72 hours at room temperature.

Assessment of Anti-fungal Properties

Agar disc diffusion method

Antifungal activity of the sample was determined by disc diffusion method on sabouraud dextrose agar (SDA) medium. Sabouraud dextrose agar (SDA) medium was poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension.

Fluconazole (15 mg) is taken as positive control. The extracts and positive control of 20 µl each were added in sterile discs with each disc containing increasing concentrations as 500 µg/ml, 750 µg/ml and 1000 µg/ml and placed in SDA plates. The plates were incubated at 28ºc for 24 hrs. After the initial culture of the extracts, to assess the synergistic activity the fixed combination of Carica papaya leaf extract and flucanazole were used and cultured again. The antifungal activity was determined by measuring the diameter of zone of inhibition (mm) around the disk, which was measured by vernier caliper.

Minimum inhibitory concentration assay (MIC)

Sample preparation

One mg of plant extract powder was taken and mixed with 1 ml of DMSO obtaining the concentration of 1 mg/ml

MIC Determination:

This assay determines the studied agent's capability to inhibit the growth of known micro-organism. Minimum Inhibitory concentration is achieved by passing the sample through the method of successive dilution. One ml of sterile potato dextrose agar broth was distributed to 8 tubes and was submitted to autoclave under constant pressure at the temperature of 121ºc. After the broth reaches room temperature, 1 ml of diluted sample was added in tube1. Then 1 ml was transferred from tube 1 to tube 2. This transfer was repeated successively until it reaches tube 8. 100 µl of Candida albicans cultures were added to all the tubes from 1 to 8. Incubation was done at 37^0C for 24 hrs. After incubation, the turbidity was observed. MIC, the concentration of higher dilution tubes in which the absence of fungal growth occurred, was noted.

Results

Agar disc diffusion method

The antifungal activity was not demonstrated with aqueous extract of papaya leaf extract with increasing concentrations. In ethanolic extract of plant, demonstrated the antifungal activity against Candida albicans, of which highest concentration showed significant activity with zone diameter of 12.55±0.21 mm (Table 1).

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Table 1: Zone of inhibitions of test and standard drug

S. no.	Test	Zone of inhibition (mm) (Mean±SD)			
		1000 (µg/ml)	750 (µg/ml)	500 (µg/ml)	Fluconazole (1mg/ml)
1	Papaya leaf ethanol extract	12.55±0.21	10.22±0.5	9.06±0.66	13.88±0.78
2	Papaya leaf ethanol extract + Fluconazole	14.1± 0.52	9.1±0.78	8.4±0.77	13.81±0.89
3	Papaya leaf aqueous extract	-	-	-	15±0.5

The synergistic activity with Fluconazole and plant extract showed highly significant antifungal activity with zone of inhibition of 13.88±0.78mm when compared with the standard drug (fluconazole)

Table 2: Minimum inhibitory concentrations of test and standard

Sample	Minimum inhibitory concentration (µg/ml)
Fluconazole (drug)	500
Papaya leaf ethanol extract	300
Papaya leaf ethanol extract+Fluconazole	130
Papaya leaf aqueous extract	-

Minimum inhibitory concentration

In this test, the plant extract showed moderate antifungal activity with MIC of 300 µg/ml when compared to the control. This test also demonstrated the synergistic activity of the antifungal property of the extract with MIC of 130 µg/ml (Table 2). The aqueous extract did not show any activity.

Discussion

Recent years emphasize the need for antifungal agent in the current situation to overcome the eminent crisis due to increased incidence of resistance and antifungal treatment failures. The major causative factor for the development of this crisis owes largely to increase in the immune compromised population and need for prolonged therapy in situations such as HIV patients, organ transplantation and cancer chemotherapy.[7-9]

In this current study, the antifungal activity of the papaya leaf ethanolic extract was clearly demonstrated in all concentrations, when compared to standard. This result was seen in both disc diffusion assay and minimum inhibitory concentration which was in agreement to a study in which revealed that the papaya leaf extract exhibited antifungal property in well diffusion method.[10] Our study showed that the concentration of ethanolic extract effectively suppresses the mycelia growth of Candida albicans and this effect was found to increase with concentration of ethanolic extract. Although, synergistic activity of the Carica papaya leaf extract with the standard drug Fluconazole has not been studied previously. The current study reveals that the papaya leaf extract markedly reduces the MIC, which indicates that the minimum amount of the test components is needed to inhibit the Candida growth, when used along with Fluconazole. Also, the zone diameter was increased when used in combination, stipulating increased sensitivity of the organism to the test compound when compared to the

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individual plant extracts. Considering these results, there is a strong synergistic antifungal activity between ethanolic papaya leaf extract and fluconazole.

The phytochemical constituent of the medicinal plants plays a major role in its therapeutic potential. In a study the phytochemical components of Carica papaya have been studied and they showed many active principles such as alkaloids, carbohydrates, saponins, glycosides, proteins and amino acids, phytosterol, flavinoids, terpinoids and tannins in various extracts. This study also showed that the ethanolic extract contained all the active principles found in the plant extract but the aqueous plant extract showed only alkaloids.[11] In our study, this may attribute to no activity of aqueous plant extract and presence significant antifungal activity in ethanolic plant extract. Also, it may be safe to conclude that the increasing activity with increasing concentrations may be due to the presence of more amount of active principle in higher concentration of the ethanolic extract of Carica papaya. However, further research is needed to unveil the mechanism of action and the specific active component of the extract contributing to the antifungal activity.

From this study, we found that the Carica papaya leaf extract has an antifungal activity and has synergistic effect when used with fluconazole. Further, in-vivo studies with other fungi will assess the potential use of these compounds for extended therapeutic applications.

Conclusion

From this study, we can safely conclude that the Carica papaya leaf extract has a significant antifungal property and exhibit synergistic effect when used with fluconazole. Therefore, this can be considered as a potential agent against human pathogenic fungi in future after meticulous research. This preliminary study was an attempt with positive results and a bridge for future research to develop a potential agent to overcome the emerging public health crisis.

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