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Research Article

Unveiling the Therapeutic Potential of Carica Papaya Leaf Extracts a Study on Antifungal and Anti-Inflammatory Activities

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ABSTRACT

Background: Traditional medical plants have been known to society for a long time. Other than easily available and inexpensive, they are able to cure diseases with few side effects compared to modern medicine. The use of papaya leaves in this study was not only because they contained various chemical compounds that could exert their pharmacological effects, but also the presence of various compounds, which are indicated as Antifungal and Anti-Inflammatory.

Objective: The present research tried to study the antifungal and anti-inflammatory effects of Carica papaya (Red Lady Variety) leaf extracts.

Methods: Freshly harvested Carica papaya leaves of the Red Lady Variety were collected and identified by the herbarium of the Department of Botany at Karachi University. After air-drying, they were chopped, ground into fine powder and extracted with n-Hexane and acetone in a Soxhlet apparatus using 10 grams and 82 grams respectively for drying sample. Extracts after concentration on a rotary vacuum evaporator were stored in a desiccator for further studies and applications. In vitro, agar tube dilution protocol for antifungal activity and oxidative burst assay using chemiluminescence technique for anti-inflammatory.

Results: The in- vitro antifungal Percentage of linear growth inhibition exhibited by n-hexane was between 24% and 40% on different fungal species tested, including Trichophyton rubrum (25%), Candida albicans (40%), Aspergillus niger (25%), Microsporum canis (26%), Fusarium Ini (24%), Candida glabarata (30%) and Aspergillus fumigatus (35%). On the other hand, the acetone extract was able to exhibit growth inhibition rates which were slightly lower, ranging from 20% to 26%. The inhibition rates were as follows: Trichophyton rubrum (23%), Candida albicans (26%), Aspergillus niger (23%), Microsporum canis (25%), Fusarium Ini (20%), Candida glabarata (23%), and Aspergillus fumigatus (23%). In-vitro anti-inflammatory, when tested at 100 µg/mL concentration, it was found that n-hexane leaf extract was able to exhibit 43.6% inhibition of inflammation but was still considered to be less active. Likewise, acetone leaf extract did not show any measurable inhibition when subjected to similar conditions and therefore this also ranked as inactive.

Conclusion: The antifungal inhibition rates were between 23% and 40%, meaning some potential but lower efficiency compared to standard antifungal agents. In the same line, anti-inflammatory activity was moderate where the n-hexane extract only achieved a 43.6% inhibition and the acetone extract had no significant effects at 100 µg/ml.

Keywords: Red Lady, Carica Papaya, Antifungal, Anti-Inflammatory, Acetone And N-Hexane.

INTRODUCTION

Researches have shown that there are many antifungal medicines in the form of the mouth and applied to the skin. Some of these medicines are azoles, polyenes, allylamines and griseofulvin (1 Aneke et al., 2018). Nevertheless, the efficiency of those treatments varies, and in some instances, they fail to work more than 40% of the time, possibly because of resistance (1 Aneke et al.,

2018). This is an urgent call for green alternatives without much effects or toxicity. Papaya fruits have been noted to contain effective antibacterial agents which could be used for drug design purposes in pharmaceutical industries (2 Hariono et al., 2021; 3 Sharma et al., 2022). Additionally, studies carried out in China point out that papaya seed essential oil exhibits great anticandidal activities and hence Carica papaya is among the potential natural antifungal resource (4 He et al., 2017).

From microarray analysis in various biological reactions such as inflammatory diseases associated with tissue damage or microorganisms causing infections leading to symptoms like suffering, hot sensation, swelling, as well as dysfunctioning cells or organs which affects one's day-to-day activities (5 1). Most common synthetic medications like steroids and non-steroidal anti-inflammatory drugs (NSAIDs) are utilized as standard treatment options. However, NSAIDs may cause various adverse effects such as kidney damages fats accumulations (edema) hypertension and gastrointestinal tract hemorrhaging (6 2), while corticosteroids have been linked with joint and tissue dysfunction (7 3). Hence there is an increasing demand for indigenous alternatives that can be used safely over a longer period of time (8 4). As a result, herbal medicines which are less expensive have started gaining attention amongst individuals looking for ways to manage their symptoms naturally (9 5). In particular, Carica papaya leaves appear promising in terms of anti-inflammatory properties. Studies have shown that papaya leaf extract inhibits interleukin-1 β (IL-1β) levels during inflammatory processes (10 7), and reduces oxidative stress which contributes to tissue damage (11 8). Papaya leaf extract has an IC50 of 172.3µg in antidenaturation test (12 Kumar et al., 2021), while papaya leaf juice at concentration of 0.72 ml/100 g body weight was found to alleviate inflammation induced by carrageenan (13 10).

Medicinal plants are the frequently employed as antimicrobial agents for combating fungi and have been utilized traditionally. Carica papaya Linn, a fruit originates its family members Caricaceae, is renowned worldwide for its nutritional and therapeutics values. The different parts of the Carica papaya plant have been exploited for remedial consumptions since ancient times (14 Singh et al., 2020). The ripe fruit is used to treatment chronic forms of skin indurations, sinuses, Chronic skin ulcers, bleeding piles and dyspepsia in various parts of world, stomachic, digestive, diuretic, sedative and tonic (14 Singh et al., 2020). A latest study revealed that this plant possessed many medical benefits such as antibacterial property, antiviral property, anticancer property, the ability to reduce sugar in blood (hypoglycemic effect) as well as anti-inflammatory actions. Papaya leaves are used to for the treatment of malaria. Carica papaya leaves enclose caprine, a component that kills micro-organisms. In addition to these Carica papaya leaves contains phenolic components including protocatechuic acid, p-coumaric acid, 5, 7-dimethoxycoumarin, caffeic acid, kaempferol, quercetin, and chlorogenic acid (15 Aruljothi et al., 2014).

MATERIAL AND METHOD

Study Design: A preclinical study employing in-vitro methods was carried out for six months, in the Department of Pharmacology and Therapeutics located at Baqai Medical College.

Plant Collection and Authentication: Leaves were collected from the Karachi University Garden and authenticated at the Botany Department herbarium (G.H. No. 97627).

Plant Material Extraction: The extraction of Carica papaya 'Red Lady' was carried out at the Industrial Analytical Centre of H.E.J. Research Institute of Chemistry, Karachi, Pakistan. To start, five kilograms of fresh green Carica papaya 'Red Lady' variety were thoroughly cleaned with tap water to eliminate dirt and distilled water rinse to eliminate impurities. After air-drying, they were chopped, ground into fine powder and extracted with n-Hexane and acetone in a Soxhlet apparatus using 10 grams and 82 grams respectively for drying sample. The process was given a period of 72 hours in which the solvent vapour passed through the sample repeatedly then condensed to give out extracts that were collected thereafter. The extracts were then concentrated under reduced pressure at 60 °C using a Buchi Rotavapor R-200 before being dried and kept in a refrigerator for further analyses. This technique helped to extract all components in an efficient manner thereby making them ready for subsequent studies (16).

Agar Tube Dilution Protocol for Antifungal Activity

METHODOLOGY

The stock solution was made by dissolving 24 mg of crude extract and 12 mg of a pure compound in 1 ml of sterile DMSO. This was done to create test samples. A slightly acidic pH of 5.5-5.6 with a 2% glucose or maltose concentration has been achieved by mixing 32.5 grams of Sabouraud dextrose agar medium with 500 ml distilled water which promote fungal growths. The ingredients dissolve in the mixture after steaming it and dispensing it into 4 ml aliquots in screw-capped tubes that are then autoclaved at 121°C for 15 minutes. After cooling down to 50°C, 66.6 µl from the stock solution is added to each tube resulting in final concentrations of 400 µg/ml for the crude extract and 200 µg/ml for the pure compound. The tubes are inclined solidifying at room temperature. Each tube was inoculated with a 4 mm diameter piece of fungus taken from sevens' days old culture. In the non-mycelial growth, an agar surface streaking method is employed instead. Moreover, additional growth media supplemented DMSO are utilized as negative controls while reference antifungal drug are used as positive controls. The incubation period is 3-7 days at 27-29°C with culture observation twice a week.

To quantify fungal development in the media supplemented with compounds, linear growth (mm) is evaluated and growth inhibition computed using the following formula:

% Inhibition = 100 × (1 - linear growth in test / linear growth in control) (17).

Oxidative Burst Assay using Chemiluminescence Technique: Chemiluminescence assay was performed with luminol enhancement. To be succinct, 25 µL of diluted whole blood HBSS++ (Hanks Balanced Salt Solution with calcium chloride and magnesium chloride) [Sigma, St. Louis, USA] was mixed with 25 µL of three different concentrations of compounds (1, 10, and 100 µg/mL) that were analyzed three times; thus, each concentration had three replicates. Control wells contained HBSS++ and cells but no compounds. The assay took place in white half area 96 well plates [Costar, NY] that were put into a luminometer thermostat chamber for 15 minutes at 37 ºC [Labsystems, Helsinki, Finland]. After incubation, 25 µL of serum opsonized zymosan (SOZ) [Fluka, Buchs, Switzerland] and 25 µL of the intracellular reactive oxygen species probe, luminol [Research Organics, Cleveland, OH, USA], were added to each well, except the blank wells (which contained only HBSS++). Luminometer measured ROS (Reactive Oxygen Species) levels expressed as relative light units (RLUs). Ibuprofen was used as assay standard with IC50 value of 11.2 ± 1.9 (18).

RESULTS

Table#1 Antifungal Activity N-Hexane and Acetone Leaves Extracts of Carica Papaya 'Red Lady' Variety

Name of fungus	n-Hexane Leaves extract			Acetone Leaves extract			Std. drugs	MIC (µg/mL)
	Sample Linear Growth (mm)	Control Linear Growth (mm)	% Inhibition Linear Growth (mm)	Sample Linear Growth (mm)	Control Linear Growth (mm)	% Inhibition Linear Growth (mm)		
Trichophyton rubrum	100	100	25	100	100	23	Miconazole	70
Candida albicans	100	100	40	100	100	26	Miconazole	110.8
Aspergillus niger	100	100	25	100	100	23	Amphortericin B	20
Microsporum	100	100	26	100	100	25	Miconazole	98.4

canis								
Fusarium Ini	100	100	24	100	100	20	Miconazole	73.25
Candida glabarata	100	100	30	100	100	23	Miconazole	110.8
Aspergillus fumigatus	100	100	35	100	100	23	Amphortericin B	100

Inhibition of 0–40%: no activity; 40–60%: low activity; 60–70%: moderate activity; 70–100%: significant activity; LG: linear growth (mm)

In this study, we evaluated the antifungal potential of n-hexane and acetone leaf extracts from the Carica papaya 'Red Lady' against various fungi. The results are presented in Table #1. Percentage of linear growth inhibition exhibited by n-hexane was between 24% and 40% on different fungal species tested, including Trichophyton rubrum (25%), Candida albicans (40%), Aspergillus niger (25%), Microsporum canis (26%), Fusarium Ini (24%), Candida glabarata (30%) and Aspergillus fumigatus (35%). On the other hand, the acetone extract was able to exhibit growth inhibition rates which were slightly lower, ranging from 20% to 26%. The inhibition rates were as follows: Trichophyton rubrum (23%), Candida albicans (26%), Aspergillus niger (23%), Microsporum canis (25%), Fusarium Ini (20%), Candida glabarata (23%), and Aspergillus fumigatus (23%). Compared to some standard antifungal drugs like Miconazole and Amphotericin B, these percentages are comparatively higher for different fungi. For example, Miconazole had MIC values of 70 µg/mL against Trichophytonrubrum and up to 110.8 µg/mL against Candida albicans whereas Amphotericin B had MIC values of 20µg/mL against Aspergillus niger and 100µg/mL against Aspergillus fumigatus respectively. Thus, both Carica papaya leaf extracts have less antifungal ability than traditional medicines

Table#2 Antiinflammatory Activity N-Hexane and Acetone Leaves Extracts of Carica Papaya 'Red Lady' Variety

S. No	Extract/Std. Drug	Conc. (µg/ml)	% Inhibition	IC_{50} ± S.D
1	**n-Hexane Leaves extract of Carica papaya 'Red Lady' variety**	100	43.6	Inactive
	Acetone Leaves extract of Carica papaya 'Red Lady' variety	100	-	Inactive
	Ibuprofen	73.2		11.2 ± 1.9

Table #2 summarizes the evaluation of the anti-inflammatory activity of n-hexane and acetone leaf extracts from Carica papaya 'Red Lady'. When tested at 100 µg/mL concentration, it was found that n-hexane leaf extract was able to exhibit 43.6% inhibition of inflammation but was still considered to be less active. Likewise, acetone leaf extract did not show any measurable inhibition when subjected to similar conditions and therefore this also ranked as inactive. In comparison, standard anti-inflammatory drug, ibuprofen had much more pronounced effects with 73.2% inhibition and an IC50 value of 11.2 ± 1.9 µg/mL. This significant difference indicates that though Carica papaya leaf extracts have some level of anti-inflammation property their efficacy is much lower compared to that of ibuprofen. Therefore, though n-hexane extract seems to be more promising, acetone extract may not possess notable anti-inflammatory characteristics.

DISCUSSION

In this work, the antifungal activity of the n-hexane and acetone leaf extract of the 'Red Lady' variety of Carica papaya against a number of fungal strains was evaluated. Although some of the extracts exhibited inhibitory effects, the antifungal activities of both extracts were relatively low compared to standard drugs such as Miconazole and Amphotericin B. The range of the inhibition rate of 23% to 40% for these extracts observed in this study thus reflects their low efficacy in the prevention of fungal growth and, hence, their limited potential as antifungal agents. This might be attributed to the specific chemical composition of extracts, which is likely to be less potent or selective against the tested fungi in this study. Furthermore, the activity of extracts did not exceed the degree considered as significant inhibition of 70-100%, which indicates that extracts do not possess any considerable antifungal activity. These results agree with the conclusion for many studies using natural plant extracts that their antifungal activity was variable and sometimes modest. Similarly, in 2024, Hina Yasin et al. reported that antifungal activity was tested by different fungal cultures against common strains such as Trichophyton rubrum, Aspergillus niger, Microsporum canis, Fusarium lini, Candida glabarata, and Aspergillus fumigatus. It is found out that in the case of ML, AqL, and MB extracts, this effect appeared to be negligible (19). While, in 2009 Ahmad B et al. observed that the crude methanolic extract of O. Griffithii demonstrated moderate antifungal activity against both Aspergillus flavus, 55%, and Fusarium solani, 40%. In contrast, the chloroform fraction showed better activity against the growth of both A. flavus, 59%, and Fusarium solani, 60% (20). However, Aljuhani S et al., in 2024, reported the methanolic extract of the papaya fruits to have strong antifungal activity—zone of inhibition of 37 mm, a minimum inhibitory concentration of 1,000 µg/mL, and minimum fungicidal concentration of 1,900 µg/mL. The results of the MTT assay showed there was low cytotoxicity at concentrations of 20 µg/mL, 50 µg/mL, 100 µg/mL, 150 µg/mL, and 200 µg/mL. Moreover, the IC50 outcome showed that there is significantly decreased cell viability upon treatment with an increased concentration of the extract. In addition, morphologically, the papaya extract induced striking morphological changes in M. canis hyphae and spores (21).

The current study evaluates the anti-inflammatory activity of n-hexane and acetone leaf extracts of Carica papaya 'Red Lady' variety. The results obtained showed weak activity for both extracts. The n-hexane extract showed a 43.6% inhibition at 100 µg/ml. However, it was considered inactive since its effect is relatively very low compared to the standard drug. The acetone extract did not exhibit any inhibitory activity at the same concentration; hence, it was also considered inactive. In contrast, the standard anti-inflammatory drug Ibuprofen showed a significantly higher 73.2% inhibition with an IC50 value of 11.2 ± 1.9 µg/ml, thus proving much more effective. Therefore, these results provide an indication that although there is some potential in extracts of Carica papaya, their anti-inflammatory activity was far lower than that of Ibuprofen, pointing to further research and optimization necessary for improving their therapeutic efficacy.

In 2023, Khan NY described that the antiinflammatory activity of the whole plant methanolic extract of A. oxycedri (WAOME), WAOHF, and the aqueous fraction (WAOAF) was inactive (18). On the other hand, Kamilla L. in 2021 showed that the methanol extract of papaya exhibited significant antiinflammatory effects, with a maximum inhibition of 74.29% at 800 ppm and the most effective concentration at 200 ppm showing 62.19% inhibition (22). Furthermore, Wijesooriya A. A. in 2019 noticed that the aqueous seed extract of the Sri Lankan Red Lady variety of papaya (AESP), at strengths of 75.0-150.0 µg/mL, delivered 15.5%-22.7% membrane stabilization protection to human red blood cells, compared to 50.8%-58.4% protection with aspirin (23).

CONCLUSION

From the results, it is shown that the n-hexane and acetone leaf extracts of Carica papaya 'Red Lady' had minimal antifungal and anti-inflammatory activities. The antifungal inhibition rates were between 23% and 40%, meaning some potential but lower efficiency compared to standard antifungal agents. In the same line, anti-inflammatory activity was moderate where the n-hexane extract only achieved a 43.6% inhibition and the acetone extract had no significant effects at 100 µg/ml.

RECOMMENDATIONS

Other extraction methodologies and solvents should be tried to extract the highest bioactive compound from Carica papaya leaf extracts. Detailed chemical analysis may result in the identification and isolation of active compounds. Combination of such plant extracts with any other antifungal agent or as different plant parts may increase their potential. Alternative extraction procedures and solvents need to be explored for greater anti-inflammatory activity. The chemical analysis at the back has to be properly done to identify the active components, refine and optimize their concentrations for greater efficacy.

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