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Research Article

Antibacterial Activity of Leaves and Stem Extract of *Carica papaya* L.

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ABSTRACT

It was reported that the extracts of papaya leaves could inhibit the growth of some bacterial pathogens. Antibacterial activity of *Carica papaya* leaf extracts on pathogenic bacteria was observed in this study. Papaya leaves were extracted by using maceration method and three kinds of solvents: ethanol and ethyl acetate. Papaya leaf and stem extracts were tested against both Gram positive and Gram Negative bacteria such as *Staphylococcus aureus, Streptococcus pneumonia, Bacillus cereus, Salmonella typhi, Escherichia coli* and *Pseudomonas aeroginosa* by diffusion method. The extract demonstrated higher activities against all the Gram negative bacteria than Gram positive bacteria tested, with the highest activity (16 mm zone of inhibition) demonstrated against *Salmonella typhi*. Increase in temperature enhanced the activity of the extracts, while alkaline pH decreased the activity. The Minimum Inhibitory Concentration (MIC) of the extracts ranged between 50-200 mg/ml. Preliminary phytochemical analyses showed that the extracts contain alkaloids, tannins, saponins and phenols. *Carica papaya* may be used for the treatment of gastroenteritis, uretritis, otitis media, typhoid fever and wound infections.

Keywords: *Carica papaya*, Minimum Inhibitory Concentration, Gastroenteritis.

INTRODUCTION

The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs[1]. Infectious diseases are the world's major threat to human health and account for almost 50,000 deaths every day[2]. The situation has further been complicated with the rapid development of multidrug resistance by the microorganisms to the antimicrobial agents available. *Carica papaya*, belongs to the family of Caricaceae and several species of Caricaceae have been used as remedy against a variety of diseases[3]. Papaya plant (*Carica papaya* L.) is widely found in Indonesia. Almost all parts of the plant can be utilized by humans for food or for medicinal purposes [4, 5]. Its fruits, leaves and flowers are edible. Its roots can be used as medicine for renal and urinary bladder problem, and its seeds have anthelmintic activity[6]. Papaya leaf extracts have phenolic compounds, such as protocatechuic acid, p-coumaric acid and caffeic acid[7]. *Carica papaya* plants produce natural compounds in leaf bark and twig tissues that possess both highly anti – tumour and pesticidal properties. It was suggested that a potentially lucrative industry based simply on production of plant biomass could develop for production of anti – cancer drugs, pending Food and Drug Agency approval and natural (botanical) pesticides[8]. The papaya fruit, as well as all other parts of plant, contain a milky juice in which as active principle known as papain is present. The juice has been in use on meat to make it tender[9]. The seed is used for intestinal worms when chewed. The root is chewed and the juice swallowed for cough, bronchitis and other respiratory diseases. The unripe fruit is used as a remedy for ulcer and impotence[10]. This research was done to observe the antibacterial activity of papaya leaf extracts against pathogenic bacteria.

MATERIALS AND METHODS

Processing of plant samples

Plant materials were collected from in an around Sundarakkottai, Mannargudi, Thiruvarur District. The fresh roots and leaves were harvested and properly washed in tap water, and then rinsed in

sterile distilled water. The root and leaves was dried in the hot air oven at 40º C for 3 days. The dried roots and leaves were pulverized, using sterile laboratory mortar and pestle, to obtain a powered form. These were stored in airtight glass containers protected from sunlight until required for analysis.

Preparation of extracts

The leaves and root powder was extracted with ethanol and ethyl acetate of 95% in soxhlet extractor 72 hours. After exhaustive extraction, the leaves extract and root extract were filtered and concentration with the help of rotary evaporator [11].

Phytochemical screening

The portion of the dry extract was subject to the Phytochemical screening using various method[12]. Phytochemical screening was performed to test for alkaloids, saponin, tannins, falvanoids, Carbohydrate and Glycosides.

Experimental microorganism

The experimental organisms were isolated from clinical samples from hospital patients from Government Hospital, Mannargudi, Thiruvarur District. Purity plates of each of the bacterial isolates were obtained by culturing on their respective selective media. Biochemical tests were performed to re-identify and confirm the identity of the isolates. Fresh plates of the test bacteria were made from the isolate cultures obtained on agar slants. Discrete colonies of fresh cultures of the different bacterial isolates were then picked and suspended in 5 ml Nutrient broth and incubated for 24 hours at 37º C prior to antimicrobial susceptibility testing.

Identified organisms

Gram negative strains: *Escherichia coli*, *Pseudomonas aeroginosa* and *Salmonella typhi*. Gram positive strains: *Staphylococcus aureus* and *Bacillus subtilis*.

Determination of antimicrobial activity

Antibacterial activity of the aqueous and organic extracts of the palnt sample was evaluated by the agar well diffusion method[13]. 0.2 ml of seeded broth culture containing 10^{-6} to 10^{-7} cfuml-1 of the test organism was inoculated in solidified agar plates. Two or three wells were made in agar layer of each petridish by a steel borer. To these the aliquots of 100 ml of extract dilutions, reconstituted in 50% ethanol and ethyl acetate organic solvent extracts and distilled water at concentrations of 250, 200, 150 and 100 mg/ml were applied in each of the wells in culture plates previously seeded with the test organisms. The cultures were incubated at 37º C of 24 hours. The antibacterial potential of test compound was determined on the basis of diameter of zone of inhibition around the wells[14, 15].

Determination of Minimum Inhibitory Concentration (MIC)

MIC of extracts was determined using turbidity method in nutrient broth medium. The experiment was conducted according to serial dilution method. The suspension of seeded broth was made by transferring 2 ml of the seeded broth to 100 ml of the 0.9% w/v of the sterilized saline solution. The stock solution of test compounds were prepared at concentration of 50 – 200 mg/ml. 0.1 ml normal saline suspension was added to each assay tube. The procedures were conducted under strict aseptic conditions. The inoculated tubes were kept at 37º C for 24 hours for bacterial assay. After incubation period, tubes were removed and observed for any deposits and shaken to suspend bacteria that might have been settle down. MIC values were determined by checking for the absence of visual turbidity[16].

RESULTS

Phytochemical analysis of *C.papaya*

Pytochemical screening of *Carica papaya* leaves showed the presence of Alkalaoids, Carbohydrates, Saponins, Glycosides, Phenolic compounds, Flavinoids and Tannins (Table 1). The presence of Saponins, Glycosides, Flavinoids showed greater intensity of their presence in ethanol, ethlyacetate and water extract.

Evaluation of antibacterial potential of *C.papaya*

The ethanolic and ethyl acetate extracts of leaves and root of *C. papaya* were screened for their antimicrobial activity against different strains of Gram negative (*Escherichia coli*, *Pseudomonas aeroginosa* and *Salmonella typhi*) and Gram positive strains (*Staphylococcus aureus* and *Bacillus subtilis*). The antibacterial action was shown in the form of zone of inhibition as given in table 2. The antibacterial action of leaves was more than the root, moreover both extracts showed dose dependent activities. In addition to having good activity against other bacteria, when compared to aqueous and ethyl acetate extract the ethyl acetate extract of leaves exhibited strong activity against *S. typhi* having zone of inhibition 12 mm, 14 mm and 18 mm at the dose of 150, 200 and 250 mg/ml respectively. While the significant activity of the root was observed against *S.typhi* having zone of inhibition 10 mm, 12 mm and 14 mm at the dose of 150, 200 and 250 mg/ml respectively. The ethanolic extract leaves and roots moderately to kill all the bacterial pathogens than aqueous extract of leaves and root.

Evaluation of Minimum Inhibitory Concentration (MIC) of *C. papaya*

Table 3 shows the results of MIC determination on the test organisms. While the MIC values ranging between 100 – 200 mg/ml were demonstrated

against the rest of the test bacteria. The MIC of ethyl acetate extract of leaves against *S.aureus, S.pneumoniae, E.coli* and *P.aeruginosa* was 150 mg/ml. The MIC of ethyl acetate extract of root against *S.aureus, S.pneumoniae, E.coli* and *P.aeruginosa* was 100 mg/ml respectively. The increase of antibiotic resistance of microorganisms to conventional drugs has necessitated the search for new, efficient and cost effective ways for the control of infectious diseases.

DISUSSION

The presence of bioactive substances have been reported to confer resistance to plants against bacteria, fungi and pests and therefore explains the demonstration of antibacterial activity by the plant extracts used in this study[17]. The results of this study showed that the organic extracts were more effective than aqueous extracts and the ethyl acetate extracts demonstrated the highest activity. This may be due to the better solubility of the active components in organic solvents[18]. Among the gram – positive and gram – negative bacteria tested, gram – negative bacteria were more susceptible to the extracts. This result, however, is at disparity with an earlier report indicating that plant extracts are more active against gram- positive bacteria than gram-negative bacteria[19]. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents[20, 21]. High MIC may be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds. Temperature stability of plant extracts has been reported earlier[22]. This study demonstrated that the herbal medicine can be as effective as modern medicine to combat pathogenic microorganisms. Using different purification, isolation and characterization methods, antimicrobial principals can be obtained and thus the activity of antimicrobial compounds can be improved for further pharmaceutical uses.

Table 1: Phytochemical analysis of *Carica papaya* leaf Extract

Phytochemicals	Test performed	Water extract	Ethanol Extract	Ethyl acetate extract
Alkaloids	Dragenodroff's test	+	+	+
Carbohydrates	Molish test	-	+	+
Saponins	Chloroform and H2SO4 test	-	+	+
Glycosides	Molish test	-	+	+
Phenolic compounds	Ferric chloride test and Lead acetate test	-	-	+
Flavinoids	Shinoda test	-	+	+
Tannins	Neutral Fecl3	-	-	-

+ Positive, - Negative

Table 2: Antibacterial activity of leaf and root extracts of Carica papaya on the test organisms

Organisms	Leaf Extract (mg/ml) / zone of inhibition (mm)												Root Extract (mg/ml) / zone of inhibition (mm)											
	100			150			200			250			100			150			200			250		
	WE	EE	EAE	WE	EE	EAE	WE	EE	EAE	WE	EE	EAE	WE	EE	EAE	WE	EE	EAE	WE	EE	EAE	WE	EE	EAE
E.coli	0	8	8	0	8	8	0	6	8	0	10	12	0	2	2	0	2	8	0	4	4	0	6	8
P.aeruginosa	0	10	8	0	6	10	0	8	8	0	12	12	0	2	2	0	4	2	0	4	4	0	6	8
S.typhi	0	8	6	0	10	12	0	8	14	0	12	18	0	2	8	0	4	10	0	4	12	0	6	14
S.aureus	0	6	6	0	8	10	0	6	10	0	10	14	0	2	4	0	4	4	0	8	8	0	4	10
B.subtilis	0	6	8	0	6	10	0	6	12	0	8	14	0	0	2	0	2	4	0	8	6	0	4	8

WE: Water Extract; EE: Ethanol Extract; EAE: Ethyl Acetate Extract

Table 3: Minimum Inhibitory Concentration (MIC) of leaf and root extracts of Carica papaya on the test organisms

Organisms	Leaf Extract - MIC (mg/ml)			Root Extract – MIC (mg/ml)		
	WE	EE	EAE	WE	EE	EAE
E.coli	+++	100	150	+++	50	100
P.aeruginosa	+++	50	150	+++	50	100
S.typhi	+++	100	100	+++	100	50
S.aureus	+++	50	150	+++	50	100
B.subtilis	+++	50	150	+++	50	100

+++: Profuse Growth; WE: Water Extract; EE: Ethanol Extract; EAE: Ethyl Acetate Extract

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