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The efficacy of *Carica papaya* leaf extract on some bacterial and a fungal strain by well diffusion method

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ABSTRACT

Objective: To investigate the antimicrobial activity and phytochemical screening Ethanol, methanol, Ethyl acetate, acetone, chloroform, Petroleum ether, hexane, hot water, and extracts of *Carica papaya*. **Methods:** The aim of the present study was to evaluate the qualitative analysis of phytochemicals and antimicrobial activity of various solvent extracts of *Carica papaya*. The antimicrobial activities of different solvent extracts of *Carica papaya* were tested against the Gram-positive and Gram-negative bacterial strains and fungus by observing the zone of inhibition. The Gram-positive bacteria used in the test were *Staphylococcus aureus*, *Bacillus cereus* and *Micrococcus luteus*, and the Gram-negative bacteria were *Escherichia coli*, and *Klebsiella pneumoniae*, fungus like *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Candida tropicalis*, *Cryptococcus neoformans*, and *Candida kefyr*. **Results:** It was observed that ethanol, methanol, ethyl acetate, acetone, chloroform, petroleum ether, hexane and aquas extracts showed more activity against bacteria and fungus. The chloroform extract of *Carica papaya* showed more activity against *Micrococcus luteus*, zone of diameter 15.17 ± 0.29 mm and acetone extract of *Carica papaya* showed more activity against *Candida albicans*, zone of diameter 11.23 ± 0.25 mm compared to other solvent extracts. **Conclusions:** In this study chloroform extract in bacteria and acetone extract in fungus showed a varying degree of inhibition to the growth of tested organism, than Ethanol, methanol, Ethyl acetate, Petroleum ether, hexane and hot water extracts. The results confirmed the presence of antibacterial and antifungal activity of *Carica papaya* extract against various human pathogenic bacteria. Presences of phytochemical and antimicrobial activity are confirmed.

1. Introduction

Carica papaya, belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases^[1] Originally derived from the southern part of Mexico, *Carica papaya* is a perennial plant, and it is presently distributed over the whole tropical area. In particular, *Carica papaya* fruit circulates widely, and it is accepted as food or as a quasi drug. Many scientific investigations have been conducted to evaluate the biological activities of various parts of *Carica papaya*, including fruits, shoots, leaves, rinds, seeds, roots or latex.

The leaves of papaya have been shown to contain many

active components that can increase the total antioxidant power in blood and reduce lipid peroxidation level, such as papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates^[2]. In spite of the concurrent use of the extract of *Carica papaya* with prescription oral hypoglycemic agents in some patients^[3, 4] there is a dearth of literature on the effects of the extract on activity of oral hypoglycaemic agents.

Carica papaya plants produce natural compounds (annonaceous acetogenins) 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^[5]. The high level of natural self-defence compounds in the tree makes it highly resistant to insect and disease infestation^[6]. *Carica papaya* L. leaf tea or extract has a reputation as a

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tumour–destroying agent[7]. The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present. Aside from its value as a remedy in dyspepsia and kindred ailments, it has been utilized for the clarification of beer. The juice has been in use on meat to make it tender[8]. 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[9]. The present study was carried out to test the antibacterial efficacy of the leaves extract of *Carica papaya* against bacterial spp.

2. Materials and methods

All the chemicals and reagents used were from C.D.H and Ranchem. Glass wares used were from Borosil. The media and broth used for microbial culture were from Hi–Media Pvt. Limited, Bombay, India.

2.1. Collection of Plant

Fresh leaves of *Carica papaya* leaf were collected during June–July of 2010 in and around Arakkonam, Tamilnadu were authenticated by Department of Botany. The voucher specimens were kept in the Department of Botany in C. Abdul Hakeem College, Melvisharam, Vellore, Tamilnadu, India.

2.2. *Carica papaya* leaves extract preparation

All the laboratory works are done in Microlabs, Institute of Research and Technology, Arcot, Tamil Nadu, India. The collected plant leaves were washed thoroughly 2–3 times with running water and with distilled water. The leaves were air–dried under shade. The leaves were crushed to make possible fine powder with the help of mortar and pestle and stored for further analysis. Then this powdered samples (100g/100ml) in hot water, ethanol, methanol, chloroform, Ethyl acetate, Petroleum ether, hexane and acetone extracts for Overnight at room temperature. Soxhelt apparatus are used for this extraction. The extract from three consecutive soaking are pooled and evaporated under pressure.

The crude samples were subjected to phytochemical screening for the presence of amino acids, proteins, saponins, triterpenoids, flavonoids, carbohydrates, alkaloids, phytosterols, glycosidal sugars, protein, tannins, and phenols.

2.3. Phytochemical screening of the extract

The portion of the dry extract was subjected to the Phytochemical screening using the method adopted by Trease, Evans and Harbourne[10, 11]. Phytochemical screening

was performed to test for alkaloids, saponin, tannins, flavanoids, steroids, sugars and cardiac glycosides.

2.4. Antimicrobial study

The bacterial spp. used for the test were *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Micrococcus luteus*, (*M. luteus*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumonia* (*K. pneumonia*) The fungus spp used for the test were *Aspergillus niger* (*A. niger*), *Aspergillus flavus* (*A. flavus*), *Candida albicans* (*C. albicans*), *Candida tropicalis* (*C. tropicalis*), *candida kefyr* and *Cryptococcus neoformans* All the stock cultures were obtained from Microlab, Institute of Research and Technology, Vellore, Tamilnadu, India. The microorganisms were grown overnight at 37°C in Mueller–Hinton Broth at pH 7.4[12, 13].

2.5. Culture media and inoculums preparation

Nutrient agar /broth (Himedia, India.) were used as the media for the culturing of bacterial strains. Loops full of all the bacterial cultures were inoculated in the nutrient broth and incubated at 37°C for 72 hrs and Potato dextrose agar / and potato dextrose broth (Himedia, India) were used as the media for the culturing of fungal strains. Loops full of all the fungus cultures were inoculated in the potato dextrose broth (PDA) and incubated at room temperature for 72 hrs.

2.6. Testing for antibacterial activity

The extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotic Ciprofloxacin (10 µg/mL) in–vitro by well diffusion method[14–16]. The cup–plate agar diffusion method was employed to assess the antibacterial activity of the prepared extracts[17]. 20 ml of the inoculated nutrient agar were distributed into sterile petri dishes. The agar was left to set and in each of these plates, 5 mm in diameter, were cut using a sterile cork borer No. 4 and the agar discs were removed[18]. Alternate cups were filled with 20 µL of each extracts using microtiter–pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 hours. The respective solvents were used as controls. The diameters of the growth inhibition zones were measured at 24 hours of incubation averaged and the mean values were tabulated.

2.8. Antifungal activity

The extracts were also screened for their antifungal activity in comparison with standard antibiotic ketoconazole (10 µg/mL) in–vitro by well diffusion method[14–16]. Lawn culture was prepared using the test organism on potato dextrose broth (PDA). The inoculated plates were kept aside for a few

minutes. Using well cutter, four wells were made in those plates at required distance. Using sterilized micropipettes 30 μ L of different solvents with selected *Carica papaya* leaf extract was added in to the well. The plates with yeast like fungi were incubated at 37°C for overnight. The plates with mold were incubated at room temperature for 48 hrs. The activity of the extract was determined by measuring the diameters of zone of inhibition. For each fungal strain, controls were maintained where pure solvents were used instead of (*Carica papaya* leaf) extracts.

2.9. Statistical analysis of data

The data obtained were subjected to ANOVA test to determine the significance extracts in antimicrobial activity of *Carica papaya* leaf. The values are expressed in mean \pm SEM,

3. Results

Phytochemical screening of *Carica papaya* leaves showed the presence of Alkaloids, Carbohydrates, saponins, glycosides, Proteins& aminoacids, Phytosterol, Phenolic compounds, flavonoids, Terpinoids Terpinoids, Tannins (Table 1). The presence of Phytosterol in *Carica papaya* was very prominent in all the extracts. The saponins, Glycosides, Proteins&aminoacids, Flavonoids, Terpinoids, showed greater intensity of their presence in Ethanol, methanol, Ethyl alcohol and acetone extraction than other.

The results of antibacterial activity are given in the Table 2, which clearly show that all the extracts have shown antibacterial activity equivalent to that of standard against the entire tested organisms. Ethanol, methanol, Ethyl acetate, acetone, chloroform and hotwater extracts have shown better activity against all the six microorganisms.

Table 1. Preliminary phytochemical analysis of *Carica papaya* leaf .

Phytochemicals	Test performed	Ethanol extracts	Methanol extract	Ethyl acetate	Acetone extracts	Chloroform	Petroleum ether	Hexane	Aquas extract
Alkaloids	Dragendorff's test	+	-	+	+	+	+	+	+
Carbohydrates	molish test	+	-	+	-	+	+	-	-
Saponins	Chloroform and H ₂ SO ₄ test	+	+	+	+	-	-	-	-
Glycosides	molish test	+	+	+	+	-	+	-	-
Proteins& aminoacids	Millon's Test	+	+	+	+	-	-	-	-
Phytosterol	Liebermann–Burchard's Test	+	+	+	+	+	+	+	-
Phenolic compounds	Ferric chloride test and Lead acetate test	-	-	+	+	+	+	-	-
Flavinoids	Shinoda test	+	+	+	+	-	-	-	-
Terpinoids	Noller's test	+	+	+	+	-	+	-	-
Tannins	Neutral FeCl ₃	-	-	-	-	+	+	+	-

(+) Positive (-) Negative.

Table 2. Inhibition zone diameter of extracts against bacteria. Antibacterial activity of different extracts of *Carica papaya* leaf against Differents organisms (Mean \pm SEM) (mm).

Organism	Ethanol	Methanol	Ethyl Acetate	Acetone	Chloroform	Petroleum either	Hexane	Hot water	ciproflaxacin
<i>Escherichia coli</i>	8.30 \pm 0.26	7.17 \pm 0.15	9.17 \pm 0.15	8.07 \pm 0.12	13.17 \pm 0.29	-	-	-	23.50 \pm 0.50
<i>Micrococcus luteus</i>	8.23 \pm 0.25	6.00 \pm 0.00	7.07 \pm 0.12	-	15.17 \pm 0.29	-	-	-	16.97 \pm 0.45
<i>Pseudomonas aeruginosa</i>	8.23 \pm 0.21	6.30 \pm 0.15	10.20 \pm 0.20	10.83 \pm 0.29	10.07 \pm 0.12	-	-	-	25.0 \pm 0.50
<i>Bacillus cereus</i>	9.20 \pm 0.26	8.17 \pm 0.15	9.17 \pm 0.29	8.20 \pm 0.20	-	-	-	-	21.83 \pm 0.29
<i>Klebsiella pneumoniae</i>	6.17 \pm 0.15	-	8.07 \pm 0.12	8.17 \pm 0.15	12.17 \pm 0.29	-	-	-	14.83 \pm 0.29
<i>Staphylococcus aureus</i>	8.20 \pm 0.20	7.00 \pm 0.00	7.17 \pm 0.29	6.0 \pm 0.00	8.07 \pm 0.12	-	-	7.0 \pm 0.21	29.83 \pm 0.76

Table 3. Inhibition zone diameter of extracts against fungus.

Organism	Ethanol	Methanol	Ethyl Acetate	Acetone	Chloroform	Petroleum either	Hexane	Hot water	ketoconazole
<i>Aspergillus niger</i>	7.07 \pm 0.12	6.0 \pm 0.00	8.03 \pm 0.15	6.10 \pm 0.10	5.07 \pm 0.12	-	-	-	7.40 \pm 0.53
<i>Aspergillus flavus</i>	6.20 \pm 0.20	5.07 \pm 0.12	8.07 \pm 0.31	10.17 \pm 0.29	6.07 \pm 0.12	-	-	-	8.23 \pm 0.25
<i>Candida albicans</i>	8.23 \pm 0.25	9.30 \pm 0.26	7.0 \pm 0.00	10.23 \pm 0.25	5.93 \pm 0.12	-	-	-	8.83 \pm 0.29
<i>Candida tropicalis</i>	10.0 \pm 0.20	8.0 \pm 0.20	5.07 \pm 0.12	9.07 \pm 0.12	7.13 \pm 0.32	-	-	-	10.10 \pm 0.36
<i>Cryptococcus neoformans</i>	7.0 \pm 0.20	8.0 \pm 0.00	5.07 \pm 0.12	6.07 \pm 0.12	6.0 \pm 0.20	-	-	-	13.17 \pm 0.29
<i>Candida kefyr</i>	6.0 \pm 0.00	5.0 \pm 0.00	6.17 \pm 0.15	5.03 \pm 0.6	5.07 \pm 0.12	-	-	-	11.17 \pm 0.29

Antifungus activity of different extracts of *Carica papaya* leaf of against differents organisms (Mean \pm SEM) (mm).

Ethanol extract was more effective against *B.cereus*. Methanol extract was more effective against *B. cereus* and *E.coli*. Ethyl acetate extract was more effective against *P.aeruginosa*, *E. coli* and *B. cereus*. Acetone extract was more effective against *P.aeruginosa* and *B. cereus*. Chloroform extract was more effective against *Micrococcus luteus* and *E. coli*. Petroleum ether and Hexane extract was noeffective against bacteria. Hot water extract was more effective against *S. aureus*.

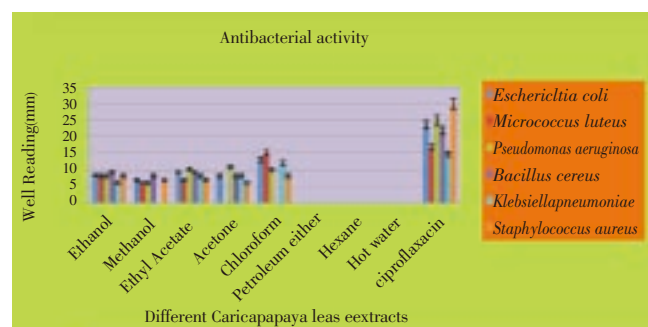


Figure 1.

The results of antifungal activity are given in the Table 3, which clearly show that all the extracts have shown antifungal activity against the entire tested organisms. Ethanol, methanol, Ethyl acetate, acetone, chloroform extracts have shown better activity against all the five microorganisms. Ethanol extract was more effective against *C. tropicalis*. Methanol extract was more effective against *C.albicans*, *C.tropicalis* and *C.neoformans*. Ethyl acetate extract was more effective against *A.flavus*, and *A.niger*. Acetone extract was more effective against *C.albicans*, and *A.flavus*. Chloroform extract was more effective against *C.tropicalis* and *A.flavus*. Petroleum ether extract, Hexane extract and Hot water extract was noeffective against fungal strain.

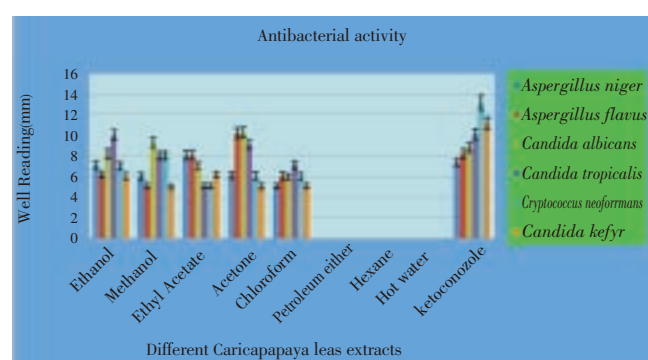


Figure 2.

4. Discussion

The Therapeutic value of medicinal plants lies in the various chemical constituents in it. The bioactivity of plant extracts is attributed to phytochemical constituents. For instance, plant rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water soluble compounds

thereby killing the bacteria by directly damaging its cell membrane[19]. Flavonoids are a major group of phenolic compounds reported for their antiviral[20], antimicrobial and spasmolytic properties. Alkaloids isolated from plant are commonly found to have antimicrobial properties[21]. The presence of saponins supports the fact that pawpaw leaf has cytotoxic effects such as permealization of the intestine as saponins are cytotoxic[22]. It also gives the leaves the bitter taste. Saponin has relationship with sex hormones like oxytocin. Oxytocin is a sex hormone involved in controlling the onset of labour in women and the subsequent release of milk[22]. Another important action of saponins is their expectorant action through the stimulation of a reflex of the upper digestive tract[5]. Alkaloids are the most efficient therapeutically significant plant substance. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties[23]. They show marked physiological effects when administered to animals. The presence of alkaloids in the leaves shows that these plants can be effective anti-malaria, since alkaloids consist of quinine, which is anti-malaria[8]. The cardiac glycosides therapeutically have the ability to increase the force and power of the heart-beat without increasing the amount of oxygen needed by the heart muscle. They can thus increase the efficiency of the heart and at the same time steady excess heart beats without strain to the organ[24]. Deficiency of ascorbic acid is associated with pains in the joint and defect in skeletal calcification, anaemia, manifestation of scurvy haemorrhage from mucous membrane of the mouth and gastrointestinal tract[25]. This function of ascorbic acid accounts for its demand for normal wound healing. There is also an interesting ability of ascorbic acid as an antioxidant, to prevent or at least minimize the formation of carcinogenic substances from dietary material[26]. As a result of the presence of ascorbic acid in *carica papaya* leaves, the plant can be used in herbal medicine for the treatment of common cold and other diseases like prostate cancer. Other vitamins though in trace amount are essential for body metabolism[27].

This study has shown the phytochemicals and antimicrobial activity. It is concluded that the plant extract possess microbial activity against tested organisms. The zone of inhibition varied suggesting the varying degree of efficacy and different phyto constituents of herb on the target organism. The antimicrobial activity of the plants may be due to the presence of various active principles in their leavies. Further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs.

Conflict of interest statement

We declare that we have no conflict of interest.

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