St. Joseph's Journal of Humanities and Science
ISSN: 2347 - 5331
http://sjctnc.edu.in/6107-2/

Antimicrobial Studies of Plant Parts of *Carica papaya* against Pathogens

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Abstract

The plant parts (seed, root &leaf) of *Carica papaya* were collected from Kuchipalayam, Villupuram district. The collected plant parts were washed with tap water to remove the dust particles, shade dried and grounded the samples. Powdered seed, root and leaf parts of *Carica papaya* were individually extracted with solvents such as ethanol, methanol, acetone and water. The antibacterial activity of the plant parts (seed, root, leaf) extracts of *Carica papaya* were tested against the 4 ATCC cultures. The seed ethanol extract of *Carica papaya* showed maximum inhibitory activity against E coli (ATCC-8739) (16mm) and the root ethanol extract of *Carica papaya* showed maximum activity against *S aureus*(ATCC-25923) (13mm). GC/MS analysis was carried out for the *Carica papaya* seed extract in Indian Institute of Food Processing Technology (Tanjore) and ten compounds were identified such as Benyl nitrile, Thiocyanic acid, Phenylmethyl ester, n-Hexadecanoic acid, Oleic acid, Octadecanoic acid, Glycerol 1-Palmitate, β-Monoolein, 17-Octadecynoic acid, 16-Hydroxyhexadecanoic acid and β-Sitosterol.

Key words: *Carica papaya*, ethanol, methanol, acetone and Gas chromatography-Mass spectrometry.

INTRODUCTION

Carica papaya is a small, fast growing tree, which grows from approximately 3 to 12 meters long. The fruit of *Carica papaya* is smooth skinned, yellow to greenish brown in colour, the yellow soft ''Custard like'' pulp having sweet flavour and contain dark centered seeds within it [1-8]. The leaves are large, 50-70 cm in diameter.The phytochemicals are present in leaves, stem, bark, root, rhizome, flowers and seeds.

The fruit of *Carica papaya* contain Protein, Fat, Fiber, Carbohydrates, Amino acid, Citric acid, Malic acid, Minerals such as Calcium, Iron, Vitamin-C, Thiamine, Riboflavin, Niacin and Carotene.The volatile compounds are benzylisothiocyanate, cis and Trans 2, 6-dimethyl-3, 6 epoxy-7 octen-2-ol, alkaloids and carpaine.Seed containsFatty acids, crude protein, crude fiber, papaya oil, Carpaine, Caricin, Glucotropacolin and a Myrosin enzyme.Leaves contain Alkaloids, Carpain,

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Pseudocarpain, Dehydrocarpain I and II, Choline, Carposide, Vitamin C and E.Root contains Carposides and an enzyme Myrosin.

The skin, flesh and seeds of *Carica papaya* have antibacterial effects against numerous bacteria such as *Staphylococcus aureus, Bacillus cereus, Escherichia coli*, *Enterobacter cloacae*, *Proteus vulgaris, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa* and *Shigella flexneri*.The root aqueous extract of *Carica papaya* is used to treat Syphilis .*Carica papaya* fruit is rich in nutrient source and the compound of danielone is a phytoalexin have high antifungal acvivity against *Collectotrichum gloesporiodes*[10].The seeds of *Carica papaya* have antiparasitic activity against *Trichomonas vaginalis*, *Entamoeba histolytica* and *Dirofilaria immitis*. Bioactive compounds chymenopapain and papain are present in leaves of *Carica papaya* which is active against malaria.

The green fruits of *Carica papaya* is active against hepatitis .The leaves of *Carica papaya* contain Carpaine, which has high antioxidant activity. Seeds of *Caricapapaya* contain bio active compounds such as flavonoids which is responsible for anticancer and anti oxidant activity. The fruit of *Carica papaya* are rich in β-carotene which prevents cancer and the aqueous leaf extract of *Carica papaya* have anti-tumor activity. [10]. The leaf extracts of *Carica papaya* have larvicidal properties against *Aedes aegtypti* mosquito which is the vector of dengue. The leaves of *Carica papaya* have wound-healing activity and it also prevent the multiplication of wound infection causing bacteria [9].

Multidrug resistant microbial pathogens are a major concern for health problem and increasing morbility and mortality rates in developing countries . The herbal medicine leads to develop better drugs against multidrug resistant microbial pathogens . The plant parts of *Carica papaya* have many antimicrobial activities against many pathogens which can be used to treat many diseases. In the present study the solvent extracts of plant parts (seed, root and leaf) of *Carica papaya* was studied for its bioactive potential against 4 ATCC cultures.

MATERIALS AND METHODS

Collection of Plant Parts of *Carica Papaya*

The plant parts such as leaf, root, seeds of *Carica papaya* were collected from Kuchipalayam, Villupuram district, Tamilnadu.

Processing of Plant Samples

The plant material were collected and cleaned with water to remove the soil and dust particles on the surface of the samples. The leaf, root and seed samples of *Carica papaya* were shade dried in a dark place at room temperature for few days (upto the samples get shade dried) [7]. The dried plant samples were ground into a fine powder and stored in clean airtight plastic containers for further use.

Preparation of Plant Extracts

The prepared powder sample of *Carica papaya* leaf, root and seeds were mixed with different solvents like ethanol, methanol, acetone and water individually [4]. Then they were kept at room temperature for 72 hours. Each mixture was stirred for every 24 hours using glass rod. These mixtures were then filtered through Whatmann no.1 filter paper. Exrtaction procedure was done further twice for extraction of the bioactive compounds. The filtrate was collected in a separate container and concentrated by evaporating the solvent.

Preparation of Plant Extract Loaded Sterile Discs

Stock solution of the plant extract were prepared (100 mg/ml) and the sterile disc were allowed to dipped in the extracts for few minutes for proper absorption, after which they were removed and allowed to dry at room temperature. After drying they were used for checking antimicrobial activity [6].

Antibacterial Activity of Plant Parts Extract of *Carica Papaya*

The disc diffusion technique was used to determine the antibacterial activity of the extract. The solvent extracts (ethanol, methanol, acetone and water) of leaf, root and seed samples of *Carica papaya* were prepared and tested against pathogenic organisms *Escherichia coli* (ATCC-8739), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853) and *Salmonella typhimurium* (ATCC-14028).

Muller Hinton agar was poured into the sterile Petri plates and allowed to set. The 4 hours cultures of test isolates were swabbed aseptically on the agar plates. The *Carica papaya* extract discs were then gently placed on the plates at equal interval. Then the plates were incubated at 37 C for 24 hours. The diameter of the zone of inhibition was measured in mm after 24 hours.

The plant part extract which showed inhibitory activity in 100mg/ml concentration was further studied for inhibitory activity by preparing different stock concentrations such as 75mg/ml, 50mg/ml, 25mg/ml and 10mg/ml. The activity of plant extracts were studied by well diffusion method using Muller Hinton agar plates.

GC/MS Analysi

Capillary column 30m × 0.25 mm coated with 0.25 μM film of HP-5.Sample elution using 50: 1 helium was used as carrier gas at 1.0 ml min^{-1}.Column temperature, 100˚C for 1 minutes increased 10˚C min^{-1} to 275˚C $min^{-1}$for 20 minutes.Time taken for chromatography per sample was 40 minutes.

Analysis of the Phytocomponents in *Carica Papaya* Seed using GC-MS Technique

One micro litre of the filtrate was injected into the GC-column. Then the sample get evaporated and carried away by the carrier gas, helium and it got segregated into individual fractions. The sample fraction coming out of the column was let into the mass detector and the mass spectrum of each component was recorded. The mass spectrum of the unknown component was compared with the known spectrum was accomplished using data base dictionaries.

Identification of Components

The database in the National Institute Standard and Technology (NIST) has been used for the interpretation on GC – MS. The spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST library. Then the structure,molecular formula, molecular weight of components were identified accordingly.

RESULT AND DISCUSSION

Antibacterial activities of *Carica papaya* plant parts such as roots,leaves and seeds were carried out (Plate-1). Among the 4 solvent seed extracts of *Carica papaya*, ethanol extract showed maximum inhibition against *E coli* (ATCC-8739) (16mm) (Table-1)(Plate-2).Among the 4 solvent leaf extract of *Carica papaya,* methanol and acetone extract showed only trace activity against *S typhimurium*(ATCC-14028)and *E coli* (ATCC-8739). Among the 4 solvent root extract of *Carica papaya,* methanol and acetone extract showed only trace activity against *S typhimurium*(ATCC-14028)and *E coli* (ATCC-8739).

Plate-1-Carica papaya plant parts (Roots, Leaf & Seed)

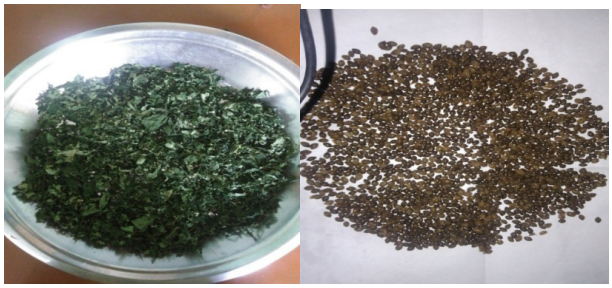
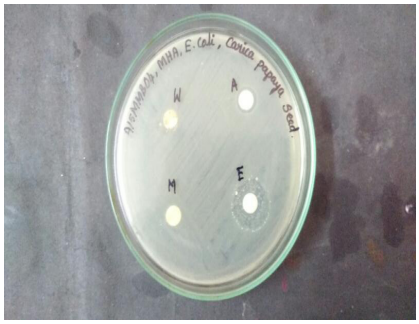


Table-1: Antibacterial Activity of Carica papaya seed extract against ATCC cultures

S. No.	ATCC CULTURES	Zone of inhibition in mm			
		ETHANOL	METHANOL	ACETONE	WATER
1	*Escherichia coli* (ATCC-8739)	**16**	NA	**9**	NA
2	*Staphylococcus aureus* (ATCC-25923)	NA	NA	8	T
3	*Pseudomonas aeruginosa* (ATCC-27853)	NA	NA	NA	NA
4	*Salmonella typhimurium* (ATCC-14028)	NA	NA	NA	NA

T –Trace activityNA- No Activity

Plate-2 : Antibacterial Activity of Carica papaya seed extract against E coli (ATCC-8739)



E –Ethanol extract M –Methanol extract
A-Acetone extract W-Water extract.

GC/MS Analysis for Seed of Carica Papaya

GC/MS analysis was carried out for the *Carica papaya* seed extract in Indian Institute of Food Processing Technology (Tanjore) and ten compounds were identified such as Benyl nitrile, Thiocyanic acid, Phenylmethyl ester, n-Hexadecanoic acid, Oleic acid, Octadecanoic acid, Glycerol 1-Palmitate, β-Monoolein, 17-Octadecynoic acid, 16-Hydroxyhexadecanoic acid and β-Sitosterol(Figure-1).

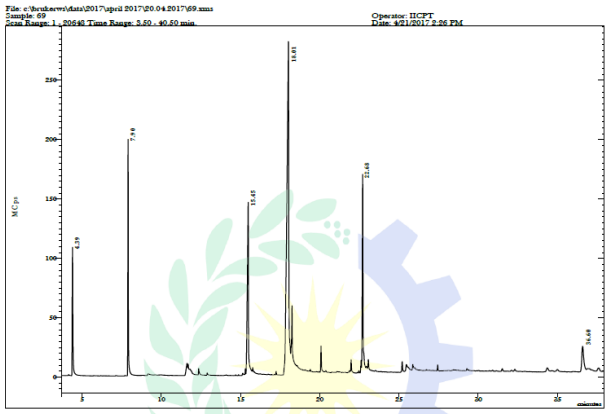


Fig. 1: GC- MS/MS Chromatogram of Carica papaya seed extract

[1] found out antimicrobial activity of aqueous, ethanol and acetone extract of fresh and dried leaves of *Carica papaya* against three strains of *E coli* (ATCC 2392), *E coli* (ATCC 25922) and *E coli* (ATCC 35218); *K pneumoniae* (ATCC 700603), *Paeruginosa* (ATCC 27853), *P vulgaris* (ATCC 13315), *S aureus*(ATCC 29213), *S aureus* (ATCC 55620), *S aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and *S pyogens* (ATCC 8662).They revealed that organic extracts were more effective than aqueous extracts. The maximum zone of inhibitionwas obtained against *S aureus* (ATCC 25923) (28mm).

In the present study, the antibacterial activity of plant parts (seed, root, leaf) extracts of *Carica papaya* in different solvents (ethanol, methanol, acetone and water) were carried out and the results showed that seed ethanol extract of *Carica papaya* showed maximum inhibitory activity against *E coli* (ATCC-8739) (16mm), The ethanol root extract of *Carica papaya* showed moderate activity against *S aureus* (ATCC-25923) (13mm) and *P aeruginosa* (ATCC-27853) (9mm). The acetone seed extract of *Carica papaya* showed moderate activity against *E coli* (ATCC-8739) (9mm) and *S aureus*(ATCC-25923) (8mm).

Igwe [6] carried out GC/MS analysis of isopropanol leaf extract of *Carica papaya* and identified the bioactive compounds such as hexahydro-1-OH-naphtho (1,8a-b)oxiren-2(3H)-one (2.17%), 3,7-dimethyl oct-7-en-1-ol (8.08%), 3-methyl-4-(phenylthio)-2-enyl-2, 5-dihydrothiophene-1, 1-dioxide (11.78%), Cyclopentaneandecanoic acid methyl ester (12.02%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (37.78%) and 9-octadecenamide (28.18%).

Harini *et al.,* [5] carried out GC/MS of n-hexane leaf extract of *Carica papaya* variety CO7. Their result showed that the phytochemicals such as Clausamine G, Galactitol, Ibogamine (anticancer property), Garveatin D and N-Methylaspartic acid as antimicrobial metabolites.

In the present study GC/MS analysis was carried out for the *Carica papaya* seed extract in Indian Institute of Food Processing Technology (Tanjore) and ten compounds were identified such as Benyl nitrile, Thiocyanic acid, Phenylmethyl ester, n-Hexadecanoic acid, Oleic acid, Octadecanoic acid, Glycerol 1-Palmitate, β-Monoolein, 17-Octadecynoic acid, 16-Hydroxyhexadecanoic acid and β-Sitosterol.

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