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ANTIMICROBIAL ACIVITIES OF *CARICA PAPAYA* L.

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Abstract

The *Carica papaya* plant materials such as leaf, fruit (and seed) were collected and allowed to drying in dark place and ground in electric chopper. The powdered plant materials were filled separately in the thimble and extracted successively using a soxhlet extractor with distilled water, acetone, chloroform and ethanal. All the extracts were subjected to systematic phytochemical screening for the presence of phytochemical contituents. This indicates the presence carbohydrates, protein, vitamin C, tannin,alkaloids, flavanoids, steroids and saponin. Antimicrobial activities of all the extract were determined by well diffusion method. In this observation, the leaf of *Carica papaya* exhibits significant inhibitory activity against all test pathogens, in all plant material, ethanol extracts showed maximum activity. The fruit sample was further studied by FT-IR, it shown 18 functional groups compounds in between the spectra 400-4000 nm.

Key words : Flavanoids, Soxhlet extractor, Carica papaya L., medicinal plants.

Introduction

The papaya, *Carica papaya* L., is a member of the small family Caricaceae allied to the passifloraceae. As a dual or multipurpose, early bearing, space conserving, herbaceous crop, it widely acclaimed, despite its susceptibility to nature enemies. Thought the exact area of origin is unknown, the papaya believed native to tropical America. perhaps in southern Mexico and neighbouring central America. It has been speed throughout the tropical countries in the world. In Kanyakumari district, *Carica papaya* has grown in all home gardens. Everthough, pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has also increased (Gislene *et al*., 2000). The frequency of life-threatening infection caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries (Al-Bari *et al*., 2008).

Natural remedies from medicinal plants are considered to effective to safe alternative treatment of various different diseases because most of the bacteria have developed resistance against commercially avilableantibiotics. Antibiotics show some side effects like allergic reactions, disturbances of normal flora of intestine. Therefore, there is a need to develop alternative antimicrobial drugs for the treatments of infectious diseases (Tambekal *et al*., 2012).

The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies (Zy *et al*., 2005). Bacterial resistance to antimicrobial drugs is a worldwide problem that has emerged even among the common poultry pathogens. Now-a-days, the use of antibiotics to control diseases is producing adverse toxicity to the host organs, tissues and cells. The toxicity produced by the antimicrobial agents can be cured or prevented or antagonized using herbs (Amin and Kapadnis, 2005). For a long time, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties (Adriana *et al*., 2007). The local use of natural plants as primary health remedies, due to their pharmacological properties, is quite common in Asia, Latin America and Africa (Bibitha *et al*., 2002).

The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbours an inexhaustible source of active

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ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Ahmad, 2001).

Phytochemicals are compounds that occur naturally in plants. They contribute to the color, flavor and smell of plants. In addition, they form part of a plant's natural defense mechanism against diseases. Their therapeutic values to human health and disease prevention have been reported (Okwu, 2004). There is no plant that does not have medicinal value. The active components are normally extracted from all plant structures, but the concentrations of these components vary from structure to structure. However, parts known to contain the highest concentration of the principles are preferred to therapeutic purposes and it can either be the leaves, stems, barks, roots, bulks, corms, rhizomes, woods, flowers, fruits or the seeds (Kafaru, 1994). The phytochemical analysis were performed for the presence of alkaloids, glycosides, specific glycosides like saponin glycosides, anthraquinone glycosides, cardiac glycosides, cyanogenetic glycosides, tannins and phenolic compounds, flavonoids, proteins and amino acids, sterols, triterpenoids, carbohydrates, fats, fixed oils (Dey and Ghosh, 2010). Papaya leaves have been reported to contain carpaine, which has high antioxidant content that may be helpful for the prevention of atherosclerosis, diabetic heart disease, heart attacks and strokes. Also, itimproves the immune system and prevents illnesses such as recurrent ear infections, colds and flu (Nwinyi *et al*., 2010). The plant is also described in a documented property forms and it act as analgesic, amebicide, antibacterial, cardiotonic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic and vermifuge. It is distributed throughout Asia, Nigeria etc (Afolayan, 2003). The latex from the leaves has been used as antihelmints and for the treatment of infections of bacterial origin (Fajimi *et al*., 2001).

Materials and Methods

Sample collection for antimicrobial activity

Fresh leaves, fruit and seeds of *Carica papaya* were collected from Thuckalay, Kanyakumari district. Leaves were picked out from the branch and cut into small pieces. Fruit was sliced into small pieces and the seeds were collected from the healthy, matured fruits. All the samples were immediately transported to the laboratory for further processing.

The entire collected samples were subjected to surface cleaning by rinsing the samples with sterile water, in order to remove dust particles present on the plant materials. The debris free plant materials were allowed to drying in a dark place at room temperature for few days (upto the samples get shade dry). The dried plant samples were ground in electric chopper to get fine powder form for further use.

Preparation of plant extracts

The prepared powder sample of *Carica papaya* leaf, fruit and seedswere subjected to soxhlet extraction using distilled water (aqueous extract), acetone, chloroform and ethanol. Each 5 grams of dried, powder of plant material was filled separately in the thimble and extracted successively with 60ml of solvents using a soxhlet extractor for three hours. After solvent evaporation, each of these solvent extract was weighed and preserved in room temperature until further use.

Qualitative analysis phytochemical constituents

All the plant extracts were subjected to systematic phytochemical screening for the presence of chemical constituents (Sofowra, 1993 and Harborne, 1973).

Tests for carbohydrates (Benedict's test)

Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Tests for proteins (Biuret test)

3 ml of each test solution was added to 4% NaOH and few drops of 1% $CuSO_4$ solution into separate tubes. The tubes were observed for violet or pink colour formation.

Tests for vitamin C

1 ml of 2% w/v solution was diluted with 5 ml of water. 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside and 2 ml of diluted sodium hydroxide solution were added. Then 0.6 ml of hydrochloric acid was added dropwise and stir, the yellow colour turns blue it's indicates positive results.

Tests for alkaloids (Wagner's test)

2-3 ml filtrate was taken into separate tubes. To that few drops of Wagner's reagent was added and observed reddish brown precipitate.

Detection of flavonoids

Lead acetate Test : The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

Tests for tannins

With 2-3 ml test solution, 5% $FeCl_3$ solution was added and observed for deep blue-black colour reactions.

Tests for steroids (Salkowski reaction)

To 2 ml of sample, 2 ml chloroform and 2 ml concentrated H_2SO_4 were added and observed chloroform layer for red color and acid layer for fluorescence.

Test for phenolic compounds (Ferric chloride test)

The extract was diluted to 5 ml with distilled water. To that a few drop of neutral 5% ferric chloride solution was added. A dark green color indicates the presences of phenolic compounds.

Test for saponins

The fruits samples were diluted with distilled water and made into 20 ml. The suspension was shaken well in graduvatedcylinder for 15 minutes, 2 cm layer of foam indicates the presences of saponins.

Anti-microbial activity assay

Four bacterial pathogenic strains (namely *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Staphyloccocus aureus*) and three fungal strains (namely *Aspergilus niger*, *Spergilus fumigatus* and *Penicillium* sp) were used in this investigation. The media used for antibacterial test were Nutrient Broth. The test bacterial strains were inoculated into nutrient broth and incubated at 37^{0}C for 24hrs. After the incubation period, the culture tubes were compared with the turbidity standard. Fungal inoculums were prepared by suspending the spores of fungus (as previously cultured) in saline water mixed thoroughly, made tubidity standard and used.

Bioassay was carried out by Agar well diffusion method. Fresh bacterial culture of 0.1 ml having 108 CFU was spread on nutrient agar (NA) plate using swab. The fungal strains also the same but the medium was Potato dextrose agar (PDA). Wells of 6 mm diameter were punched off into medium with sterile cork borer and filled with 50µl of plant extracts by using micro pipette in each well in aseptic condition. Plates were then kept in a refrigerator to allow pre-diffusion of extract for 30 minutes. Further the plates were incubated in an incubator at 37^{0}C for 24 hours and 28-30^{0}C for 3-4 days for bacterial and fungal cultures, respectively. The antimicrobial activity was evaluated by measuring the zone of inhibition.

Fourier Transform Infrared Spectrophotometer (FTIR) analysis

ATR model FTIR Spectrophotometer (Bruker Co., Germany) was used for the analysis of the dried fruit of *Carica papaya.* The spectrum (400-4000 nm) was recorded using Attenuated Total Reflectance (ATR) technique beach measurement.

Results and Discussion

Papaya belongs to the genus *Carica* of the family *Caricaceae* with forty eight species, of all the best known. It is cultivated all over the world, papaya fruit is very popular with farmers in general because it requires area per plant, easy to cultivate and provides more income per hectare.

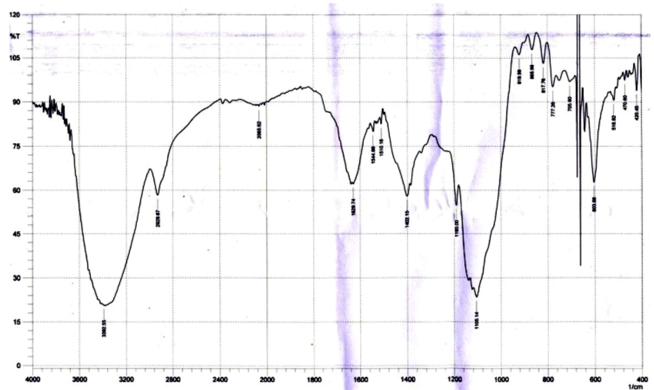
The phytochemical screening of *Carica papaya* plant materials were carried out and the results were recorded. In the present study, all the tested plant materials (leaf, seed and fruit) extracts indicate the presence of carbohydrates, protein, vitamin C, akaloids, flavonoids, tannin, steroids, phenolic compounds and saponins. The presence of ascorbic acid in *Carica papaya* leaves indicates that the plant can be used in herbal medicine for the treatment of common cold and other diseases like prostate cancer (Njoku and Akumefula, 2007). The antibacterial activity of the Maducusatropurpura plants may be attributed to the presence of bioactive compounds such as phenols, saponin, steriods, alkaloids and flavonoids (Murugan and Mohan, 2012).

Alkaloids are the most efficient therapeutically significant plant substance (Igboko, 1983) and the alkaloid was present in our samples also. The biological functions of flavonoids include protection against allergies, inflammations, platelets aggregation microbes, ulcer, viruses and tumours (Okwu and Okwu, 2004). Alkaloids isolated from plant are commonly found to have antimicrobial properties (Jose *et al*., 2005). They 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 (Njoku and Akumefula, 2007). Flavonoids represent the common and widely distributed group of plant phenolics. They are free radical scavengers and super antioxidants and potent which prevent oxidative cell damage and have strong anticancer activity (Salah *et al*., 1995).

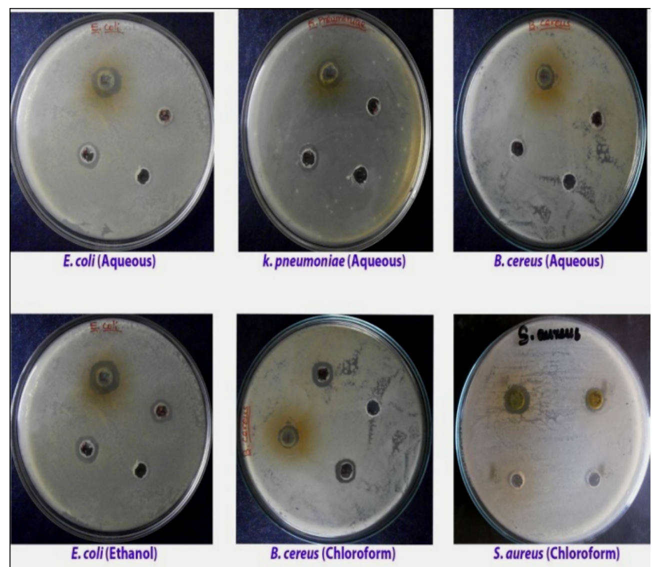
The presence of tannins in the *Carica papaya* can support its strong use for healing of wounds, ulcers, hemorrhoids, frost-bites and burns in herbal medicine (Igboko, 1983). Tannins are metal chelators and can form complexes with macro molecule. Through this process essential substrates, co-factor and enzymes of micro-organism are depleted leading to cell death. The presence of phenolic compounds in the seed of *Carica papaya* shows that the seed may have antimicrobial potential. This explains its use in treating diarrhoea, typhoid fever and some other intestinal problem. This is because phenols and phenolic compounds have been extensively used in disinfections and it remains the standard with which other bactericides are compared (Oakenful, 1981). The saponins constituents are responsible for the possession of haemolytic property. Tannins have astringent properties which hasten the healing of wounds and inflamed mucous membrane (Igboko, 1983 and Maduiyi, 1983). The presence of saponins supports the fact that pawpaw leaf has cytotoxic effects such as permealization of the intestine as saponins are cytotoxic (Okigbo *et al*., 2009).

Infectious diseases are a major cause of morbidity and mortality in India. The number of multiple drug resistant strains and the appearance of the strains with reduced susceptibility to antibiotics are continuously increasing. The antimicrobial activities of *Carica papaya* plant material (leaf, fruit and seeds) with different solvents were investigated in the present study to identify the presence of bioactive substances which 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 (Srinivasan *et al*., 2001).

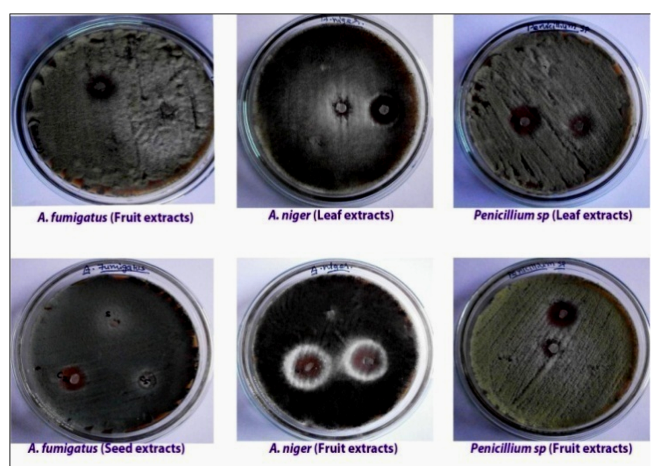
In the present study, the aqueous extracts of *Carica papaya* leaf showed inhibitory activity against *E. coli* (12mm), *K. pneumoniae* (12mm), *B. cereus* (10mm) and *A. fumigatus* (7mm); Acetone extract of leaf exhibits inhibitory activity against *E. coli* (10mm), *K. pneumoniae* (12mm), *S. aureus* (8mm), *B. cereus* (11mm) and *A.niger* (8mm); the chloroform extract of the leaf showed inhibitory action against all test bacterial and one fungal strain except *A. niger*; the ethanol extract of the leaf showed activity on all test microorganisms. In bacterial pathogen, *E.coli* and *K. pneumoniae* were highly (14mm) inhibited and fungus *A.fumigatus, penicilliumsp* (8mm), *A. niger* (9mm). The aqueous extracts of *Carica papaya* fruit showed activity only on *E. coli* and *K.* pneumoniae (11mm). The acetone extract of the fruit exhibits inhibitory activity against *E. coli* (8mm), *K. pneumoniae* (7mm) and *B. cereus* (10mm); the chloroform extract of the fruit showed inhibitory action against all test bacterial and one fungal strain (*A. niger*-7mm); the ethanol extract showed activity on all test microorganisms. In bacterial pathogen, *E. coli* and *Staph. aureus* were highly (12mm) inhibited, incase of fungus *A. fumigatus* (7mm), *A. niger* and *Penicillium* (8mm) were inhibited. The chloroform extract of *Carica payapa* seed showed inhibitory activity against *E. coli* (11mm), *K. pneumoniae* (9mm), *S. aureus* (8mm), *B. cereus* (10mm), *A. fumigatus* (8mm), *A. niger* (7mm) and *Penicillium* sp (9mm). The ethanol extract of the seed showed inhibitory activity against *E. coli* (11mm), *K. pneumoniae* (12mm), *S. aureus* (10mm), *B. cereus* (10mm), *A. fumigatus* (8mm), *A. niger* (8mm) and *Penicillium* sp (8mm). This situation provided the impetus



FTIR chromatogram of *Carica papaya* fruit.



Antibacterial activity of *Carica papaya.*



Antifungal activity of *Carica papaya*.

Table 1 : Phytochemical constituents of *Carica papaya* leaf extracts.

S. no.	Chemical constituents	Aqueous	Acetone	Chloroform	Ethanol
1.	Carbohydrates	+	+ +	+	
2.	Protein	+	+ -	+	
3.	Vitamin C	+	- -	-	
4.	Alkaloids	+	+ +	+	
5.	Flavonoids	-	+ -	+	
6.	Tannins	-	- +	-	
7.	Steroids	+	+ +	-	
8.	Phenolic compunds	-	+ +	-	
9.	Saponins	-	+ -	+	

'+' presence of compound, '-'absence of compound.

Table 2 : Phytochemical constituents of *Carica papaya* fruit extracts.

S. no.	Chemical constituents	Aqueous	Acetone	Chloroform	Ethanol
1.	Carbohydrates	+	+	+	+
2.	Protein	+	+	-	+
3.	Vitamin C	+	-	-	-
4.	Alkaloids	+	+	+	+
5.	Flavonoids	-	+	+	+
6.	Tannins	-	-	+	+
7.	Steroids	+	+	+	+
8.	Phenolic compunds	-	+	+	+
9.	Saponins	-	-	+	+

'+' presence of compound, '-' absence of compound.

Table 3 : Phytochemical constituents of *Carica papaya* seed extracts.

S. no.	Chemical constituents	Aqueous	Acetone	Chloroform	Ethanol
1.	Carbohydrates	+	+	+	+
2.	Protein	+	+	-	+
3.	Vitamin C	+	-	-	-
4.	Alkaloids	+	+	+	+
5.	Flavonoids	+	+	+	+
6.	Tannins	+	-	+	+
7.	Steroids	+	+	+	-
8.	Phenolic compunds	-	+	+	+
9.	Saponins	-	-	+	+

'+' presence of compound, '-' absence of compound.

Table 4 : Zone of inhibition of *Carica papaya* leaf extracts.

S. no.	Test organisms	Aqueous	Acetone	Chloroform	Ethanol
	Bacterial strains				
1.	*Escherichia coli*	12	10	12	14
2.	*K. pneumoniae*	12	12	12	14
3.	*Staph. aureus*	-	8	10	11
4.	*Bacillus cereus*	10	11	10	12
	Fungal strains				
1.	*Aspergillus fumigatus*	7	-	8	8
2.	*Aspergillus niger*	-	8	-	9
3.	*Penicillium* sp.	-	-	9	8

Zone of inhibition in 'mm', '-'no inhibition.

Table 5 : Zone of inhibition of *Carica papaya* fruit extracts.

S. no.	Test organisms	Aqueous	Acetone	Chloroform	Ethanol
	Bacterial strains				
1.	*Escherichia coli*	11	8	12	12
2.	*K. pneumoniae*	11	7	12	10
3.	*Staph. aureus*	-	-	11	12
4.	*Bacillus cereus*	-	10	9	9
	Fungal strains				
1.	*Aspergillus fumigatus*	-	-	-	7
2.	*Aspergillus niger*	-	-	7	8
3.	*Penicillium* sp.	-	-	-	8

Zone of inhibition in 'mm'; '-'no inhibition

Table 6 : Zone of inhibition of *Carica papaya* seed extracts.

S. no.	Test organisms	Aqueous	Acetone	Chloroform	Ethanol
	Bacterial strains				
1.	*Escherichia coli*	8	10	11	11
2.	*K. pneumoniae*	-	10	9	12
3.	*Staph. aureus*	-	-	8	10
4.	*Bacillus cereus*	-	8	10	10
	Fungal strains				
1.	*Aspergillusfumigatus*	-	-	8	8
2.	*Aspergillusniger*	-	7	7	8
3.	*Penicillium sp.*	-	7	9	8

Zone of inhibition in 'mm'. '-'no inhibition.

to the search for new antimicrobial substances from various sources like medicinal plants. It is important to investigate scientifically these plants, which have been used in traditional medicines as potential sources of novel antimicrobial compounds (Hema *et al*., 2013).

Maximum antibacterial activity in methanol extract (Vinod *et al.*, 2013).

The results obtained from the above data represents the fact that the plant materials with organic solvents were exhibited significant anitimicrobial activities, especially the solvent chroloform and ethanol showed inhibitory activities against all test pathogens. In those two solvents, ethanol extracts were showed good significant inhibitions. The same results were observed by Okoye (2011), the ethanolic extract of the seeds of *CARICA PAPAYA* had greater zones of inhibition than the aqueous extract. This implies that the ethanolic extract of the seeds had more potency than the aqeous extract against the four test fungi *A.niger, P. notatum, F. oxysporum*and *C. Albicans*. This may be due to the better solubility of the active components in organic solvents (de Boer *et al*., 2005). The difference in the composition of the crude extracts is likely to be due to the varying degrees of solubility of the active constituents in the four solvents used. Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent (Marjorie, 1999). Other studies reported that most active constituents are mainly aromatic or saturated organic compounds which have better solubility in organic solvents (Marjorie, 1999). Based on the limited spectrum of activity of the other extracts compared with the ethanol extracts, it suggests that the active component is more soluble in ethanol than in the other solvents (Okunola A. Alabi *et al.,* 2012). Also the gram negative bacteria were more susceptible than the gram positive bacterium especially *E. coli* and *K. pnuemoniae.* This result was supported by Anibijuwon and Udeze (2009) in their study also. Among the Gram-positive and Gram-negative bacteria tested against the root extract of *C. papaya*, the Gram-negative bacteria were more susceptible especially *P. aeruginosa*to the extracts.

The efficacy of treatments with *C. papaya* is dependent on the quantity of the different chemical substances present in the preparation. The quantity of chemical substances varies in the fruit, latex, leaves, and roots and varies with the extraction method, age of the plant part, and the cultivar and sex of the tree (Wagh *et* *al*., 1993) Although the mechanism of action of this extract is not understood, it has been proposed that its action against the bacteria and fungi may be due to the inhibition of cell wall formation in the cell resulting in a leakage of cytoplasmic constituents by the bioactive components of the extract (Bais *et al*., 2002). Phytochemical compounds such as tannin coagulate the wall proteins,saponins facilitated the entry of toxic material or leakage of vital constituents from the cell (Onwuliri and Wonang, 2005). Flavonoids inhibit the activity of enzymes (Dathak and Iwu, 1991) by forming complexes with bacterial cell walls, extracellular and soluble proteins (Kurtz *et al*., 1995).

Conclusion

The present study concluded that, the preliminary screening of phytochemical constituents results demonstrated the presence of various bioactive metabolites. The antimicrobial activity results showed the inhibitory activity of *Carica papaya*, the demonstration of antimicro bial activity of *Carcia papaya* against the test organisms has provided a scientific basis for its local usage as a medicinal plant also it is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity. The results of the study also supports the traditional application of the plant and suggests that the plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs for the treatment of gastroenteritis, uretritis, otitis media and wound infections. Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antibacterial from this plant are the future challenges.

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