

## Evaluation of Biomedical Importance of Papaya Pulp

V. Balamurugan\*, J. Ramamoorthy, A. Sabaridasan

PG and Research Department of Biotechnology, Sri Vinayaga College of Arts and Science,  
Ulundurpet, Villupuram, Tamil Nadu, India

### Abstract

*The present study describes the preparation of petroleum ether, ethyl acetate, and methanol extract from Carica papaya; further the extracts revealed dose dependent anti-oxidant potential. Among the extracts, ethyl acetate extract showed more percentage of scavenging than other two extracts. The screening of antimicrobial activity for three extracts revealed antimicrobial potency towards 12 test organisms. Among these extracts, petroleum ether shows more activity against 12 microbes compared with other two extracts. The petroleum ether extract showed the presence of alkaloids, coumarin, sugars, terpenoids, flavonoids, protein glycosides and carboxylic acid. This result is same in ethyl acetate and methanol extract. All three extracts when screened showed marginal anti-cancer activity against breast cancer cell line (MCF7). Thus the present investigation revealed that Carica papaya holds potent anti-oxidant, antimicrobial, phytochemical constituents and marginal anti-cancer activity.*

**Keywords:** Antimicrobial activity, Carica papaya, phytochemical analysis, scavenging activity

\*Author for Correspondence E-mail: balakal78@gmail.com

### INTRODUCTION

Papaya is high source of enzyme papain which effective against cancer. Papain is an endolytic plant cysteine protease enzyme isolated from papaya (*Carica papaya* L.) latex. It preferentially cleaves peptide bonds involving basic amino acids, particularly arginine, lysine and residues following phenylalanine. The unique structure of papain gives its functionality that helps to understand how this proteolytic enzyme works and useful for a variety of purposes [1]. Papain breaks fibrin coat of cancer cell wall. So ultimately it helps against the cancer. Papaya has larger stores of cancer fighting lycopene [2]. According to some authors, lycopene is a member which helps in overcoming the toxic manifestations of cancer cells [3, 4]. The papaya leaf may potentially serve as a good therapeutic agent for protection against gastric ulcer and oxidative stress [5]. However, the vast potentialities of plants as a source for antimicrobial drugs with reference to antibacterial agent motivated the present systematic investigation to screen the aqueous and methanolic root extracts of *Carica papaya*

for its antimicrobial activity [6]. Another set of plates was cultured to estimate the effect of combination therapy using the herbal drug together in varied concentrations with the standard drugs [7]. Recent researches have shown the composition of a chloroform seed extract of *C. papaya* determined by GC-MS [8].

Even though, phytochemical profiling of young leaves of *Carica papaya* revealed the presence of pharmacologically active phyto-compounds, alkaloids, phenolics, flavonoids and also, amino acids [9]. In the present study, the preliminary screening of phytochemical constituents results demonstrated the biomedical importance of papaya pulp.

### MATERIALS AND METHODS

The papaya was purchased from market Cuddalore, Tamil Nadu. Care was taken to select the firm and mature fruit without any damage of the fruit.

#### Processing of Plant Material

The papaya was washed under tap water and the pulp and peel were separated. The pulp and

peel was cut into small pieces and then dried under sunlight. After drying, the pulp and peel were ground well using mechanical blender into fine powder and transferred into air-tight container with proper labeling for further use.

### Solvent Extraction Process

The solvent extraction was done on papaya pulp and papaya peel for various processes. The samples were mixed with 100 ml of petroleum ether; in each 25 g of sample for low polar and middle polar using ethyl acetate, and high polar was mixed with 80% methanol. Then, the samples were centrifuged and maintained at 50°C for drying.

### Anti-Oxidant Assay

The free radical scavenging activity was measured using DPPH by the method of Blois MS 1958. A 0.1 mM solution of DPPH in ethanol was prepared and 2.96 ml of this solution was added to 0.4 ml of various quantities and the reference compound, after 30 min, absorbance was measured at 517 nm. BHA was used as a reference material. Percent inhibition was calculated by comparing the absorbance values of control and samples.

### Antimicrobial Assay

Agar well diffusion method was used to evaluate the antimicrobial activity of plants or microbial extracts. The agar plate (Muller Hinton agar) surface was inoculated by spreading a volume of the microbial inoculums (12 different strains) over the entire agar surface. Then, five holes were punched aseptically with a sterile tip and a volume of 100 µl of the three extract solutions at 10 mg/ml concentration were introduced into the well. Then, agar plates were incubated under suitable conditions depending upon the test microorganisms. The antimicrobial agent (extract) diffused in the agar medium and inhibited the growth of the microbial strain tested. The diameter of zone of inhibition was measured as:

$$\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{Test}}}{A_{\text{control}}} \times 100$$

### Phytochemical Analysis

The phytochemical analysis was tested from papaya pulp by various tests. The presence of

alkaloids, coumarin, phenol, sugars, terpenoids, tannins, flavonoids, protein, fat, glycosides, steroids and carboxylic acid was found.

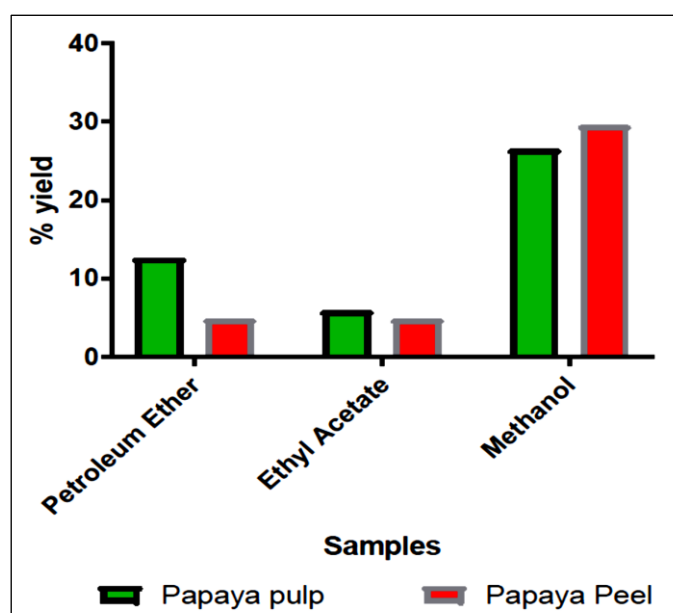
### Anticancer Potential of Papaya Extract

The breast cancer cells (MCF7) were plated separately using 96 well plates with the concentration of  $1 \times 10^5$  cells/well in DMEM media with 1X antibiotic antimycotic solution and 10% fetal bovine serum (Himedia, India) in CO<sub>2</sub> incubator at 37°C with 5% CO<sub>2</sub>. The cells were washed with 200 µl of 1X PBS, then the cells were treated with various test concentration (25, 50, 100, 200, and 250 µg/ml) of compound in serum free media and incubated for 24 h. The medium was aspirated from cells at the end of the treatment period. 0.5 mg/ml MTT prepared in 1X PBS was added and incubated at 37°C for 4 h using CO<sub>2</sub> incubator. After incubation period, the medium containing MTT was discarded from the cells and washed using 200 µl of PBS. The formed crystals were dissolved with 100 µl of DMSO and thoroughly mixed. The development of color intensity was evaluated at 570 nm. The formazan dye turned to purple blue color. The absorbance was measured at 570 nm using microplate reader.

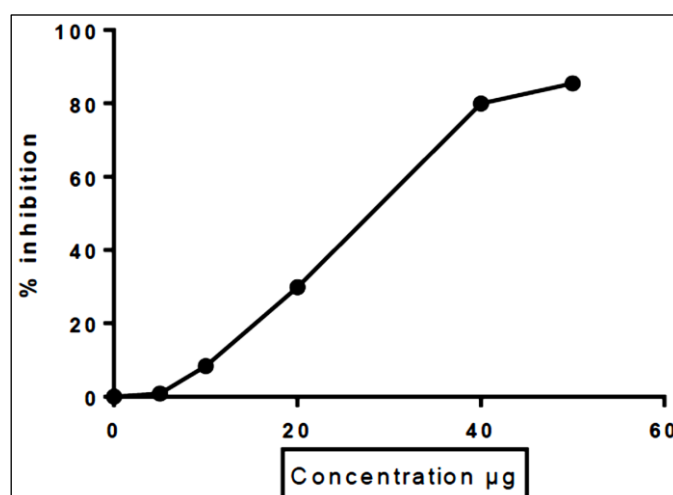
## RESULT

### Anti-oxidant Assay

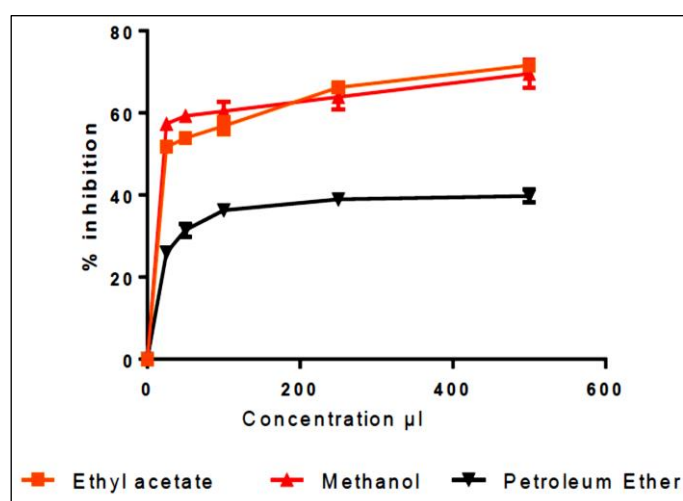
The percentage of scavenging was calculated for all three extracts (petroleum ether, ethyl acetate, and methanol) of *C. papaya* pulp and peel at five different concentrations (25, 50, 100, 250, 500 µg) and plotted as graphs (Figures 1–4). The positive controls (ascorbic acid) percentage of scavenging was also plotted. From the result obtained, all three extracts have anti-oxidant property. The percentage of scavenging increases with increase in extracts concentration. Therefore, anti-oxidant property of this pulp and peel was great only at higher concentrations. On comparing with petroleum ether and ethyl acetate, methanol extract shows more percentage of scavenging. Thus, the results obtained show that the pulp and peel exhibit anti-oxidant property in all three extracts.



**Fig. 1:** Total Yield of Papaya Pulp and Papaya Peel.



**Fig. 2:** Percentage of Scavenging for Ascorbic Acid (Control) in Five Different Concentrations.



**Fig. 3:** Percentage of Scavenging for Three Extracts in Five Different Concentrations of Papaya Pulp.

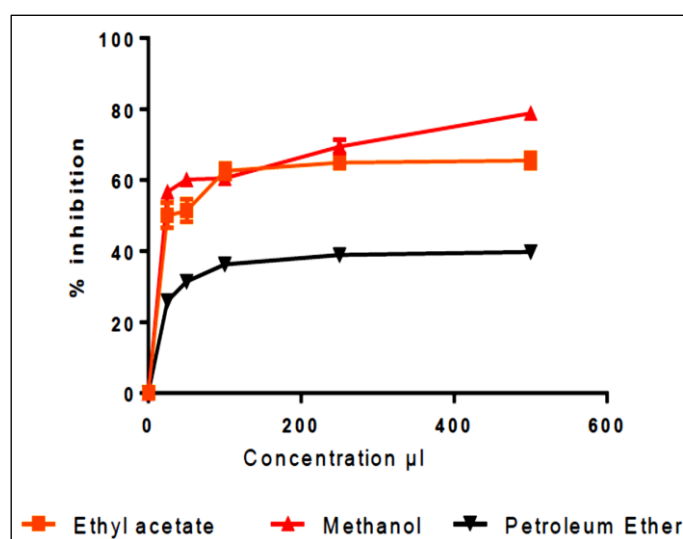


Fig. 4: Percentage of Scavenging for Three Extracts in Five Different Concentrations of Papaya Peel.

Table 1: Antimicrobial Efficacy of Papaya Pulp and Peel Extract of Three Different Compounds.

Compounds (Inhibition in mm)		Microbial Strains											
		<i>A. niger</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>E. coli</i>	<i>E. faecalis</i>	<i>E. auroges</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. zoopiepidermis</i>	<i>V. cholera</i>	<i>S. mutant</i>
Petroleum ether	Antibiotic	34	22	21	26	31	20	24	30	29	25	21	32
	Papaya pulp	23	10	13	12	21	10	12	11	11	10	20	14
	Papaya peel	12	11	11	-	13	-	12	10	10	15	12	17
Ethyl acetate	Antibiotic	35	21	21	26	28	19	26	25	30	22	31	29
	Papaya pulp	11	-	10	-	10	14	-	11	-	19	13	-
	Papaya peel	10	-	-	-	11	12	-	-	-	10	14	-
Methanol	Antibiotic	32	18	21	29	24	22	27	-	30	20	-	33
	Papaya pulp	10	-	-	-	-	-	-	-	10	10	-	12
	Papaya peel	-	-	10	11	-	10	-	-	16	-	-	-

### Antimicrobial Assay

The agar plate was incubated overnight at 37°C and zone of inhibition diameter was measured for all ten plates. The zone of inhibition was measured in millimeter; the results are summarized in Table 1. All three extracts possess antimicrobial activity and their strength of inhibition is unique to different strains tested. Petroleum ether extract shows more antimicrobial activity against *Aspergillus niger*, *Bacillus subtilis*, *C. albicans*, *E. coli*, *E. faecalis*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *S. zoopiepidermis*, *Vibrio cholera* and *S. mutant*, than the ethyl acetate and methanol extract.

Ethyl acetate extract shows more antimicrobial activity against *Aspergillus niger*, *C. albicans*,

*E. auroges*, *P. aeruginosa*, *E. faecalis*, *S. zoopiepidermis* and *Vibrio cholera*. Methanol extract shows antimicrobial activity against *Aspergillus niger*, *Candida albicans*, *E. coli*, *E. faecalis*, *E. auroges*, *S. aureus*, *S. zoopiepidermis* and *S. mutant*.

### Phytochemical Assay

The chemical constituents of this plant were analyzed through many chemical tests. The petroleum ether, ethyl acetate, and methanol, extracts were tested individually for twelve chemical tests to determine the presence of alkaloids, coumarin, phenol, sugars, terpenoids, tannins, flavonoids, protein, fat, glycosidase, salkowski, and carboxylic acid as summarized in Table 2.

**Table 2:** Phytochemical Profile of Papaya peel and pulp with petroleum ether, ethyl acetate and methanol extract.

Phytochemical Profile	Petroleum ether		Ethyl acetate		Methanol	
	Positive	Negative	Positive	Negative	Positive	Negative
Alkaloids	Peel	Pulp	Peel	Pulp	Peel	Pulp
Coumarin	Peel, Pulp		Peel, Pulp		Peel, Pulp	
Phenol		Peel, Pulp	Peel	Pulp		Pulp
Sugars	Peel, Pulp		Peel, Pulp		Peel	Pulp
Terpenoids	Peel, Pulp		Peel, Pulp		Peel, Pulp	
Tannins		Peel, Pulp		Peel, Pulp		Peel, Pulp
Flavonoids	Peel, Pulp		Peel, Pulp		Peel, Pulp	
Protein	Peel	Pulp		Peel, Pulp	Peel, Pulp	
Fat		Peel, Pulp		Peel, Pulp		Peel, Pulp
Glycosides	Peel, Pulp		Peel	Pulp	Peel	Pulp
Salkowski		Peel, Pulp		Peel, Pulp		Peel, Pulp
Carboxylic acid		Peel, Pulp		Peel, Pulp	Peel, Pulp	

## DISCUSSION

The anti-oxidant activity was determined using DPPH assay. Different concentrations of the three extracts were estimated for anti-oxidant property and all three extracts showed anti-oxidant activity as their percentage of scavenging was significant, especially at higher concentration. The DPPH acts as free radical and extract quenching of DPPH was directly seen as reduction in color change. Ethyl acetate extract showed more anti-oxidizing activity than other two extracts. The extracts can be used to treat diseases resulting from the presence of many free radicals like aging and wrinkles [10]. It showed that ethyl acetate extract of this plant showed high anti-oxidant activity than methanol extract and petroleum ether extract. It was used  $\beta$ -carotene linoleic acid model and 1, 1-diphenyl-2-picrylhydrazyl model.

The ethyl acetate extract showed antioxidant activity of 64.8% in  $\beta$ -carotene linoleic acid model and 61.6% in 1, 1-diphenyl-2-picrylhydrazyl model. The three extracts were checked for antimicrobial activity using well diffusion method. In this present study, the petroleum ether extract has shown high zone of inhibition in *A. niger*, *B. subtilis*, *C. albicans*, *E. Coli*, *E. faecalis*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *S. zoopiepidermis*, *V. cholerae* and *S. mutant*. Ethyl acetate extract has shown a high zone of inhibition in *A.*

*niger*, *B. subtilis*, *C. albicans*, *E. Coli*, *E. faecalis*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *S. zoopiepidermis*, *V. cholerae* and *S. mutant*. Methanol extract has shown a high zone of inhibition in *A. niger*, *C. albicans*, *E. coli*, *E. faecalis*, *E. auorges*, *S. aureus*, *S. zoopiepidermis* and *S. mutant*. When compared the zone of inhibition with the standard drugs like ciprotoxin; the plant extracts have shown almost equal to the standard drug. The above parameter supports the strong scientific basis for the use of these plants in traditional treatment of microbial diseases.

The phytochemical constitutes of the three extracts (petroleum ether, ethyl acetate, methanol) of *Carica papaya* were found individually. The tests indicate the presence of alkaloids, coumarin, sugars, terpenoids, flavonoids, protein, glycosides and carboxylic acid. Flavonoids and alkaloids are secondary metabolites and find application in various biological fields. The presence of phytochemicals is more concentrated form of extract. The diluted extract should not be used while extracting phytochemicals. Other phytochemicals like fat, phenols, tannins, steroids were absent according to the result. According to Tiwari *et al.*, the following phytochemicals are extracted from the stem: Triterpene d-amyrin acetate, aliphatic acid, hexadecanoic acid, stilbene glucoside trans-



resveratrol-3-O-glucoside,  $\delta$ -amyrene, d-amyrene,  $\beta$ -sitosterol, kaempferol, quercetin and resveratrol [11]. The anti-cancer activity was also determined. Breast cancer cell lines (MCF7) were used and cytotoxicity was studied using all three extracts. The result showed that there was maximum 28% reduction in cell viability at highest concentration tested. Ethyl acetate extract showed more toxicity to breast cancer cells than other two extracts. The lowest cell viability against normal cells was 80% (ethyl acetate extract) while the cell viability against breast cancer cells was 75% (ethyl acetate extract). The ethyl acetate extract had marginal anti-cancer activity but it is enough to treat the human body. A drug should reduce at least 50% of cell viability in order to treat humans.

## CONCLUSION

In the present study, examination of biomedical essential of papaya pulp extracts when screened shows marginal anti-cancer activity against breast cancer cell line. Thus, the present investigation revealed that *Carica papaya* holds potent anti-oxidant, antimicrobial, phytochemical constituents and marginal anti-cancer activity.

## REFERENCES

1. Menard, Kumagai E, Kondo A. Aqueous Extract of *Carica papaya* Leaves Exhibits Antitumor Activity and Immune Modulatory Effects. *J Ethnopharmacol*. 1990; 127: 760–67p.
2. Mitchel. Potential Medicinal Properties of *Carica papaya* Linn, A Mini Review. *Int J Pharm Pharm Sci*. 1970; 6(2): 1–4p.
3. Stahl K, Sathoshi I, Chikao M. Aqueous Extract of *Carica papaya* Leaves Exhibit Anti Tumour Activity and Immunomodulatory Effects. *J Ethnopharmacol*. 1992; 27: 760–767p.
4. Khachik D, Pauli A, Natisteat, Rosemary L. Cynogenic Allosides and Glucosides from *Possoftora edulis* and *Carica papaya*. *Phytochemistry*. 2002; 60: 873–882p.
5. Indran M, Mahmood A, Kuppusamy UR. Protective Effect of *Carica papaya* Leaf Extract against Alcohol Induced Acute Gastric Damage and Blood Oxidative Stress in Rats. *West Indian Med J*. 2008; 57(4): 323–6p.
6. Caludia Marica, Kondo A, Iwata S, et al. Aqueous Extract of *Carica papaya* Leaves Exhibits Anti-Tumor Activity and Immunomodulatory Effects. *J Ethnopharmacol*. 2010; 127(3): 760–7p.
7. Adejuwan AO, Agboje E, Abdullah M. *Carica papaya* Increases Regulatory T Cells and Reduces IFN- $\gamma$ +CD4+T Cells in Healthy Human Subjects. *Mol Nutr Food Res*. 2011; 55(5): 803–6p.
8. Perez Zavala. Sensitive and Relevant Model for Evaluating Anti-Inflammatory Activity Papaya Latex-Induced Rat Paw Inflammation. *J Pharmacol Toxicol Methods*. 2011; 28(1): 15–9p.
9. Akhila S, Vijayalakshimi NG. Wound Healing Properties of *Carica papaya* Latex: *in vivo* Evaluation in Mice Burn Model. *J Ethnopharmacol*. 2015; 121(2): 338–41p.
10. Otsuki N. Aqueous Extract of *Carica papaya* Leaves Exhibits Anti-Tumor Activity and Immunomodulatory Effects. *J Ethnopharmacol*. 17 Feb 2010; 127(3): 760–7p.
11. Prashant Tiwari, et al. Antimicrobial Activity Evaluation of the Root of *Carica papaya* Linn. *Int J PharmTech Res*. 2011; 3(3): 1641–48p.

### Cite this Article

V. Balamurugan, J. Ramamoorthy, A. Sabaridasan. Evaluation of Biomedical Importance of Papaya Pulp. *Research & Reviews: A Journal of Biotechnology*. 2017; 7(3): 14–19p.