

Antimicrobial and antioxidant properties of peel and seed extract of *Carica papaya* L.

Vasundra & Upma Dutta*

Division of Microbiology, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu (SKUAST-J), Chatha, Jammu & Kashmir, India

Received 27 August 2023; Revised 20 February 2024

Carica papaya is a widely consumed fruit in many tropical areas of the world, but its peel and seeds are generally discarded as waste material. However, peels and seeds consist of various natural bioactive compounds that are responsible for many medicinal and therapeutic properties and can be further used in various industries for functional foods, pharmaceuticals, and cosmetic products. Since ancient times, the entire plant of *Carica papaya* L., including the peel, seeds, leaves, etc., has been used as traditional medicine. Here, we studied the phytochemical constituents, antimicrobial and antioxidant activities of papaya waste (peel and seeds) samples obtained from the local market in Jammu district of Jammu & Kashmir. Antimicrobial assessment using the agar-well diffusion method revealed the substantial efficacy of both the extracts. At a concentration of 100 µg/mL, both papaya peel and seed ethanolic extracts exhibited substantial zones of inhibition against *Escherichia coli* (16.00±1.00 mm and 19.40±1.15 mm), *Bacillus cereus* (21.50±1.00 mm and 08.00±1.00 mm), *Klebsiella pneumoniae* (25.50±1.00 mm and 20.00±1.00 mm) and *Staphylococcus aureus* (14.40±1.15 mm and 7.50±1.00 mm), respectively. The minimum inhibitory concentration (MIC) was determined using the macro broth dilution method, indicating that the seed extract had a maximum MIC of 100 µg/mL against all test pathogens, surpassing the peel extract. The qualitative analysis showed that phytochemicals including tannins, saponins, alkaloids and phytosteroids were absent in both the extracts. The bioactive potential of papaya peel and seeds is quantified by determining their total phenol content and total flavonoid content. The papaya seeds exhibited TPC of 62.45 mg GAE's/gdw and a TFC of 47.84 mg QE's/gdw, followed by papaya seeds. The peel extract demonstrated significant antioxidant potential, exhibiting remarkable DPPH radical scavenging activity (IC₅₀ value: 55.50 µg/mL) and β-carotene/linoleic acid assay (IC₅₀ value: 75.80 µg/mL), surpassing the seed extract. This study concludes that papaya waste can be a good source of antimicrobial and antioxidant agents.

Keywords: Antimicrobial, Antioxidant, Functional foods, Nutraceutical, Papaya waste

Carica papaya L. is a well-known and economically significant fruit tree that grows in subtropical and tropical regions of the world and its fruits are eaten as fruits, vegetables or prepared foods throughout the world¹. The papaya belongs to a small family known as Caricaceae, which consists of only four genera in the world. There are four species in India that belong to the genus *Carica* Linn, with *C. papaya* Linn being the most frequently cultivated species². Papaya cultivation in India is prominently carried out in key states, namely Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, and Chhattisgarh. The total papaya cultivation area spans 149 thousand hectares across the country, yielding a combined production of 5949 thousand metric tons³. Also, numerous scientific studies have been carried out to assess the biological

functions of their various parts, such as fruits, seeds, roots, shoots, rinds, leaves or latex⁴.

The standard papaya fruit is composed of the following components: pulp (79.5%), skin (12%) and seeds (8.5%). Fruit wastes, like the peel from some fruits, have an even higher concentration of beneficial active ingredients than other plant components. Papaya peel and seeds are usually discarded as waste material that can be utilized for medicinal purposes⁵. The extraction of bioactive phytochemicals and their use in a variety of industries, including the food, cosmetics, and pharmaceutical industries, is the most effective and financially advantageous method of managing fruit wastes⁶. Many significant chemicals that might be the building blocks of contemporary medications to treat a variety of ailments could be found by phytochemical screening⁷. Studies have revealed the presence of important phytochemical constituents in fruit peel⁸. Fruit peels contain far more bioactive phytochemicals

*Correspondence:
E-Mail: upmaduttamicro@gmail.com

than the edible portion of the fruit⁹. Pharmacological studies also demonstrated antibacterial, antioxidant and anticancer properties¹⁰. Antimicrobial activity of seeds was documented against *Trichomonas Vagina listrophozoties*¹¹. Both the seed and pulp were reported to show bacteriostatic properties against several entero-pathogens, such as *Proteus vulgaris*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Salmonella typhi*¹². The water extract from the mixture of seed, peel and flesh of unripe *Carica papaya* also inhibited the Gram negative and positive pathogens. In addition to having antibacterial properties, researchers have found that *C. papaya* can reduce a number of adverse effects caused by synthetic antimicrobial drugs¹³.

In the present study, we investigated the antioxidant, and antimicrobial components from the papaya peel and seeds against infection causing microbes and their phytochemical analysis to discover new sources of natural antioxidants from waste products.

Material and Methods

Collection of papaya waste sample

The papaya fruits (Var. Red Lady) were collected from the local market of Jammu district of J & K. The collected papaya samples were brought to the laboratory, Division of Microbiology at Sher-e-Kashmir University of Agricultural Sciences and Technology, where the samples were peeled off and seeds were removed manually. The peels were cut into small pieces, with each piece measuring approximately 3-6 cm and the seed sample size measuring 1-2 cm. Then, the peel and seeds were thoroughly washed with distilled water. The collected waste was dried at room temperature (27°C) for 4-5 days. The dried samples were subjected to grinding to get a fine powder and stored in the refrigerator for further use.

Extraction from papaya waste

The extracts were prepared by dissolving 10 g powder of each sample with 90 mL of ethanol (1:9) separately in round bottom flasks and incubated at 37°C and 150 rpm for 24 h in a shaking incubator. The resulting liquid extract was then concentrated using a rotary evaporator after being separated from the solid residue by filtration using a Whatman No. 1 filter paper¹⁴. Then, the dried crude extracts were dissolved in dimethyl sulphoxide to a concentration of 1 mg/mL and stored at 20°C for further study.

Evaluation of antimicrobial properties of papaya waste

Agar well diffusion method

The antimicrobial properties of papaya peel and seeds were assessed using Agar well diffusion method against four different human pathogenic cultures i.e., *E. coli* (MTCC40), *B. cereus* (MTCC12856), *K. pneumoniae* (MTCC13294), and *S. aureus* (MTCC15029) procured from IMTECH- Chandigarh, India. Molten nutrient agar (NA) was poured into petri plates and allowed to set. A sterile swab is used to evenly distribute human pathogenic cultures over the nutrient agar medium. Then, the four wells were created using a 6 mm cork borer in the agar plates, and samples were filled with the help of a pipette. The plates were incubated at 37±2°C for 24 h and zones of inhibition were recorded with the help of scale¹⁵. DMSO was used as a negative control and streptomycin sulphate was used as a positive control.

Determination of Minimum Inhibitory Concentration (MIC) of papaya waste

Macro broth dilution method

MIC is defined as the lowest concentration of the antimicrobial agents at which no observable growth of microorganisms or turbidity could be seen. The inoculum of each test bacterium was freshly prepared by incubating the cultures overnight. Each microbial inoculum's concentration was adjusted according to the turbidity of the 0.5 McFarland standard. Both the extracts at different concentrations of 20, 40, 60, 80, 100 and 120 µg/mL were mixed with 5 mL of sterile nutrient broth, and then 20 µL of each bacterial inoculum were added to the test tubes. Test tubes were incubated at 37°C for 24 hours and then turbidity was observed. The nutrient broth with microbial inoculum was used as a positive control and only the nutrient broth was used as a negative control¹⁶.

Qualitative phytochemical analysis

For qualitative phytochemical analysis of papaya peel and seeds, the following tests were carried out¹⁷.

Test for carbohydrates

One mL of Molisch's reagent and a few drops of sulphuric acid were added to 2 mL of peel and seed extract. The presence of carbohydrates can be detected by their changing colour to purple or red.

Test for tannins and saponins

Two mL of 5% ferric chloride was added to 1 mL of peel and seed extract. The presence of tannins can be detected by their changing colour to greenish black or dark blue.

Two mL of distilled water was added to 2 mL of peel and seed extract and shaken in a graduated cylinder for 15 minutes lengthwise. The presence of saponins can be detected by the formation of a 1 cm layer of foam.

Test for flavonoids and alkaloids

One mL of sodium hydroxide was added to 2 mL of peel and seed extract. The presence of flavonoids can be detected by their changing colour to yellow.

Two mL of conc. hydrochloric acid was added to 2 mL of peel and seed extract, followed by the addition of a few drops of Mayer's reagent. The presence of alkaloids can be detected by their changing colour to green or white precipitates.

Test for quinines and glycosides

One mL of concentrated sulphuric acid was added to 1 mL of peel and seed extract. The presence of quinones can be detected by the formation of a red colour.

Three mL of chloroform and 10% ammonia solution were added to 2 mL of peel and seed extract. The presence of glycosides can be detected by the formation of a pink colour.

Test for terpenoids and phenols

Two mL of chloroform and concentrated sulphuric acid were carefully added to 0.5 mL of peel and seed extract. The presence of terpenoids can be detected by the formation of red - brown colour at the interface.

Two mL of distilled water was added to 1 mL of peel and seed extract followed by a few drops of 10% ferric chloride. The presence of phenols can be detected by the formation of a blue or green colour.

Tests for steroids and phytosteroids

One mL of chloroform followed by a few drops of conc. sulphuric acid was added to 1 mL of peel and seed extract. The presence of steroids and phytosteroids can be detected by the appearance of brown rings and bluish rings, respectively.

Quantitative phytochemical analysis

Determination of total phenolic content of papaya waste

Total phenolic content was quantified using Folin-Ciocalteu's method¹⁸. The various concentrations of the extract were dissolved in distilled water and mixed with 2 mL of FC reagent diluted with water and the mixture was incubated at 37°C for 10 min. Then, 3 mL of sodium carbonate solution was added. The solution was vortexed for 2 min and kept in the

dark room for 30 min for incubation. Later, the absorbance was read at 760 nm using a UV-Vis spectrophotometer. Using the gallic acid calibration curve as a standard, the values were obtained for the concentration of total phenols and expressed as mg of GAE's/gdw of extract¹⁹.

Determination of total flavonoid content of papaya waste

The total flavonoid content was determined by an aluminum chloride colorimetric assay²⁰. 2 mL of 2% aluminum chloride in ethanol were mixed with different concentrations of extract diluted with ethanol (1 mg/ml). After 5 min, 2 ml of sodium nitrite was added. Then, the mixture was kept at 37°C for incubation for 30 min. The absorbance was measured at 415 nm in a spectrophotometer against a blank. Using the quercetin calibration curve as a standard, the values were obtained for the concentration of total flavonoid and expressed as mg of QE's/gdw of extract.

DPPH free radical scavenging assay

Radical scavenging activity of extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was determined spectrophotometrically²¹. About 2 mL of ethanolic solution of DPPH radical was mixed with different concentrations of both peel and seed extract (i.e., 40, 60, 80 and 100 µg/mL) in different test tubes. The reaction mixture was shaken vigorously and allowed to stand at 37°C for 30 min. The absorbance was read spectrophotometrically at 517 nm. The radical scavenging activity was calculated using the following equation:

$$\text{RSA}\% = [(A_0 - A_1 / A_0) * 100]$$

where A_0 = absorbance of the control and A_1 = absorbance of the sample. Extract concentrations providing 50% inhibition (IC_{50}) were calculated from the plot of inhibition (%) against extract concentrations²².

β -Carotene/Linoleic acid test

A solution of β -carotene was prepared by mixing 2 mg of β -carotene in 10 mL of chloroform. Then, 2 mL of this solution were pipetted into a 100 mL of round bottom flask. After completely evaporating the chloroform, 25 µL of linoleic acid, 200 mg of Tween 40 emulsifier and 100 mL of distilled water with vigorous shaking for 30 minutes were added. About 2 mL aliquots of this mixture were transferred into different test tubes containing different concentrations (i.e., 40, 60, 80 and 100 µg/mL) of both the extracts. Then, the test tubes were shaken and incubated at 37°C. The zero - time absorbance was measured at

470 nm using a spectrophotometer and also after 120 min of incubation²³. Same procedure with BHT, with same concentration and a blank of ethanol were repeated. Antioxidant activity was calculated using the following equation:

$$\%AA = [1 - (A_0 - A_T) / (A_{00} - A_{0T})] * 100$$

where A_0 and A_{00} are the zero time absorbance for samples and control, respectively. A_T and A_{0T} are absorbance measured for samples and control after incubation for 120 min, respectively. The extract concentration providing 50% antioxidant activity (IC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration. BHT was used as standard²⁴.

Statistical analysis

All the above-mentioned experiments were carried out in triplicate, and the data were expressed as Mean \pm SD using Microsoft Excel. The regression analysis i.e., (Pearson’s correlation coefficient) between the experimental data was performed using Microsoft Excel statistical functions.

Results

Crude extract yield from samples

The collected papaya samples, i.e. peel and seeds were dried at 27°C and then subjected to grinding to get a fine powder. The powders of both samples were extracted using ethanol as a solvent. The papaya peel and seeds produced 1.92 and 1.58 g of crude extract per 100 g of sample powder, respectively.

Evaluation of antibacterial activity of crude extract of papaya peel and seeds

The crude extract of papaya peel and seeds was screened for their antibacterial activities against different human pathogens at different concentrations by using the agar well diffusion method and the results are depicted in Table 1 and Fig. 1. The results revealed that at 50 $\mu\text{g/mL}$ concentration, both the extracts of papaya didn’t show any zone of inhibition against all the human pathogens. At 80 $\mu\text{g/mL}$ concentration, the papaya peel showed 10.50 \pm 1.00, 04.00 \pm 0.20 and 11.00 \pm 0.50 mm zones of inhibition against *B. cereus*, *K. pneumoniae*, and *S. aureus*, respectively. However, no inhibition was recorded against *E. coli* on the other hand, the papaya

Table 1 — Antibacterial activity of crude ethanolic extract of papaya peel/seeds

Bacterial strains	Concentration ($\mu\text{g/mL}$)			Positive control	Negative control
	50	80	100		
<i>Escherichia coli</i>	0.00 \pm 0.00/0.00 \pm 0.00	0.00 \pm 0.00/5.00 \pm 0.50	16.00 \pm 2.00/9.40 \pm 1.15	19.00 \pm 1.00/18.00 \pm 1.05	No growth
<i>Bacillus cereus</i>	0.00 \pm 0.00/0.00 \pm 0.00	10.50 \pm 1.00/4.50 \pm 0.50	21.50 \pm 2.00/8.00 \pm 1.00	18.60 \pm 2.00/14.00 \pm 1.00	in both
<i>Klebsiella pneumoniae</i>	0.00 \pm 0.00/0.00 \pm 0.00	4.00 \pm 0.20/7.00 \pm 0.20	25.50 \pm 2.00/20.00 \pm 1.00	22.60 \pm 2.00/20.00 \pm 4.00	extracts
<i>Staphylococcus aureus</i>	0.00 \pm 0.00/0.00 \pm 0.00	11.00 \pm 0.50/9.00 \pm 0.20	14.40 \pm 1.15/7.50 \pm 1.00	16.80 \pm 1.50/16.10 \pm 0.08	

[Data expressed as Mean \pm Standard Deviation (n=3); Negative control: DMSO; Positive control: Streptomycin sulphate]

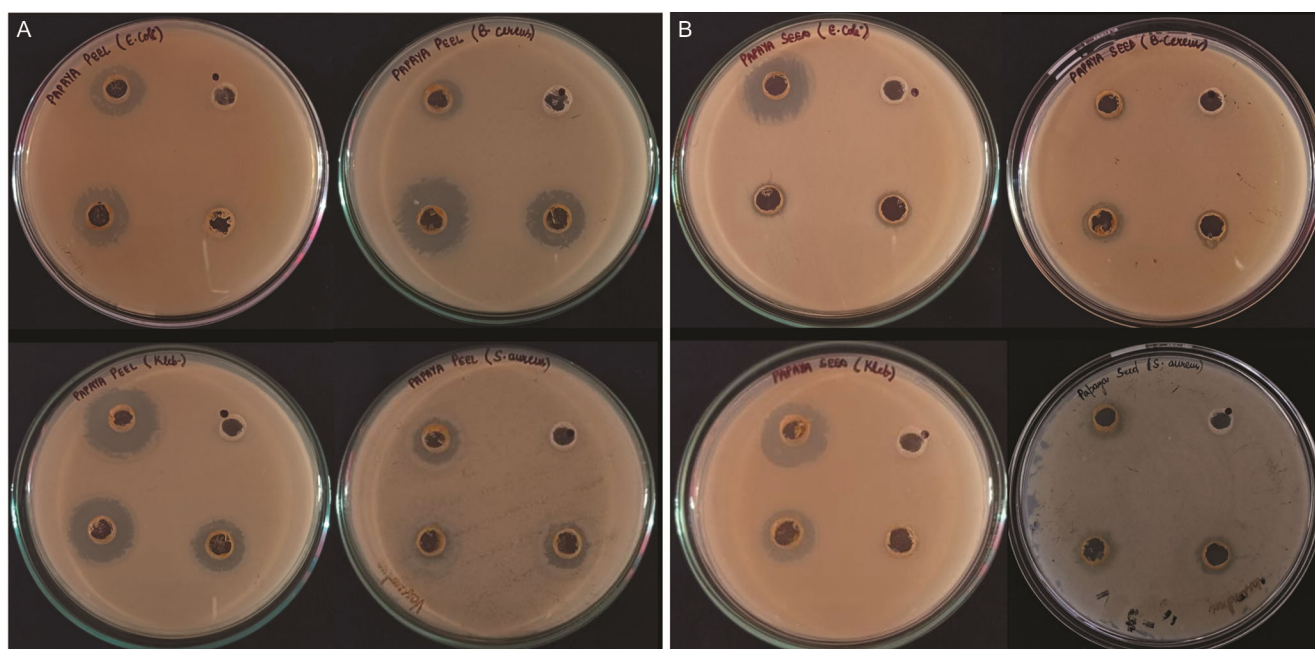


Fig. 1 — Antimicrobial activity of papaya peel and seeds against *E. coli*, *B. cereus*, *K. pneumoniae* and *S. aureus* at 100 $\mu\text{g/mL}$

seed showed 05.40 ± 5.00 , 04.50 ± 5.00 , 07.00 ± 2.00 and 09.00 ± 2.00 mm zone of inhibition against *E. coli*, *B. cereus*, *K. pneumoniae* and *S. aureus*, respectively. At $100 \mu\text{g/mL}$ concentration, the maximum zone of inhibition was recorded. The papaya peel showed 16.00 ± 1.00 , 21.50 ± 1.00 , 25.50 ± 1.00 and 14.40 ± 1.15 mm zone of inhibition against *E. coli*, *B. cereus*, *K. pneumoniae* and *S. aureus*, respectively. In contrast, papaya seed showed 19.40 ± 1.15 , 08.00 ± 1.00 , 20.00 ± 1.00 and 7.50 ± 1.00 mm zone of inhibition against *E. coli*, *B. cereus*, *K. pneumoniae* and *S. aureus*, respectively. Notably, the maximum zone of inhibition was observed against *K. pneumoniae* in the papaya peel extract, while the least zone of inhibition was recorded against *S. aureus* in the papaya seed extract at the $100 \mu\text{g/mL}$ concentration. *K. pneumoniae* exhibited the highest level of inhibition against both extracts. No inhibition was recorded by the negative control (DMSO) and the positive control (Streptomycin Sulphate) of papaya peel and seeds showed inhibition for *E. coli* (19.00 ± 1.00 mm; 18.00 ± 1.05 mm) ; *B. cereus* (18.60 ± 2.00 mm; 14.00 ± 1.00 mm) ; *K. pneumoniae* (22.60 ± 2.00 mm; 20.00 ± 4.00 mm) ; *S. aureus* (16.80 ± 1.50 mm; 16.10 ± 0.08 mm), respectively.

Determination of minimum inhibitory concentration (MIC) of papaya waste

The results of the minimum inhibitory concentration of the crude extract of papaya peel and seeds against the selected pathogenic strains by using macro broth dilution method are depicted in Table 2. The MIC values of the ethanolic extract of papaya peel against *E. coli*, *B. cereus*, and *S. aureus* were $120 \mu\text{g/mL}$, and $100 \mu\text{g/mL}$ against *K. pneumoniae*. On the other hand, for the ethanolic extract of papaya seeds, the MIC values were $100 \mu\text{g/mL}$ against all the strains. It can be inferred from the results that papaya seeds are more effective than papaya peel as an antimicrobial agent against all the tested pathogenic strains.

Qualitative phytochemical analysis

The crude extract of papaya peel and seeds was screened for the phytochemical constituents, and the

results depicted in Table 3 and Fig. 2 revealed that there is the presence and or absence of certain phytochemicals in both samples. The phytochemicals including carbohydrates, flavonoids, quinones, glycosides, terpenoids, phenols and steroids, were present, whereas tannins, saponins, alkaloids and phytosteroids were absent in both extracts i.e., papaya peel and seeds.

Determination of total phenolic content of papaya peel and seeds

The result of the total phenolic content of papaya peel and seeds was examined using Folin-Ciocalteu's reagent which is expressed in terms of gallic acid equivalent (the standard curve equation: $y = 0.0046x + 0.0675$) and is represented in Table 4. The values obtained for the concentration of total phenols are

Table 2 — Minimum inhibitory concentration of crude extract of papaya peel and seeds

Bacterial strains	MIC ($\mu\text{g/mL}$)	
	Peel	Seed
<i>Escherichia coli</i>	120	100
<i>Bacillus cereus</i>	120	100
<i>Klebsiella pneumoniae</i>	100	100
<i>Staphylococcus aureus</i>	120	100

Table 3 — Preliminary phytochemical screening of peel and seed ethanolic extracts

	Peel extract	Seed extract
Carbohydrates	+	+
Tannins	-	-
Saponins	-	-
Flavonoids	+	+
Alkaloid's	-	-
Quinones	+	+
Glycosides	+	+
Terpenoids	+	+
Phenols	+	+
Steroids	+	+
Phytosteroids	-	-

['-' Indicates Absence and '+' Presence]

Table 4 — Total phenol and total flavonoid content of papaya peel and seeds

Extract	TPC (mg GAE's /gdw)	TFC (mg QE's /gdw)
Papaya peel	51.39 ± 0.003	34.92 ± 0.004
Papaya seed	62.45 ± 0.014	47.84 ± 0.024

[Data expressed as Mean \pm Standard Deviation (n=3)]

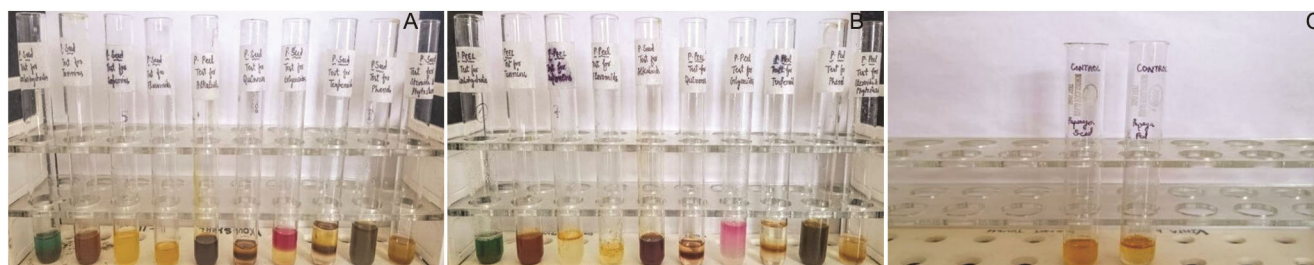


Fig. 2 — Preliminary phytochemical screening of (A) papaya peel (B) seeds extract (C) its Control

expressed as mg of GAE's/gdw of extract. All parts of *Carica papaya* are substantial sources of polyphenols, but the total amount varied considerably. The total amount was 51.39 mg of GAE's/gdw in papaya peel whereas in papaya seeds, the total amount was 62.45 mg of GAE's/gdw. The total phenolic content (TPC) was higher in the extract of papaya seeds than in the peel.

Determination of total flavonoid content of papaya peel and seeds

The result of total flavonoid content (TFC) of papaya peel and seeds was examined using the aluminum chloride method, which is expressed in terms of quercetin equivalent (the standard curve equation: $y = 0.0039x + 0.0325$) and is represented in Table 4. The values obtained for the concentration of total flavonoids are expressed as mg of QE's/gdw of extract. The total amount of flavonoids varied significantly, and the papaya seed extract contained about 34.92 mg of QE's/gdw whereas in papaya seeds, the total amount was 47.84 mg of QE's/gdw. The total flavonoid content was highest in the extract of papaya seeds, followed by peel.

Evaluation of antioxidant properties of papaya peel and seeds DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The crude extract of papaya peel and seeds were evaluated for their antioxidant properties using a DPPH free radical scavenging assay. During performing this assay, radical scavenging activity (RSA%) in DPPH was observed in concentrations of 20, 40, 60, 80 and 100 $\mu\text{g/mL}$ was 25, 40.76, 56.34, 64.8 and 82.11%, respectively, in the extract of papaya peel whereas in the extract of papaya seeds, the scavenging activity is 20.38, 31.73, 44.8, 52.69 and 69.03%, respectively. The results of radical scavenging activity (RSA) are represented in Fig. 3A in which the IC_{50} value of papaya peel is 55.50 $\mu\text{g/mL}$, and papaya seed is 70.10 mg/mL , which means that papaya peel showed maximum inhibition compared to the seeds.

β -Carotene/Linoleic acid test

The crude extract of papaya peel and seeds was evaluated for their antioxidant properties using β -carotene /linoleic acid test. During performing this assay, antioxidant activity (AA%) was observed in concentrations of 20, 40, 60, 80 and 100 $\mu\text{g/mL}$ was 25.17, 38.09, 46.2, 50.34 and 61.22%, respectively, in the extract of papaya peel and 9.52, 20.4, 30.6, 36.05 and 48.29%, respectively, in the extract of papaya seed. The results of antioxidant activity (AA%) are

represented in Fig. 3B. The IC_{50} value of papaya peel is 75.80 $\mu\text{g/mL}$, and papaya seeds is 104.20 mg/mL , which means that papaya peel showed maximum inhibition compared to the seeds.

Pearson's correlation analysis between experimental data

The correlation of total phenol and total flavonoid content with DPPH free radical scavenging activities and the β -carotene/linoleic acid test of papaya peel revealed that both TPC and TFC of papaya peel were strongly positively correlated with them i.e., $R^2 = 0.986$ and $R^2 = 0.983$; $R^2 = 0.964$ and $R^2 = 0.954$ whereas the correlation of total phenol and flavonoid content with DPPH free radical scavenging activities and β -Carotene/Linoleic acid test of papaya seeds demonstrated that both TPC and TFC of papaya seeds are strongly positively correlated with them, i.e. $R^2 = 0.982$ and $R^2 = 0.975$; $R^2 = 0.975$ and $R^2 = 0.971$.

The correlation of TPC and TFC of papaya peel with the MIC of the tested bacterium demonstrated that both TPC and TFC of papaya peel are positively correlated with MIC of *E. coli*, *B. cereus*, *K. pneumoniae* and *S. aureus*, respectively, i.e. $R^2 = 0.83$, $R^2 = 0.90$, $R^2 = 0.87$, $R^2 = 0.96$ and $R^2 = 0.83$, $R^2 = 0.92$, $R^2 = 0.86$, $R^2 = 0.96$ whereas the correlation of TPC and TFC of papaya seeds with the

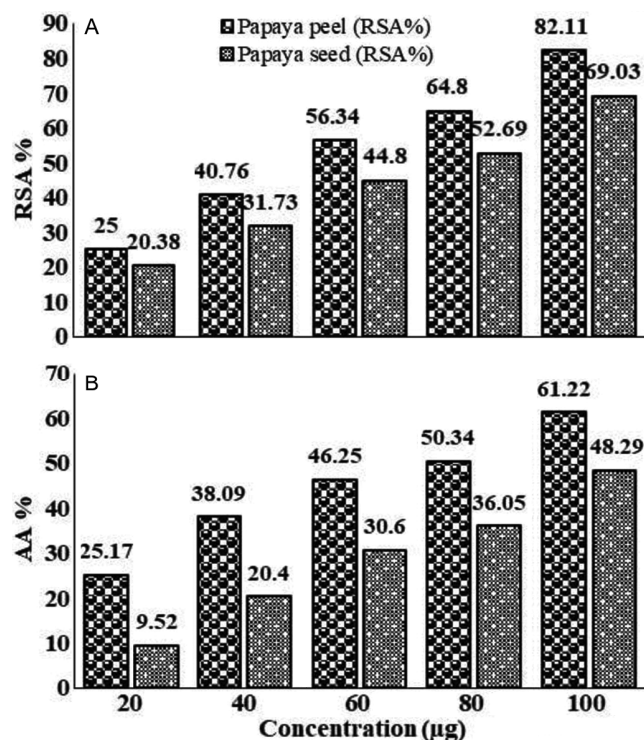


Fig. 3 — (A) DPPH assay of papaya peel and seeds (B) Carotene/Linoleic acid test of papaya peel and seeds

MIC of the tested bacterium are shown in Figs 3 and 4 which also demonstrated that both TPC and TFC of papaya peel are positively correlated with the MIC of *E. coli*, *B. cereus*, *K. pneumoniae* and *S. aureus*, respectively, i.e. $R^2 = 0.87$, $R^2 = 0.98$, $R^2 = 0.93$, $R^2 = 0.940$ and $R^2 = 0.85$, $R^2 = 0.97$, $R^2 = 0.92$, $R^2 = 0.93$.

Discussion

The present investigation evaluated that *Carica papaya* waste possesses medicinal properties, is mainly consumed for its high nutritive value, and used for pharmaceutical purposes in the treatment of various ailments. Various studies have revealed that it possesses medicinal properties like anti-inflammatory hypoglycemic, anti-fertility, abortifacient, hepatoprotective, wound healing, and recently, its anti-hypertensive and antitumor activities have also been established. The papain enzyme derived from papaya has demonstrated its ability to assist in protein digestion and has a history of traditional medicinal use in treating indigestion²⁵. A number of other fruits' waste products (peel and seeds) have higher antioxidant properties than their pulp.

The collected samples of *Carica papaya* were extracted using ethanol as a solvent and conventional solvent extraction method was employed. Asghar *et al.*²⁶ also employed the same extraction method for the extraction of papaya seeds by using Ethanol as a solvent. The results revealed that the extract from papaya peel was greater than the papaya seed extract. The extraction yield of active components is significantly influenced by the solvent. These results are in accordance with previous studies conducted for the extraction of crude extract from papaya waste by²⁷.

The crude extract of peel and seeds was assessed for antibacterial properties against four different human pathogens viz., (*E. coli*, *B. cereus*, *K. pneumoniae*, and *S. aureus*) by the agar-well diffusion method and the results showed that at higher concentration (i.e., 100 µg/mL) both peel and seed extracts significantly inhibited the growth of human pathogens. Our findings are consistent with those of Akujobi *et al.*²⁸ in which the papaya peel and seeds showed inhibition against *S. aureus* and *E. coli*. Our results are in accordance with the study conducted by Rakholiya *et al.*²⁹ in which the papaya peel extract showed a varied zone of inhibition against the selected gram positive and negative bacteria, and the same results were shown in seeds in the study

conducted by Dagne *et al.*³⁰. Thus, it indicates that the peel and seeds of *Carica papaya* have good antibacterial and antioxidant properties. Sofi *et al.*³¹ reported that the papaya peel extract has antibacterial activities against gram positive and gram negative bacteria. An investigation by Li *et al.*³² revealed that papaya seeds contain Benzyl isothiocyanate (BITC) which exhibits a broad-spectrum antibacterial effect against *E. coli*, *B. subtilis*, and *A. niger*, with a minimum inhibitory concentration of 1 µL/mL, and 0.5 µL/mL for *S. enterica*, *S. aureus*, and *P. citrinum*. The antibacterial activity demonstrated by papaya waste (peel and seeds) could be due to the combined or individual effects of the active compounds present in them. Phytochemicals function as anti-bacterial components and effective binders in order to stop pathogens from adhering to human cell walls.

Both the crude extracts that showed potent antibacterial activity were further examined for their MICs by the macro broth dilution method against (*E. coli*, *B. cereus*, *K. pneumoniae* and *S. aureus*). The peel and seed extracts have shown MICs ranging from 100 to 120 µg/mL and 100 µg/mL, respectively, against the selected human pathogens. Patra *et al.*³³ reported the antibacterial activity of the papaya peels agitated with gold nanoparticles and determined their minimum inhibitory concentration (MIC) by macro broth dilution method. Santos *et al.*³⁴ determined the minimum inhibitory concentration of peel and seeds of different exotic fruits. The Pearson's correlation coefficient between the TPC and TFC of the ethanolic extract of papaya peel and seeds with their MIC against selected pathogens showed a highly positive linear correlation. Our findings are similar to those of Ispiryan *et al.*³⁵ and Mahboubi *et al.*³⁶ and who reported that there is a positive linear correlation between antimicrobial activity (i.e., MIC) and their phenolic and flavonoid contents. By using MIC analysis, the lowest concentration of fruit waste extract can be found as a promising, economical antimicrobial agent against pathogenic microorganisms.

In the present study, the qualitative phytochemical analysis was done by conducting various tests in which there was the presence of various phytochemicals except tannins, saponins, alkaloids and phytosteroids in papaya waste extract. Our results are comparable to the study conducted by Pavithra *et al.*³⁷ in which the papaya peel showed the presence of phytochemicals that exhibit strong antimicrobial

activities due to the active constituents present in them. Kanadi *et al.*³⁸. The crude seed extracts of *C. papaya* extracted using water, methanol, ethyl acetate, chloroform and *n*-hexane screened for the presence of six major phytochemicals namely flavonoids, alkaloids, saponins, cardiac glycosides, tannins and anthraquinones showed that flavonoids, alkaloids and saponins are prominently present in all the extracts in varying composition.

In the quantitative phytochemical analysis, quantification of TPC and TFC was done, in which the papaya seed extract contained its maximum content. In a similar way, Singla *et al.*³⁹ calculated the TPC and TFC of the ethanolic extract of papaya waste. Our outcomes are in accordance with those of Jeon *et al.*⁴⁰ who calculated the TPC and TFC of papaya waste extracts, and the seed extract had a higher content of both. Additionally, Zhou *et al.*⁴¹ ascertained the total polyphenolic and flavonoid contents of ethanol, petroleum ether, ethyl acetate, *n*-butanol and water extract of papaya seeds. It is recognized that papaya waste contains phenolic acids and flavonoids as secondary metabolites which hold significance due to their antioxidant properties. Noteworthy phenolic acids encompass hydroxycinnamic acids, coumaric acids, hydroxybenzoic acids, gallic acids, and vanillic acids. Additionally, significant flavonoids comprise catechin, rutin, and quercetin. It is crucial to note that the antioxidant efficacy of phenolic compounds is intricately tied to their chemical structure. Consequently, elucidating the precise mechanism of antioxidant activity goes beyond merely assessing their total phenolic contents.

Free radicals exert a significant impact on both tissue and cellular damage. Aerobic organisms mitigate the effects of oxidant compounds through their antioxidant defense system, comprising enzymatic and non-enzymatic mechanisms. The utilization of supplementary antioxidants becomes crucial in situations where endogenous antioxidants prove insufficient. The rising interest in natural antioxidants is attributable to their effects. The incorporation of natural waste materials as supplementary antioxidants to the endogenous defence system holds the potential to postpone oxidative damage. The papaya peel and seeds have demonstrated a noteworthy presence of antioxidant compounds, suggesting its potential as a source for natural antioxidants upon extraction. In our study, the results revealed that the antioxidant activities were

greater in papaya peel extract than in seed. Nurul and Asmah⁴² determined the antioxidant activities of the ethanolic extract of papaya waste by using similar methods and found good activity. Loizzo *et al.*⁴³ evaluated the antioxidant activities of *Annona cherimola* Mill. (Cherimoya) peel and pulp by using similar assays in which the ethanolic extract of peel showed strong antioxidant activities.

The Pearson's correlation coefficient between the TPC and TFC of the ethanolic extract of papaya peel and seeds with their antioxidant assays showed a highly positive linear correlation. Shofian *et al.*⁴⁴ evaluated that there was a positive correlation between the antioxidant activities of selected five fruits (Papaya, Mango, Starfruit, Muskmelon and Watermelon) with their total phenol content. The presence of a strong positive correlation may suggest that TPC and TFC are primarily responsible for antioxidant and free radical scavenging activities. Most of the free radicals are believed to be scavenged by TPC and TFC present in most fruits. The phenolic and flavonoid contents significantly increase the antioxidant activity.

Conclusion

Investigating the antimicrobial and antioxidant properties of papaya waste can yield multifaceted outcomes with promising implications. Findings of this study revealed that the antimicrobial activity of papaya peel and seed exhibited the maximum zone of inhibition against all four pathogens at a higher concentration, i.e., 100 µg/mL. Moreover, the extract of papaya peel showed more antioxidant activity than papaya seed whereas in determination of total phenolic and flavonoid content papaya seed has more content compared to papaya peel because of the presence of various bioactive compounds. Such revelations can pave the way for isolating and identifying these compounds, offering potential applications in various industries like food, pharmaceutical and cosmetic sectors. In essence, exploring the antimicrobial and antioxidant potential of papaya waste holds the promise of transforming an otherwise discarded byproduct into a valuable resource with applications in health, industry, and environmental sustainability.

Acknowledgment

This study was supported by the Division of Microbiology, Faculty of Basic Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu.

Conflict of Interest

Authors declare no competing interests.

References

- Koul B, Pudhuvai B, Sharma C, Kumar A, Sharma V, Yadav D & Jin-O J, *Carica papaya* L.: A Tropical Fruit with Benefits beyond the Tropics. *Diversity*, 14 (2022) 683. doi: <https://doi.org/10.3390/d14080683>.
- Wadekar AB, Nimbawar MG, Panchale WA, Gudalwar BR, Manwar JV & Bakal RL, Morphology, phytochemistry and pharmacological aspects of *Carica papaya*, an review. *GSC Biol Pharm Sci*, 14 (2021) 234–248. doi: <https://doi.org/10.30574/gscbps.2021.14.3.0073>.
- Agriculture at a glance 2022. (Directorate of Economics and Statistics (DES), Ministry of Agriculture and Farmers Welfare, Government of India. <https://agriwelfare.gov.in/en/AgriculturalStatisticsataGlance>.
- Dottoa JM & Abihudi SA, Nutraceutical value of *Carica papaya*: A review. *Sci Afr*, 13 (2021) 00933. doi: <https://doi.org/10.1016/j.sciaf.2021.e00933>.
- Nirmal NP, Khanashyam AC, Mundanat AS, Shah K, Babu KS, Thorakkattu P, Al-Asmari F & Pandiselvam R, Valorization of fruit waste for bioactive compounds and their applications in the food industry. *Foods*, 12 (2023) 556. doi: <https://doi.org/10.3390/foods12030556>.
- Patra A, Abdullah S & Pradhan RC, Review on the extraction of bioactive compounds and characterization of fruit industry by-products. *Bioresour Bioprocess*, 9 (2022) 14.
- Najmi A, Javed SA, Al Bratty M & Alhazmi HA, Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. *Molecules*, 27 (2022) 349. doi: <https://doi.org/10.3390/molecules27020349>.
- Chakroun I, Bouraoui Z, Ayachi T, Hosni K, Guerbèj H, Snoussi M, Jebali J & Gharred T, Phytochemical constituents and potential applications of Thomson Navel orange (*Citrus × aurantium* var. *sinensis* L.) peel extracts: Antioxidant, antimicrobial and antiproliferative properties. *Ind Crops Prod*, 206 (2023) 117597. doi: <https://doi.org/10.1016/j.indcrop.2023.117597>.
- Bhardwaj K, Najda A, Sharma R, Nurzyńska-Wierdak R, Dhanjal DS, Sharma R, Manickam S, Kabra A, Kuča K & Bhardwaj P, Fruit and Vegetable Peel-Enriched Functional Foods: Potential Avenues and Health Perspectives. *Evid Based Complement Alternat Med*, 4 (2022) 8543881. doi: [10.1155/2022/8543881](https://doi.org/10.1155/2022/8543881).
- Villacis-Chiriboga J, Elst K, Van-Camp J, Vera E & Ruales J, Valorization of byproducts from tropical fruits: Extraction methodologies, applications, environmental, and economic assessment: A review (Part 1: General overview of the byproducts, traditional bio refinery practices, and possible applications). *Compr Rev Food Sci Food Saf*, 19 (2020) 405.
- Friedman M, Tam CC, Cheng LW & Land KM, Anti-trichomonad activities of different compounds from foods, marine products, and medicinal plants: a review. *BMC Complement Med Ther*, 20 (2020) 271. doi: [10.1186/s12906-020-03061-9](https://doi.org/10.1186/s12906-020-03061-9).
- Hsu CL, Chen W, Weng YM & Tseng CY, Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. *Food Chem*, 83 (2003) 85.
- Devanesan S, Jayamala M, AlSalhi SM, Umamaheshwari S, Jacob A & Singh R, Antimicrobial and anticancer properties of *Carica papaya* leaves derived di-methyl flubendazole mediated silver nanoparticles. *J Infect Public Health*, 14 (2021) 577.
- Xu GH, Chen JC, Liu DH, Zhang YH, Jiang P & Ye XQ, Minerals, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water. *J Food Sci*, 73 (2008) 11.
- Manandhar S, Luitel S & Dahal RK, In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. *J Trop Med*, 2019 (2019) 1895340. doi: [10.1155/2019/1895340](https://doi.org/10.1155/2019/1895340).
- Li P, Zhao YM, Wang C & Zhu HP, Antibacterial activity and main action pathway of benzyl isothiocyanate extracted from papaya seeds. *J Food Sci*, 86 (2021) 169.
- Lydia E, John S, Mohammed R & Sivapriya T, Investigation on the phytochemicals present in the fruit peel of *Carica papaya* and evaluation of its antioxidant and antimicrobial property. *Res J Pharm Phytochem*, 8 (2016) 217. DOI:10.5958/0975-4385.2016.00032.7.
- Khanizadeh S, Tsao R, Rekika D, Yang R, Charles MT & Vasantha Rupasinghe HP, Polyphenol composition and total antioxidant capacity of selected apple genotypes for processing. *J Food Comp Anal*, 21 (2008) 396. doi: <https://doi.org/10.1016/j.jfca.2008.03.004>.
- Castro-Vargas HI, Baumann W, Ferreira SRS & Parada-Alfonso F, Valorization of papaya (*Carica papaya* L.) agro industrial waste through the recovery of phenolic antioxidants by supercritical fluid extraction. *J Food Sci Technol*, 56 (2019) 3055. doi: <https://doi.org/10.1007/s13197-019-03795-6>.
- Saeed A, Marwat MS, Shah AH, Naz R, Abidin SZ, Akbar S, Khan R, Navid MT, Saeed A & Bhatti MZ, Assessment of total phenolic and flavonoid contents of selected fruits and vegetables. *IJTK*, 18 (2019) 686-93. DOI: 10.56042/ijtk.v18i4.28999.
- Xie Q, Liu ZH & Li ZQ, Chemical composition and antioxidant activity of essential oil of six *Pinus taxa* native to China. *Molecules*, 20 (2015) 9380.
- Kosanic M, Rankovic B & Dasic M, Antioxidant and antimicrobial properties of mushrooms. *Bulg J Agri Sci*, 19 (2013) 1040.
- Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B & Weil JA, Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem*, 84 (2004) 551.
- Boudoukha C, Bouriche H, Elmastas M, Aksit H, Kayir O, Genc N & Senator A, Antioxidant activity of polyphenolic leaf extract from *Santolina chamaecyparissus* L. (Asteraceae) and the isolated luteolin-7-O-glucoside. *J Pharm Res Int*, 22 (2018) 1. doi: [10.9734/JPRI/2018/40726](https://doi.org/10.9734/JPRI/2018/40726).
- Babalola BA, Akinwande AI, Otunba AA, Adebami GE, Babalola O & Nwufu C, Therapeutic benefits of *Carica papaya*: A review on its pharmacological activities and characteristics of papain. *Arab J Chem*, 17 (2024) 105369. Doi: <https://doi.org/10.1016/j.arabjch.2023.105369>.
- Asghar N, Naqvi SAR, Hussain Z, Rasool N, Khan ZA, Shahzad SA, Sherazi TA, Janjua MRSA, Saeed Ahmad Nagra, Muhammad Zia-Ul-Haq & Jaafar HZ, Compositional difference in antioxidant and antibacterial activity of all parts of the *Carica papaya* using different solvents. *Chem Cent J*, 10 (2016) 5. <https://doi.org/10.1186/s13065-016-0149-0>.

- 27 Masfufatun, Yani NPW & Putri NPYK, Antimicrobial assay of papaya seed ethanol extract (*Carica papaya* Linn) and phytochemical analysis of its active compounds. *J Phy Conf Ser*, 1277 (2019) 1.
- 28 Akujobi CN, Ofodeme CN & Enweani CA, Determination of antibacterial activity of *Carica papaya* (pawpaw) extracts. *Niger J Clin Pract*, 13 (2010) 55.
- 29 Rakholiya K, Kaneria M & Chanda S, Inhibition of microbial pathogens using fruit and vegetable peel extracts. *Int J Food Sci Nutr*, 65 (2014) 733.
- 30 Dagne E, Dobo B & Bedewi Z, Antibacterial activity of papaya (*Carica papaya*) leaf and seed extracts against some selected gram-positive and gram-negative bacteria. *Pharmacogn J*, 13 (2021) 1727.
- 31 Sofi FR, Raju CV, Lakshmisha IP & Singh RR, Antioxidant and antimicrobial properties of grape and papaya seed extracts and their application on the preservation of Indian mackerel (*Rastrelliger kanagurta*) during ice storage. *J Food Sci Tech*, 53 (2016) 104.
- 32 Li P, Zhao YM, Wang C & Zhu HP, Antibacterial activity and main action pathway of benzyl isothiocyanate extracted from papaya seeds. *J Food Sci*. 186 (2021) 169-176. doi: 10.1111/1750-3841.15539.
- 33 Patra JK, Shin, HS, Yang IJ, Nguyen LTH & Das G, Sustainable Utilization of Food Biowaste (Papaya Peel) Extract for Gold Nanoparticle Biosynthesis and Investigation of Its Multi-Functional Potentials. *Antioxidants*, 13 (2024) 581. <https://doi.org/10.3390/antiox13050581>.
- 34 Santos TRJ, Santana LCLA, Antimicrobial potential of exotic fruits residues. *S Afr J Bot*, 124 (2019) 338-344. Doi: <https://doi.org/10.1016/j.sajb.2019.05.031>
- 35 Mahboubi A, Asgarpanah J, Sadaghiyani PS & Faizi M, Total phenolic and flavonoid content and antibacterial activity of *Punica granatum* L.var. Pleniflora flowers (Golnar) against bacterial strains causing foodborne diseases. *BMC Complement Altern Med*, 15 (2015) 366. doi: 10.1186/s12906-015-0887-x.
- 36 Ispiryan A, Atkociuniene V, Makstutiene N, Sarkinas A, Salaseviciene A, Urbonaviciene D, Viskelis J, Pakeltiene R & Raudone L, Correlation between Antimicrobial Activity Values and Total Phenolic Content/Antioxidant Activity in *Rubus idaeus* L. *Plants*, 13 (2024) 504. <https://doi.org/10.3390/plants13040504>
- 37 Pavithra CS, Devi SS, Suneetha WJ, Kumari BA & Rani CVD, Antioxidant activity of papaya peel and developed chapathis. *Intern J Current Micro App Sci*, 6 (2017) 636.
- 38 Kanadi AM, Yila R, Ibrahim MP, Yaradua AI and Nasir A, Proximate composition and phytochemical constituents of matured *Carica papaya* seed extract. *Asian J Res Biochem*, 9 (2021) 28-33.
- 39 Singla M, Singh A & Sit N, Effect of microwave and enzymatic pretreatment and type of solvent on kinetics of ultrasound assisted extraction of bioactive compounds from ripe papaya peel. *J Food Process Eng*, 46 (2022). doi:10.1111/jfpe.14119.
- 40 Jeon YA, Chung SW, Kim SC & Lee YJ, Comprehensive assessment of antioxidant and anti-inflammatory properties of papaya extracts. *Foods*, 11 (2022) 3211.
- 41 Zhou K, Wang H, Mei W, Li X, Luo Y, Dai H, Antioxidant activity of papaya seed extracts. *Molecules*, 16 (2011) 6179-92. doi: 10.3390/molecules16086179.
- 42 Nurul SR & Asmah R, Evaluation of antioxidant properties in fresh and pickled papaya. *Inter Food Res J*, 19 (2012) 1117.
- 43 Loizzo MR, Tundis R, Bonesi M, Menichini F, Mastellone V, Avallone L & Menichini F, Radical scavenging, antioxidant and metal chelating activities of *Annona cherimola* Mill. (Cherimoya) peel and pulp in relation to their total phenolic and total flavonoid contents. *J Food Compos Anal*, 25 (2012) 179.
- 44 Shofian NM, Hamid AA, Osman A, Saari N, Anwar F, Pak DMS & Hairuddin MR, Effect of freeze-drying on the antioxidant compounds and antioxidant activity of selected tropical fruits. *Int J Mol Sci*, 12 (2011) 4678.