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ORIGINAL ARTICLE

Thin Layer Chromatography-Mass Spectrometry Bioautographic Identification of Free Radical Scavenging Compounds and Metabolomic Profile of *Carica papaya* Linn. Fruit and Seeds using High-Performance Thin-Layer Chromatography, Gas Chromatography-Mass Spectrometry and Ultra-Performance Liquid Chromatography-Mass Spectrometry

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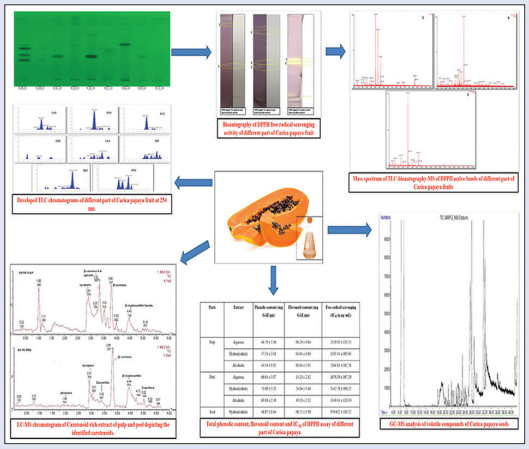
ABSTRACT

Objectives: *Carica papaya* Linn. a member of the Caricaceae family, is a tropical fruit, rich in various secondary metabolites owing to its antioxidant, anti-inflammatory, anti-diabetic, and antihelmintic properties. The study was carried out to investigate the metabolic profiling and free radical scavenging compounds in *C. papaya* fruit's pulp, peel and seeds through thin-layer chromatography-mass spectrometry (TLC-MS) bioautography. **Methodology:** Aqueous, hydroalcoholic and alcoholic extracts of fruit pulp and peel were prepared along with the hydroalcoholic and hexane extract of seeds. These were subjected to total phenol, flavonoid and free radical scavenging estimation. Qualitative and quantitative high-performance thin-layer chromatography analysis of the best extracts was performed followed by TLC-MS bio autography assay for the detection of free radical scavenging compounds. **Results:** *C. papaya* peel was noted to contain the highest phenol and flavonoid content, but the seeds showed better free radical scavenging activity. The hydroalcoholic extracts of pulp, peel, and seeds examined through TLC-bioautography showed the presence of chlorogenic acid, ellagic acid, quercetin, β-sitosterol, linoleic acid, and iso-oleic acid as potent free radical scavenging compounds. Liquid chromatography-mass spectrometry analysis showed the presence of β-carotene, lycopene, and β-cryptoxanthin in pulp and peel along with other carotenoids and benzyl isothiocyanate, linoleic acid, oleic acid, and methyl palmitate were the major compounds detected in seeds through gas chromatography-mass spectrometry analysis. **Conclusion:** This study has revealed that *C. papaya* fruit and seeds possess potent free radical scavenging compounds. Seeds which make up the waste material may be utilized in cosmetic industries as they signify rich antioxidants.

Key words: Bioautography, *Carica papaya,* free radical scavenging, liquid chromatography-mass spectrometry, metabolomic profiling

SUMMARY

- The hydroalcoholic extract of peel and pulp were found best by overall results of metabolomic profiling and 2,2-diphenyl-1-picrylhydrazyl; assay as compared to aqueous and alcoholic extract
- Quantitative estimation of polyphenolic compounds was evaluated by high-performance thin-layer chromatography
- The carotenoids were identified by ultra-performance liquid chromatography-mass spectrometry in pulp and peel as well as non-polar compounds in seed by gas chromatography-mass spectrometry. Thin-layer chromatography-mass spectrometry bioautography of *Carica papaya* fruit was reported for the first time and six metabolites were identified as a free radical scavenging compound.



Abbreviations Used: RT: Room temperature; BHT: Butylated hydroxytoluene; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ESI-MS: Electrospray ionization-mass spectrometry; GAE: Gallic acid equivalent; GC-MS: Gas chromatography-mass spectrometry; HPTLC: High-performance thin-layer chromatography; IC_{50}: Half-maximal inhibitory concentration; KOH: Potassium hydroxide; LC-MS: Liquid chromatography-mass spectrometry; NaCl: Sodium chloride; R_f: Retardation factor; R_t: Retention time; TLC-MS: Thin-layer chromatography-mass spectrometry; UPLC-MS: Ultra-performance liquid chromatography-mass spectrometry.

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INTRODUCTION

The value and importance of phytonutrients are gaining importance these days as the demand for natural metabolites in human health betterment is increasing. These phytochemicals considered essential nutrients are classified under various broad categories such as polyphenols, carotenoids, alkaloids, and glycosides. Phytonutrients increase or decrease with the age of the plant and many of them are lost during food processing too, therefore the consumption of raw fruits and vegetables is emphasized over the processed or cooked ones. *Carica papaya* Linn., a tropical fruit, is widely consumed by people all over the world for curing digestive disorders, for its antimicrobial, antimalarial, antifungal, antimetastasis, antiulcer, antimutagenic, antiallergic, anticarcinogenic, and hepatoprotective activity, in cases of inducing male and female antifertility and immunomodulatory.[1] Seeds and fruits have excellent anthelminthic and anti-amoebic activity.[2] It is rich in natural vitamins, minerals and has low calorific value making it a favorite fruit of obese people who are into weight-reducing regime. Papaya is a store-house of cancer-fighting agents such as lycopene, benzyl isothiocyanate, and many other compounds.[3]

All parts of the fruit (pulp, peel, and seeds) are known to possess different phytoconstituents in different quantities which confers different activities. The peel and pulp of the fruit possesses proteins, fats, fibers, carbohydrates, minerals, vitamins, phenols, flavonoids, carotenoids, sterols, alkaloids, volatile compounds, etc., whereas seeds possess thiocyanates, glucosinolates, fatty acids, sesquiterpenes, and enzymes beside the above-mentioned compounds.[4]

This study focuses on the determination of total phenol and flavonoid content in pulp, peel, and seeds of *C. papaya*, followed by the identification and characterization of the different class of metabolites present in ripe *C. papaya* Linn. fruit using high-performance thin-layer chromatography (HPTLC), ultra-performance liquid chromatography-mass spectrometry (UPLC-MS), and gas chromatography-mass spectrometry (GC-MS). Metabolic profiling of the fruit provides useful information for advanced biochemical and nutritional studies for human health. Pulp, peel, and seeds of *C. papaya's* ripe fruits are assessed for their ability to scavenge the free radicals to decide the antioxidant potential of the tissue and the compounds which are retrieved after thin-layer chromatography (TLC)-bioautography for antioxidant activity in the pulp, peel, and seed are identified and depicted as potent antioxidants.

METHODOLOGY

Apparatus and chemicals

HPTLC was carried out with a CAMAG TLC system (CAMAG, Muttenz, Switzerland) fitted with a WinCATs 1.2.3 software. Samples were applied with a CAMAG automatic TLC sampler and developed in a twin-trough glass chamber (24.5 cm × 8 cm × 22.5 cm). A repro star 3 with video store 2 documentation software (CAMAG) was used for the imaging and archiving the TLC chromatograms. WinCATs 1.2.3 software was used for transferring TLC plate images to the digital scanning plot. TLC precoated plates, silica 60 F_{254} 10 cm × 10 cm were used (Merck KGaA, 64271 Dermstadt, Germany). Ultraviolet (UV)-visible spectroscopy, Agilent, technologies, USA Carry 60 with Cary WinUV software was used for UV-visible spectroscopy analysis, Water's ACQUITY UPLC(™) system (Waters Corp., MA, USA) was used for mass spectrometry analysis. GC-MS instrument, Agilent, USA. MSD chemstation software was used to process data for non-polar compounds. All the chemicals and reagents used were of analytical grade and water was double distilled. 1,1-diphenyl-2-picrylhydrazyl (DPPH) (CAS Number 1898-66-4), gallic acid (CAS Number 149-91-7), rutin (CAS Number 207671-50-9) were purchased from Sigma Aldrich Co., St Louis, USA.

Plant material

Fruit pulp, peel, and seeds were separated and air-dried consecutively for 3 days followed by 4 days drying in the sun and then oven drying at 40°C–50°C temperature for 10 h with intervals in between to remove all the moisture and to prevent any microbial growth or infection.

Preparation of extract

The dried pulp, peel, and seed material were later grinded into powder for the preparation of the following extracts:

1. Aqueous, hydroalcoholic (60% alcohol), and alcoholic extracts of fruit pulp and peel along with hydroalcoholic and hexane extracts of seeds were prepared by subjecting the dried pulp, peel, and seed powder to reflux for 3 h. The filtrate was evaporated to dryness under reduced pressure and the extracts were stored at 4°C for further analysis.
2. Carotenoids rich extracts of fruit pulp and peel were prepared using liquid-liquid extraction method. The dried samples were kept in ice-cold acetone with 0.1% butylated hydroxytoluene (BHT), filtered, and evaporated. The dried material was extracted with diethyl ether (10% sodium chloride), the ether layer was collected and dried over sodium sulfate. Again the sample was mixed with 50% methanol containing 10% potassium hydroxide and petroleum ether containing 0.1% BHT and partitioned with distilled water. The collected organic layer was evaporated under reduced pressure and the extracts were stored at 4°C for further analysis.[5]

Estimation of total phenol content

Total phenolic content of aqueous, hydroalcoholic, and alcoholic extracts of *C. papaya* fruit pulp, peel, and seed was estimated using the Folin-Ciocalteu reagent method.[6] Initially, stock solution (5 mg/ml) of samples were prepared from each extract. About 0.5 ml of extract from stock solution was added 2.5 ml of 10% Folin-Ciocalteu and kept for 5 min, followed by the addition of 2.5 ml of 7.5% sodium carbonate. After incubation at room temperature for 40 min, the absorbance recorded at 765 nm. The standard calibration curve was plotted using gallic acid (5 µg/ml–100 µg/ml), the standard curve equation obtained was y = 0.0049x + 0.0597, R^2 = 0.9969, and the results were calculated using the equation:

Total phenolic content = $C_1 \times V/m$

where C_1 = Concentration of gallic acid established from the calibration curve in µg/ml, V = volume of extract in ml and m = the weight of the plant extract in mg. The result values were expressed in mg Gallic acid equivalent/g of the dry weight of the fresh sample.

Estimation of total flavonoid content

Total flavonoid content of all the extracts was stated by colorimetric assay.[7] The stock solution of 10 mg/ml of all the extracts was prepared in their respective solvents. To the flask, 0.5 ml of each sample from stock solution and 1.5 ml methanol was mixed. After 5 min, 0.1 ml aluminum chloride (10%), 0.1 ml sodium acetate, and 2.8 ml water was added. The solution was mixed and the absorbance was measured against blank at 415 nm. The standard calibration curve was plotted with various concentrations of rutin (5 µg/ml–100 µg/ml) and the results were interpreted using the standard curve equation - y = 0.0052x + 0.0041, R^2 = 0.9921. The values were expressed in mg rutin equivalent/g of dry weight.

Estimation of free radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl assay

The free radical scavenging activity of aqueous, hydroalcoholic, and alcoholic extracts of *C. papaya* fruit pulp, peel, and seed was determined using DPPH assay, describe earlier with some modification.[8] Different concentrations of the samples were prepared (10 µg/ml–1000 µg/ml) and 20 µl of each concentration of different samples along with 180 µl DPPH solution were added in ELISA plates and incubate in dark for 45 min. After incubation, the absorbance was recorded at 517 nm. Ascorbic acid was used as a positive control. The percentage of radical scavenging activity (% inhibition) was calculated by:

$$\% \text{ scavenging activity} = (A_{CONTROL} - A_{SAMPLE}/A_{CONTROL}) \times 100$$

Half maximal inhibitory concentration (IC_{50}), i.e., the amount of sample which can scavenge 50% of DPPH free radical was determined from the curve obtained after plotting % inhibitions against respective concentrations and the extracts with lowest IC_{50} in the pulp, peel, and seed, respectively, were selected.

Qualitative analysis of the extracts using high-performance thin-layer chromatography method

HPTLC fingerprinting of hydroalcoholic extracts of *C. papaya* pulp, peel, and seed was carried out to deduce the number of metabolites present in the sample. Forty mg of each extract was dissolved into their respective solvents (40 mg/ml), a volume of 5 µl each was applied on the precoated silica gel 60 F_{254} aluminum sheets by Linomat V applicator and the plate were developed in a mobile phase, toluene:ethyl acetate: formic acid (6:3:1, v/v/v). After the development of plates, it was scanned at wavelengths 254 and 366 nm by CAMAG TLC scanner III and interpretation by WinCATs software.[9]

Quantitative analysis of phenols and flavonoids using high-performance thin-layer chromatography method

Quantitative estimation of marker compounds already reported, i.e., gallic acid, quercetin, myricetin, and trans-ferulic acid was estimated by running varying volume of the mixture of the above-stated marker compounds (0.1 µl–8.0 µl) on TLC plates and plotting the graph with the concentration of each marker (ng/ml) against area occupied (AU). Using the linear curve equation generated for each marker, amount of the marker compound in hydroalcoholic extracts of pulp, peel, and seed were estimated and expressed in % w/w.[10]

Thin-layer chromatography-hyphenated bioautography for free radical scavenging activity

Identification of free radical scavenging compound was done by dipping the developed TLC plates in 0.05 mM DPPH solution, prepared in methanol. The appearance of yellow spot against the purple background confirmed the scavenging metabolite which was scraped manually from a second reproduced TLC plate identified by respective Retardation factor (R_f) value and dissolved in alcohol for mass spectroscopy. The experiment was repeated thrice for confirmation of the compound.[11] The spectrum generated was interpreted by using the European Mass Bank database, database of the National Institute of Science and Technology (NIST), and the NCBI-PubChem database for the unknown compounds.

Qualitative analysis of carotenoids in *Carica papaya* fruit pulp and peel using liquid chromatography-mass spectrometry

500 ng of Carotenoids rich extract resuspended in 1 ml of acetone was subjected to UPLC-TQD (QqQ) liquid chromatography-mass spectrometry (LC-MS) system with Aquity UPLC®BEH C_{18} column (1.7 µM, 2.1 × 100 mM). A 0.1% Formic acid (A) and Acetonitrile (B) was used as a mobile phase. The elution gradient was 90%(A):10%(B) at 0 and 1 min, 10%(A):90%(B) at 3 min, 90%(A):10%(B) at 5 and 6 min with 0.3 ml flow rate. UPLC-TQD was coupled with electrospray ionization-mass spectrometry with ESI as ion source functioning at positive mode and the ionization parameters were set as: Desolvation temperature (N) at 350°C, Capillary voltage at 3.00 KV, Cone voltage at 30 V, Dessolvation gas flow at 850 L/h, Cone gas flow at 50 L/h, Source temperature at 140°C, Column temperature at 40°C, Autosampler temperature at 10°C, Scan range at 100–1000 *m/z*. The mass spectrum of each peak obtained was interpreted by correlating the *m/z* of the metabolites mentioned in the database of NIST, NCBI-PubChem database, and European Mass Bank database.[12]

Gas chromatography-mass spectrometry analysis of non-polar compounds in the hexane extract of *Carica papaya* seeds

Hexane extract of *C. papaya* seeds was analyzed by GC-MS (Agilent Technology) with HP-5MS standard non-polar column. Carrier gas (He) flow was 1.5 mL/min. The inlet temperature was fixed at 250°C. The temperature program of the oven was as follows: 50°C initially for 2 min and then it ramped at a rate of 10°C/min to 200°C for 5 min and 5°C/min to 250°C for 10 min. The Quad Temperature was fixed at 150°C. A 2.0 µL sample was applied with a 5:1 split ratio and fully scanned at 50–700 *m/z*.[13] The spectrum generated was interpreted using the database of NIST, NCBI-PubChem database, and European Mass Bank database.

RESULTS AND DISCUSSION

C. papaya fruit was purchased from the local market (Farash Khana) of Delhi. The fruit was authenticated as per the Ayurvedic Pharmacopoeia of India.[14] The voucher specimens (BNPL/JH/M.Sc./08/2018/03) of similar material has been placed in Bioactive Natural Product Laboratory for future reference.

Total phenolic and flavonoid content estimation

Total phenol content of fruit pulp aqueous, pulp hydroalcoholic, pulp alcoholic, peel aqueous, peel hydroalcoholic, peel alcoholic and seed hydroalcoholic extract calculated was 46.70 ± 2.36 mg/g, 47.54 ± 3.01 mg/g, 43.54 ± 1.92 mg/g, 66.64 ± 3.87 mg/g, 73.68 ± 3.21 mg/g, 60.16 ± 2.48 mg/g, 46.87 ± 1.64 mg/g, respectively, whereas total flavonoid content was 6.20 ± 0.84 mg/g, 4.64 ± 0.60 mg/g, 8.84 ± 1.05 mg/g, 14.20 ± 2.82 mg/g, 24.84 ± 3.46 mg/g, 40.50 ± 2.12 mg/g, 06.55 ± 1.09 mg/g, respectively [Table 1]. Phenolics and flavonoids are responsible for antioxidant activity.[15] The large amount of phenols in peel extracts were likely in response to environmental factors, to provide protection to fruit and seeds. It was noted that total phenolic content in hydroalcoholic extracts of pulp, peel, and seed were better than the alcoholic and aqueous extracts, whereas the highest flavonoid content was estimated in alcoholic extracts of peel and pulp. The phenol content in pulp and seed was approximately the same.

Free radical scavenging activity estimation using 1,1-diphenyl-2-picrylhydrazyl assay

This method is widely used to evaluate free radical scavenging activities within a short time compared with other methods. Free radical scavenging compound reacts with DPPH, a stable free radical and convert it to 1, 1-diphenyl-2-(2, 4, 6-trinitrophenyl) hydrazine. The degree of discoloration showed the scavenging potential of a free radical

scavenging compound.[16] Free radicals and other reactive oxygen species generated in living organisms lead to many diseases including cancer, cardiovascular diseases, cataracts, asthma, hepatitis, liver injury, and immunodeficiency diseases.[17] Antioxidants function to decrease the DNA damage, diminish lipid peroxidation, maintain immune function, and inhibit the malignant transformation of cells hence, the free radical scavenging potential of all the above-stated extracts of *C. papaya* fruit were estimated through DPPH radical scavenging activity and it was compared with a known natural free radical scavenging compound, i.e., ascorbic acid. The DPPH scavenging activity of extracts increased with respect to the concentration [Table 1]. Flavonoids are highly powerful scavengers of most oxidizing molecules which include singlet oxygen and various other free radicals associated with diseases. Flavonoids suppress the reactive oxygen formation, up regulate antioxidant defense, and chelate trace elements which are involved in free radical production.[18] IC_{50} values calculated ranged from the lowest in seed hydroalcoholic extract (976.812 ± 101.32 µg/ml) corresponding to the highest free radical scavenging activity, i.e., its better ability in scavenging hydrophilic free radicals, followed by peel hydroalcoholic extract (1342.76 ± 098.25 µg/ml), peel alcoholic extract (1349.44 ± 129.30 µg/ml), pulp hydroalcoholic extract (1505.44 ± 095.64 µg/ml), peel aqueous extract (1676.39 ± 067.29 µg/ml), pulp aqueous extract (2129.02 ± 131.25 µg/ml) and highest in pulp alcoholic extract (2164.83 ± 162.58 µg/ml), respectively, whereas IC_{50} value of ascorbic acid was 29.264 µg/ml. Among the different extracts of fruit pulp, peel and seed analyzed, hydroalcoholic extract of pulp, peel, and seeds exhibited better free radical scavenging activity. Phenolic compounds conferred the oxidative stress tolerance in plant material,[19] justifying the high phenol content, although they were not significant as the scavenging activity of ascorbic acid. Better scavenging activity of seeds is found which is likely due to the presence of unsaturated fatty acids along with phenols and sterols, which are reported to be the potent free radical scavengers as well. Peel, on the other hand, contains many carotenoids, phenols, flavonoids, and sterols for defending its free radical scavenging activity. According to previous report, greater amounts of phenols and ascorbic acids have been reported in waste scalps of numerous fruits than their pulp.[20]

High-performance thin-layer chromatography Fingerprints and Quantitative estimation of polyphenolic compounds present in the extract

A total of five, seven, three, three, eight, seven, and nine bands were obtained after scanning at 254 nm in pulp aqueous, pulp hydroalcoholic, pulp alcoholic, peel aqueous, peel hydroalcoholic, peel alcoholic, and seed hydroalcoholic extracts, respectively [Table 2 and Figures 1 and 2].

On the basis of previous results, the hydroalcoholic extracts of pulp, peel, and seed were screened for further analysis. The developed HPTLC was used for simultaneous quantitative analysis of phenolic and flavonoids in pulp hydroalcoholic, peel hydroalcoholic, and seed hydroalcoholic. Earlier reported quantitative estimation of phenolic in *C. papaya* through HPLC was found to be less than that deduced in our study for these four maker compounds.[21]

The concentration of gallic acid in pulp hydroalcoholic, peel hydroalcoholic extracts was found to be 0.035% w/w and 0.33% w/w, respectively. The concentration of myricetin was calculated to be 5.95% w/w, 0.23% w/w, and 0.24% w/w, whereas the concentration of

Table 1: Total phenolic content, flavonoid content and half maximal inhibitory concentration of 2,2-diphenyl-1-picrylhydrazyl assay in different extracts of *Carica papaya* fruit pulp, peel and seeds

Parts	Extract	Phenolic contents (mg GAE/g)	Flavonoid contents (mg GAE/g)	DPPH assay (IC_{50} in µg/mL)
Pulp	Aqueous	46.70±2.36	06.20±0.84	2129.02±131.25
	Hydroalcoholic	47.54±3.01	04.64±0.60	1505.44±095.64
	Alcoholic	43.54±1.92	08.84±1.05	2164.83±162.58
Peel	Aqueous	66.64±3.87	14.20±2.82	1676.39±067.29
	Hydroalcoholic	73.68±3.21	24.84±3.46	1342.76±098.25
	Alcoholic	60.16±2.48	40.50±2.12	1349.44±129.30
Seed	Hydroalcoholic	46.87±1.64	06.55±1.09	976.812±101.32

DPPH: 2,2-diphenyl-1-picrylhydrazyl; GAE: Gallic acid equivalent; IC_{50}: Half maximal inhibitory concentration

Table 2: Metabolic profiling of *Carica papaya* fruit pulp, peel and seed in different extracts by high performance thin layer chromatography

R_f	Pulp			Peel			Seed
	Aqueous	Hydroalcoholic	Alcoholic	Aqueous	Hydroalcoholic	Alcoholic	Hydroalcoholic
0.17	–	–	–	–	–	–	+
0.19	–	+	–	+	+	–	–
0.21	–	–	–	–	–	–	+
0.27	–	–	–	–	+	–	–
0.36	–	–	–	–	–	–	+
0.43	+	+	–	+	+	–	–
0.47	–	–	–	–	–	+	–
0.50	+	+	+	–	+	+	+
0.55	–		–	–	–	–	+
0.57	–	+	–	–	+	–	–
0.59	–	–	–	–	–	–	+
0.63	+	+	+	–	+	+	+
0.68	+	+	+	–	+	+	+
0.71	+	–	–	+	–	–	–
0.75	–	–	–	–	–	+	–
0.80	–	–	–	–	–	+	+
0.83	–	+	–	–	+	+	–
Total number of metabolites	5	7	3	3	8	7	9

R_f: Retardation factor; + Present; - Absent

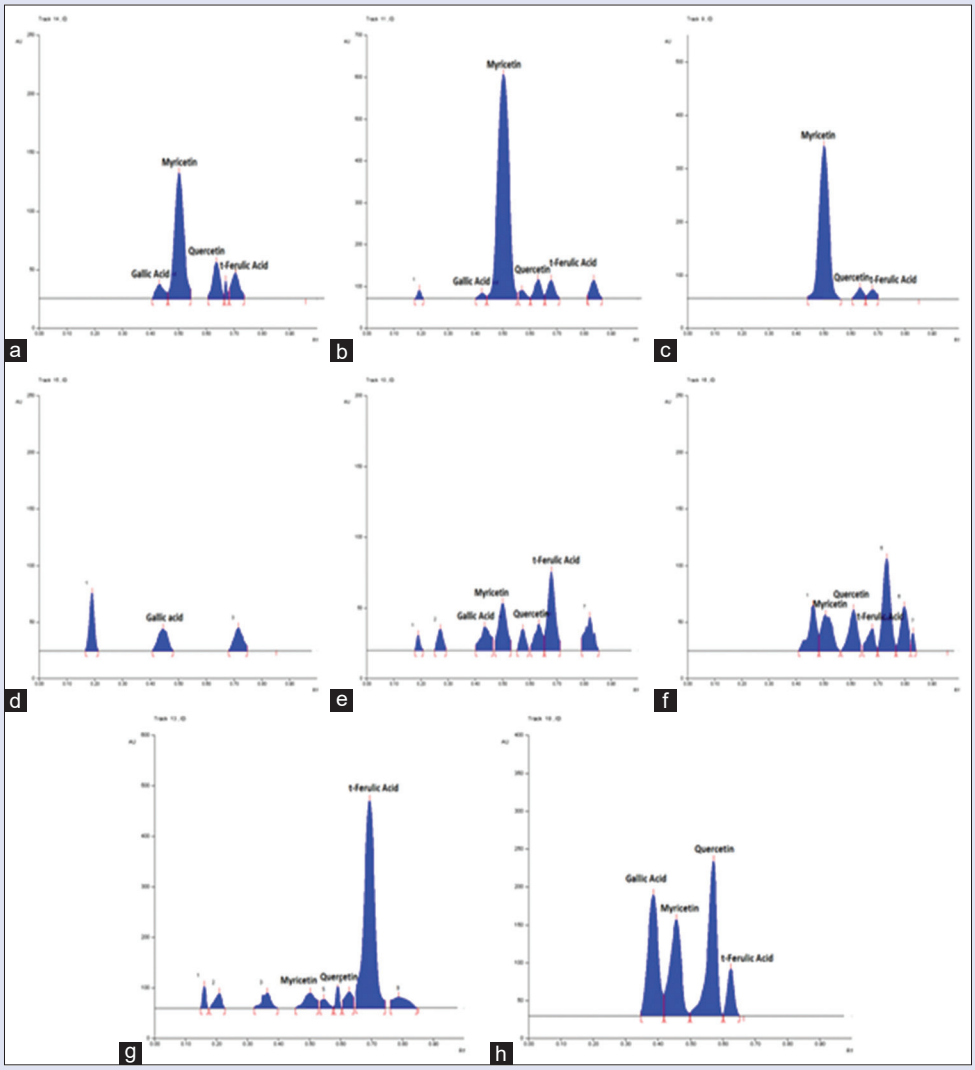

Figure 1: Developed thin layer chromatography chromatograms at 254 nm (a) pulp aqueous (b) pulp hydroalcoholic (c) pulp alcoholic, (d) peel aqueous, (e) pulp hydroalcoholic, (f) peel alcoholic, (g) seed hydroalcoholic, (h) mixed standard

quercetin was estimated to be 0.32% w/w, 0.08% w/w, and 0.15% w/w, and another phenolic marker t-ferulic acid was found to be 0.33% w/w, 0.3% w/w, and 3.86% w/w in pulp hydroalcoholic, peel hydroalcoholic, and seed hydroalcoholic, respectively. Gallic acid was not found in seed hydroalcoholic [Table 3].

Identification of free radical scavenging compounds using thin-layer chromatography-mass spectrometry bioautography

The yellow bands appeared against the purple background when the developed TLC plates of pulp, peel, and seed hydroalcoholic extracts were dipped in 0.5 mM DPPH solution [Figure 3], the spectrum analyzed after mass-spectrometry [Supplementary Figures 1-3] dictated the DPPH radical scavenging activity due to the presence of potent antioxidants identified as chlorogenic acid, β-sitosterol and ellagic acid in fruit pulp, chlorogenic acid, β-sitosterol and quercetin in fruit peel and linoleic acid and iso-oleic acid in seeds [Table 4].

These identified compounds chlorogenic acid,[22] β-sitosterol,[23] ellagic acid,[24] quercetin,[22] linoleic acid[25] are previously reported as antioxidants in the literatures.

Table 3: The concentration of gallic acid, myricetin, quercetin and trans-ferulic acid expressed in percentage w/w in different parts of fruit

Fruit part	Percentage concentration (w/w)			
	Gallic acid	Myricetin	Quercetin	*t*-ferulic acid
Pulp	0.035±0.02	5.95±0.92	0.32±0.13	0.33±0.2
Peel	0.33±0.20	0.23±0.12	0.08±0.02	0.3±0.16
Seed	Absent	0.24±0.13	0.15±0.11	3.86±0.53

Qualitative analysis of carotenoids in *Carica papaya* fruit pulp and peel using liquid chromatography-mass spectrometry

The carotenoid were identified based on the *m/z* value of the base peak as it is considered more stable than molecule ion or fragment ion peaks.[26] Carotenoids detected after UPLC-MS fingerprinting were β-carotene, lycopene, β-cryptoxanthin, ζ-carotene, phytoene, lutein, zeaxanthin, and β-cryptoxanthin laurate [Figure 4 and Table 5]. There are beneficial (protective) effects of dietary carotenoid intake, β-carotene and β-cryptoxanthin contain unsubstituted beta-ionone rings signifying for Vitamin A activity and their role as potent antioxidant. Lycopene,

Table 4: Tentatively identified free radical scavenging compounds obtained after thin layer chromatography-bioautography of pulp, peel and seed hydro-alcoholic extracts

Fruit part	Band number	R_f	*m/z*	Tentatively identified metabolite	Chemical formula	Class of metabolite	Reference ID
Pulp	1	0.57	353.48	Chlorogenic acid	$C_{16}H_{18}O_9$	Phenol	Mass bank ID: KO000468
	2	0.85	413.51	β-sitosterol	$C_{29}H_{50}O$	Phytosterol	PubChem CID: 222284
			301.38	Ellagic acid	$C_{14}H_6O_8$	Phenol	PubChem CID: 5281855
Peel	3	0.57	353.48	Chlorogenic acid	$C_{16}H_{18}O_9$	Phenol	Mass bank I.D- KO000468
	4	0.63	301.33	Quercetin	$C_{15}H_{10}O_7$	Flavanoid	Mass bank ID: PR100646
	5	0.85	413.51	β-sitosterol	$C_{29}H_{50}O$	Phytosterol	PubChem CID: 222284
Seed	6	0.54	280.30	Linoleic acid	$C_{18}H_{32}O_2$	Unsaturated fatty-acid	PubChem CID: 5280450
	7	0.59	282.38	10 octadecenoic acid	$C_{18}H_{34}O_2$	Unsaturated fatty-acid	PubChem CID: 5282760

R_f: Retardation factor; CID: Compound ID

Table 5: Tentatively identified carotenoids after ultra-performance liquid chromatography-mass spectrometry of carotenoid rich extract of pulp and peel of *Carica papaya*

Tentatively identified carotenoid in peel extract	R_t	Identified carotenoids		Molar mass	Chemical formula	Reference ID
		Pulp	Peel			
Trans β-carotene	3.66	+	+	537.55	$C_{40}H_{56}$	PubChem CID: 5280489
Lycopene	2.91	+	+	536.99	$C_{40}H_{56}$	PubChem CID: 446925
ζ-carotene	5.02	–	+	540.96	$C_{40}H_{60}$	PubChem CID: 5280788
Phytoene	1.89	–	+	544.42	$C_{40}H_{64}$	PubChem CID: 5280784
β-cryptoxanthin	4.44	–	+	554.57	$C_{40}H_{56}O$	PubChem CID: 5281235
Lutein/zeaxanthin	3.39	+	+	567.48	$C_{40}H_{56}O_2$	PubChem CID: 5281243
β-cryptoxanthin laurate	4.48	+	+	736.28	$C_{40}H_{56}O$	PubChem CID: 5281235

R_t: Retention time; CID: Compound ID; + Present; - Absent

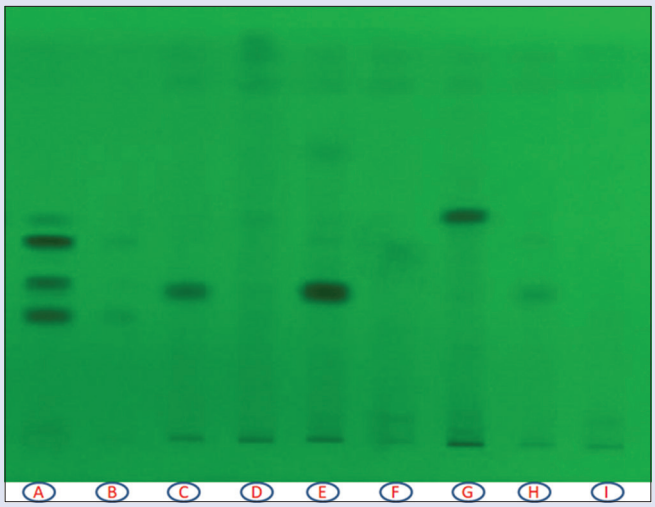

Figure 2: Developed thin layer chromatography plate photograph at 254 nm (a) mixed marker (b) blank (c) pulp alcoholic (d) peel alcoholic (e) pulp hydroalcoholic (f) peel hydroalcoholic (g) seed hydroalcoholic (h) pulp aqueous and (i) peel aqueous extract

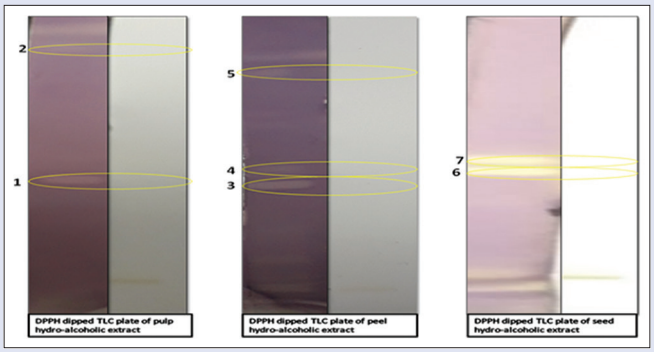

Figure 3: Yellow bands on 2,2-diphenyl-1-picrylhydrazyl dipped thin layer chromatography plates of seed (1, 2), peel (3, 4 and 5) and pulp (6, 7) extracts, indicating the 2,2-diphenyl-1-picrylhydrazyl scavenging activity

lutein, and zeaxanthin although do not contribute to Vitamin A activity but are known for their radical scavenging activity. Furthermore, lycopene provides protection against erythema formation following UV-irradiation, whereas lutein on the other hand is associated with lowering the risk for age-related mascular degeneration.[27] A positive correlation between the consumption of carotene-rich fruits and vegetables and a decreased risk of several types of cancer have been reported extensively.[28] To increase the bioaccessibility of carotenoids, carotene-rich fruits must be consumed along with milk. Hence, along with pulp of *C. papaya,* peel should also be consumed for additional benefits.

Gas chromatography-mass spectrometry analysis of non-polar compounds in the hexane extract of *Carica papaya* seeds

A total of 23 metabolites were identified after GC-MS analysis of hexane extract of seeds. Metabolites were tentatively identified by matching their mass spectrum from the NIST library. The major metabolites were benzyl isothiocyanate (retention time [Rt]-17.584), tetra-decanoic acid (Rt-23.471), methyl palmitate (Rt-27.938), hexadecanoic acid (Rt-28.706), 10-octadecenoic acid (Rt-31.657), and oleic Acid (Rt-32.097) were found in the sample. High concentration of oleic acid, iso-oleic acid, and hexadecanoic acid is usually found in the fruits of tropical and sub-tropical regions and their concentration increases with the ripening of the fruit.[29] These polyunsaturated fatty acids are also reported for their enormous antioxidant activity along with the antibacterial activity.[30] Other sesquiterpenes, thiocyanates, and fatty acids discerned were phthalic Acid (Rt-17.958), 2-naphthaoic

Table 6: Tentatively identified non-polar compounds in hexane extract of *Carica papaya* seeds through gas chromatography-mass spectrometry

Name of the compound	R_t	Molecular formula	Molecular weight	Percentage contribution
Benzyl isothiocyanate	17.584	C_8H_7NS	149.21	0.022
Phthalic acid	17.958	$C_8H_6O_4$	166.14	0.003
2-naphthaoic acid	20.447	$C_{11}H_8O_2$	172.18	0.007
1-octadecene	20.696	$C_{18}H_{36}$	252.48	0.004
Hexadecane	20.843	$C_{16}H_{34}$	226.48	0.024
3-phenyl undecane	22.49	$C_{17}H_{28}$	232.41	0.022
Benzyl isothiocyanate	22.640	C_8H_7NS	149.211	0.004
Octadecane	22.915	$C_{18}H_{38}$	254.5	0.001
Tetra-decanoic acid	23.471	$C_{14}H_{28}O_2$	228.37	0.004
4-phenyl dodecane	24.189	$C_{18}H_{30}$	246.43	0.026
Phenyl acetonitrile	24.343	C_8H_7N	117.151	0.003
Dodecane	23.786	$C_{12}H_{26}$	170.33	0.018
Decanedioic acid	24.504	$C_{10}H_{18}O_4$	202.25	0.001
3-phenyl dodecane	24.716	$C_{18}H_{30}$	246.43	0.018
Lignoceric alcohol	24.972	$C_{24}H_{48}O_2$	368.63	0.003
2-chloropropionic acid	25.009	$C_3H_5ClO_2$	108.52	0.002
Tridecane	25.961	$C_{13}H_{28}$	184.37	0.030
Methyl palmitate	27.938	$C_{17}H_{34}O_2$	270.45	0.668
Hexadecanoic acid	28.706	$C_{16}H_{32}O_2$	256.4	0.128
Docasane	29.505	$C_{22}H_{46}$	310.61	0.022
10-octadecenoic acid	31.657	$C_{18}H_{34}O_2$	282.46	2.639
Oleic acid	32.097	$C_{18}H_{34}O_2$	282.47	0.151
9-octadecenoic acid	38.84	$C_{18}H_{34}O_2$	282.46	0.002

R_t: Retention time

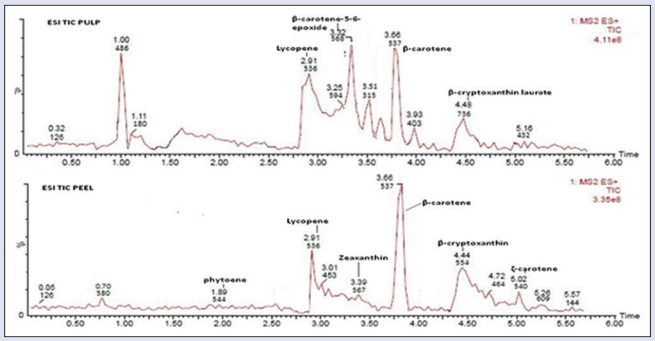

Figure 4: Liquid chromatography-mass spectrometry chromatogram of carotenoid rich extract of *Carica papaya* fruit's pulp and peel depicting the tentatively identified carotenoids

acid (Rt-20.447), 1-octadecene (Rt-20.696), hexadecane (Rt-20.843), 3-phenyl undecane (Rt-22.49), benzyl isothiocyanate (Rt-22.640), octadecane (Rt-22.915), dodecane (Rt-23.786), 4-phenyl dodecane (Rt-24.189), phenyl acetonitrile (Rt-24.343), decanedioic acid (Rt-24.504), 3-phenyl dodecane (Rt-24.716), lignoceric alcohol (Rt-24.972), 2chloropropionic acid (Rt-25.009), tridecane (Rt-25.961), docasane (Rt-29.505), and 9-octadecenoic acid (Rt-38.84) [Supplementary Figure 4; Table 6]. The degradation products of benzyl glucosinolates (benzyl isothiocyanate and phenylacetonitrile) were also obtained indicating that the papaya seeds are a source of benzyl glucosinolates.[13]

CONCLUSION

This study has revealed that *C. papaya* fruit and seeds possess potent free radical scavenging effect and the different compounds conferring it. Seeds which make up the waste material may be utilized in cosmetic industries as they signify rich antioxidant activity along with glucosinolates and its hydrolyzed products. Peels, on the other hand, can be utilized in bakery items, along with milk in deserts or consumed raw to increase carotene in-take. Overall, the whole fruit of the papaya is beneficial and may be utilized for therapeutic purpose as well as for cosmetics.

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Conflicts of interest

There are no conflicts of interest.

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