



Evaluation of Total Phenolic Content and Antioxidant Activity of *Carica papaya* Seeds: Optimization and FTIR Analysis

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ABSTRACT

Background: Medicinal plants are rich sources of antioxidants, which help combat oxidative stress in the body by neutralizing harmful free radicals. *Carica papaya* is an herbaceous, annual plant and it belongs to *Caricaceae* family. It shows various phytochemicals and acts as biological properties.

Methods: In this study, seed extract from *Carica papaya* was used to increase total phenolic content through extraction procedure involves optimizing physico-chemical parameters and Soxhlet extraction process. Finally studied antioxidant activity by reducing power assay.

Result: Total phenolic content (TPC) is extracted using a variety of solvents, solvent concentrations (%), extraction time (t), pH and particle mesh sizes (microns). The ideal conditions in this case are methanol, 80% solvent, 24 hours, pH-6 and 120 mesh size. In this extraction, the TPC content was found to be 27.83 ± 0.28 μg GAE/ml. Similarly, the TPC concentration was enhanced during the Soxhlet extraction procedure, reaching 36.16 ± 0.28 μg GAE/ml. That sample was used for antioxidant research following Soxhlet extraction process. The results revealed a reducing power ability of $79.81 \pm 0.22\%$ at the highest concentration of 2000 $\mu\text{g}/\text{ml}$ and additionally, the IC_{50} values were found to be concentration-dependent, with a value of 92.17 $\mu\text{g}/\text{ml}$ for reducing power ability.

Key words: Anti-oxidant, *Carica papaya*, Methanol, Phenolic compound, Soxhlet extraction.

INTRODUCTION

Plant extracts are rich in antioxidants, which are compounds that help protect cells from damage caused by free radicals-unstable molecules linked to aging and diseases like cancer and heart disease. These antioxidants, including polyphenols, flavonoids and vitamins like C and E, neutralize free radicals by donating electrons, reducing oxidative stress. Found in fruits, vegetables, herbs and spices, they vary in potency depending on the plant species, extraction method and environmental factors (Masaki, 2010). Their benefits include anti-inflammatory effects, improved immune function and potential prevention of chronic diseases, making them valuable in food, cosmetics and medicine. The most economically significant species in the *Caricaceae* family is the papaya (*Carica papaya*), sometimes referred to as papaw or pawpaw (Yogiraj *et al.*, 2014). It is a huge plant. It is a short-lived herbaceous plant that is indigenous to tropical areas in the Americas. Early European explorers called papayas, a fleshy fruit, tree melons, but papaya fruit is properly categorized as a berry. Papaya was brought to India in the latter part of the sixteenth century from the Philippines via Malaysia. It is a popular fruit with significant nutritional and therapeutic significance (Rinnerthaler *et al.*, 2015). The crop is incredibly profitable to grow, easy to grow and produces fruits in much less than a year. It's one of the few fruits that blooms and bears fruit all year round, which is surprising. It is a big herbaceous plant that can get as tall as 30 feet and has just one trunk (Raju *et al.*, 2015). Large, deeply lobed leaves are held on hollow petioles that are at least

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two feet long. Buds on the trunk, close to the base of the leaves, sprout flowers and fruits. The ripe, ready-to-eat papaya fruit is wonderful when it is fresh. A number of food goods, including jam and syrup, are made from fruits (Sies, 2015). Papain is a proteolytic enzyme that functions similarly to pepsin and is generated from dried fruit latex. A genetically altered (GMO) type of papaya called Rainbow was created in the early 2000s with viral resistance. One of the first GMO fruits to be produced commercially, today's papaya exports are almost entirely made up of GMO crops. The biggest papaya producer in the world is India, which is

followed by Brazil, Indonesia, Nigeria and Mexico. Only a tiny fraction of the world's total papaya production of about 0.1% is produced in the United States. The fruits are rich in carbohydrates, minerals, vitamins and lycopene, which are the strong antioxidant and most commonly associated with the tomatoes, as well as Alpha and beta carotene. This juice is utilized in the creation of a number of dyspepsia cures as well as meat tenderizers (Gella *et al.*, 2009). Papaya plant contains various phytochemicals and acts as biological properties. Phenolic compounds are significant secondary metabolites found in plants and offer multiple health benefits to humans. Typically, these substances are defined by containing at least one aromatic ring that has one or more hydroxyl groups. They may be categorized into four main groups: phenolic acids, flavonoids, stilbenes and lignans, depending on the function dictated by the number of phenol units, their carbon framework and other structural components linking these rings (Morolahun *et al.*, 2019). The diverse pharmaceutical and industrial uses of phenolic compounds make it crucial to extract and identify these naturally occurring molecules in plants to assess their health impacts and aid in food quality management, food product innovation and nutritional and health oversight. Nevertheless, extracting and purifying phenolic acids from plant matrices presents numerous challenges due to the significant influence of their structural differences on solubility, stability and separation characteristics. Additionally, they may exist in both free and bound forms alongside different components of the plant matrix (Adenowo *et al.*, 2014). Moreover, the extraction process may also experience reduced recovery of phenolics due to the elevated enzyme activity present in various plant matrices. Consequently, employing various extraction techniques and suitable extraction solvents is evidently crucial for recovering these compounds (Roshan *et al.*, 2014). Conversely, today, phenolic compounds are typically identified and measured through chromatography associated with spectroscopy. Mainly seed contain high content of phenolic compounds due to this reason, this research focused on extraction of total phenolic content (TPC) for antioxidant studies.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu (FC) reagent, methanol, sodium carbonate (Na_2CO_3), distilled water, Potassium ferricyanide ($\text{C}_6\text{N}_6\text{FeK}_3$), phosphate buffer, trichloro acetic acid and ferric chloride.

Collection of plant material

Carica papaya fruits were purchased from Modinagar, Uttar Pradesh State, India. The fruit's seeds were removed, rinsed with water and dried under the sun. The dried seeds were then ground into a powder and processed and shown in Fig 1.

Optimization parameters

Solvent, percentage solvent, extraction time, pH, particle mesh.

The process of extracting phytochemicals from plant sources is a critical stage in the investigation of natural products, laying the groundwork for the discovery, analysis and utilization of bioactive substances in areas such as medicine, agriculture and cosmetics. Extraction efficiency is greatly impacted by multiple above parameters. Optimizing these variables is a key factor in achieving maximum yielding of the target compounds.

Solvent

The solvent used in phytochemical extraction is the key factor that determines solubility, selectivity and recovery efficiency (Aybastier *et al.*, 2013). Phytochemicals differ significantly in polarity, therefore the solvent should be selected according to the polarity of the desired compounds. Polar solvents (Ethanol, Methanol, Ethyl acetate, Water) and Non polar solvents (chloroform, Benzene, Toluene, Hexane) are suitable for extraction of compounds from various plant species.

Percentage of solvent

Phytochemicals' solubility and diffusion rate are significantly influenced by the percentage of in solvent mixtures. Especially organic solvent - water mixtures frequently provide more effective extraction compared to pure solvents. This is due to water's ability to change solvent polarity and encourage the expansion of plant tissues, enhancing the penetration of solvent and the diffusion of compounds.

Extraction time

The duration of extraction influences how much phytochemicals are released from plant materials into the solvent. At first, the extraction rate rises over time as diffusion takes place quickly. Yet, once equilibrium is achieved, extended extraction periods offer minimal enhancement and could potentially result in the degradation of heat-sensitive or oxidation-prone substances (Prasad *et al.*, 2009).

pH

The pH of the solvent system directly influences the solubility and ionization of compounds. Numerous phytocompounds

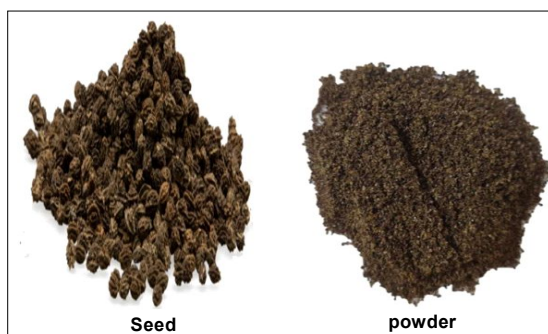


Fig 1: Collection of seed process.

are sensitive to pH and can experience oxidation or hydrolysis in strongly acidic or basic conditions. Phenolic compounds can be extracted effectively with acidic conditions that maintain stability of molecule and inhibition oxidation (Azahar *et al.*, 2017). Saponins and alkaloids on the other hand, are more easily extracted under slightly basic conditions when they are in their more soluble and non-ionized conditions. The extraction efficiency and chemical degradation are improved by adjusting the pH based on the target compound's nature. During the extraction process, buffer systems are frequently utilized to maintain a constant pH.

Particle mesh size

Another important factor that affects extraction efficiency is the particle mesh size of the dried and powdered plant material. Mainly diffusion and mass transfer rates are enhanced by smaller particle sizes, which increase the surface area exposed to the solvent (Ilaiyaraja *et al.*, 2015). Usually, a medium mesh size (50-70 mesh) provides the balance equilibrium between solvent permeability and surface area of particle.

Plant extraction

1 gram powder was placed in a conical flask and 25 ml of organic solvent (Methanol) was added. The samples were soaked before being filtered through a funnel using Whatman No. 1 paper (Nugroho *et al.*, 2017). The filtered samples were then heated to a specific temperature to allow the organic solvent to evaporate (Methanol at 65°C). Finally, a plant extract was prepared to determine TPC.

Determination of TPC

Estimation of TPC was carried out using the Folin Ciocalteu (FC) reagent method. In a test tube, 1ml of the extract was taken out. FC reagent (0.5 ml) and sodium carbonate (1 ml) were added and the total volume was adjusted to 10 ml using distilled water and the mixture was maintained at ambient temperature for a 30-minute incubation period (Yamdeu Galani *et al.*, 2017). The reaction mixture's absorbance was determined at 680 nm using a colorimeter. The calibration curve is used to calculate the TPC. Here, TPC was expressed as gallic acid equivalents (GAE).

Soxhlet extraction process

In the Soxhlet's extraction process, 10 g of papaya seed powder (100 mesh size) was placed in the thimble. A round-bottom flask containing 250 ml of 80% methanol was adjusted to pH 6 and the condenser was connected to setup thimble (Halim *et al.*, 2011). The complete setup was positioned on the heating mantle at 40°C. When the methanol solvent started boiling, its vapors ascended from the round-bottom flask to the top of the thimble. Then, the vapors condensed and dripped onto the plant powder. Subsequently, the color and flavor components of the plant powder migrated into the bottom flask via the path of distillation (Park *et al.*, 2016). As a result, we observed the cyclic process where the sample changed from colored to

colorless. The process was stopped immediately once a colorless sample appeared in the path of distillation. After four hours, the extraction sample was finally ready for TPC estimation.

Antioxidant activity

Medicinal plants are rich sources of natural antioxidants, which play a crucial role in scavenging free radicals. These antioxidants primarily include polyphenols, nitrogen-containing compounds and terpenoids (Liu *et al.*, 2017; Süntar *et al.*, 2012), known for their diverse biological activities. Efficient extraction and accurate assessment of these antioxidants are vital for identifying potential antioxidant sources and enhancing their applications in the pharmaceutical industry (Duc *et al.*, 2023).

Dilutions

Various concentrations of extract (obtained from Soxhlet extraction) were made by diluting the stock solution, as detailed in Table 1.

Reducing power assay

In this instance, gallic acid was used as a standard to evaluate the reducing ability of plant extract. 1 ml of plant extracts at different concentrations (from 50 µg/ml to 2000 µg/ml) was taken in separate test tubes and 1 ml of distilled water was added to each tube. 2.5 ml of sample in 0.2 M phosphate buffer (pH-6.6) and 2.5 ml of 1% potassium ferricyanide were put into each test tube. The mixture was heated for 20 minutes at 50°C (Wijesooriya *et al.*, 2019). Finally, 2.5 ml of 10% trichloroacetic acid was injected into each sample, which was then centrifuged for 15 minutes at 3000 rpm. After centrifugation, 2.5ml of the supernatant solution was collected and diluted with 2.5 ml of distilled water. Finally, 0.5 ml of 0.1% ferric chloride was added. At 700 nm, absorbance was measured. In this case, increased reaction mixture absorbance was used to demonstrate increased reducing power. Gallic acid was utilized as a control in this experiment.

The formula below was used to determine the percentage lowering power of plant extracts:

Reducing power ability percentage (%) =

$$\frac{(\text{Absorbance value of control} - \text{Absorbance value of test sample})}{(\text{Absorbance value of control})} \times 100$$

Table 1: Preparation of dilutions.

Concentration (µg/ml)	Extract (ml)	Distilled water (ml)	Total volume (ml)
50	0.2	9.8	10
100	0.4	9.6	10
250	1.0	9.0	10
500	2.0	8.0	10
1000	4.0	6.0	10
2000	8.0	2.0	10

FT-IR (fourier transform infrared spectroscopy) analysis

FTIR analysis was performed to identify various functional groups in the methanolic extract (dry extract) of papaya (Nayak *et al.*, 2007). This technique helps identify essential functional groups related to bioactive compounds by analyzing their unique absorption peaks. This analysis offered important insights into the chemical composition of papaya, enhancing its pharmacological relevance and potential medicinal uses.

RESULTS AND DISCUSSION**Optimization of various parameters for extraction of TPC**
Effect of different solvents

Different organic solvents were utilized to extract the maximum yield of TPC from papaya seeds. Methanol was optimum for extraction of TPC and its concentration was 14.16 ± 0.28 $\mu\text{g GAE/ml}$ is best and shown in Table 2. Methanol exhibits large levels of residual solvent polarity in various solvents (Sruthi *et al.*, 2021).

Effect of percentage solvent

In this process, percentage solvents are played a key role for extraction of TPC. 80% solvent is optimum and the concentration of TPC is 18.00 ± 0.00 $\mu\text{g GAE/ml}$ shown in Table 3. Similar to other percentages of solvents, 80% of them exhibit high polarity during TPC extraction (Maritim *et al.*, 2003).

Effect of time

In this extraction process, TPC was found to be 22.00 ± 0.50 $\mu\text{g GAE/ml}$ at 24 hrs. Here, 24 hr is an optimum time in this extraction process and shown in Table 4. Due to the plant material's solubility in the solvent used to separate or extract the phytochemicals (Mohammad Munawar *et al.*, 2019), a 24-hour extraction period is ideal for this technique (TPC).

Effect of pH

In this extraction process, TPC was found to be 25.50 ± 0.50 $\mu\text{g GAE/ml}$ at pH-6. The optimum extraction was observed at pH-6 shown in Table 5. The extraction of TPC was found to be optimum at the acidic pH level of 6 (Deepak *et al.*, 2023).

Effect of particle mesh size

In this extraction process, 120 mesh particle size (125 microns) is optimum and it was found to be 27.83 ± 0.28 $\mu\text{g GAE/ml}$ in seeds of this plant and shown in Table 6. In Particle mesh size, 120 is optimum compared to remaining sizes due to high content of surface area of plant particles (Hasham-Hisam *et al.*, 2011).

Soxhlet extraction

In the extraction process, the total phenolic content was found to be 36.16 ± 0.28 $\mu\text{g GAE/ml}$ at 40°C for 4 hours. Here the parameters of batch extraction were applied into Soxhlet extractor. Only plant flavor was extracted from seed material resulting in enhanced TPC value. This extraction process isolated the seed material's flavor, transferring it

from the thimble to the bottom flask over duration of four hours time period (Magnani *et al.*, 2014). Here, the fumes fell on the plant material and extracted the flavor of seed material and transferred into the bottom flask through distillation path.

Evaluation of *in vitro* antioxidant activity

Various free radicals and reactive oxygen species generate numerous byproducts within the human body. Oxidative damage to biomolecules such as lipids, DNA and proteins is a major contributor to several degenerative diseases (Rolo *et al.*, 2006). The ability of antioxidants to scavenge these reactive species plays a crucial role in

Table 2: Effect of different solvent for extraction of TPC from *Carica papaya* seeds.

Different solvent	TPC ($\mu\text{g GAE/ml}$)
Ethanol	11.50 ± 0.50
Methanol	14.16 ± 0.28
Ethyl acetate	09.16 ± 0.28
Water	06.16 ± 0.28

Table 3: Effect of percentage solvent for extraction of TPC from *Carica papaya* seed.

Percentage solvent (%)	TPC ($\mu\text{g GAE/ml}$)
100	14.16 ± 0.28
80	18.00 ± 0.00
60	12.50 ± 0.00
50	11.50 ± 0.50
40	10.50 ± 0.50
20	08.66 ± 0.28

Table 4: Effect of time for extraction of TPC from *Carica papaya* seed.

Time (hr)	TPC ($\mu\text{g GAE /ml}$)
12	18.66 ± 0.28
24	22.00 ± 0.50
36	14.00 ± 0.00
48	12.33 ± 0.28

Table 5: Effect of pH for extraction of TPC from *Carica papaya* seed.

pH	TPC ($\mu\text{g GAE/ml}$)
5	20.16 ± 0.28
6	25.50 ± 0.50
7	17.33 ± 0.28
8	15.16 ± 0.28
9	12.16 ± 0.28

Table 6: Effect of particle mesh size for extraction of TPC from *Carica papaya* seed.

Particle mesh (microns)	TPC ($\mu\text{g GAE/ml}$)
80 mesh (177 microns)	16.33 ± 0.28
100 mesh (149 microns)	20.66 ± 0.28
120 mesh (125 microns)	27.83 ± 0.28

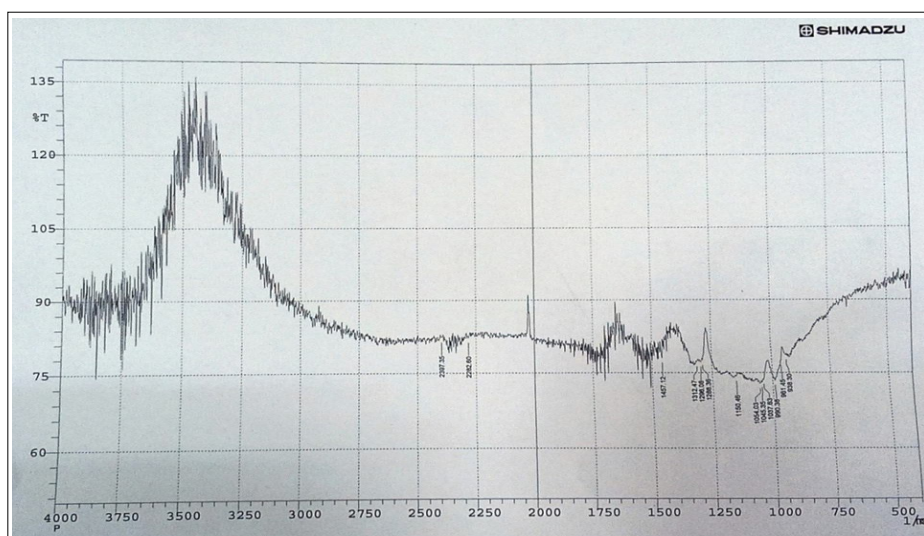


Fig 2: FTIR analysis.

Table 7: Effect of plant extract on reducing power ability.

Control=0.38

Conc. ($\mu\text{g/ml}$)	% Reducing power of Standard sample (Gallic acid)	% Reducing power of plant extract
50	21.57 $\pm$ 0.81	19.21 $\pm$ 0.45
100	27.85 $\pm$ 0.27	23.71 $\pm$ 0.24
250	39.21 $\pm$ 0.43	32.16 $\pm$ 0.43
500	59.47 $\pm$ 0.92	52.48 $\pm$ 0.40
1000	83.68 $\pm$ 0.15	73.08 $\pm$ 0.16
2000	86.84 $\pm$ 0.64	79.81 $\pm$ 0.22

managing various health conditions. The antioxidant potential of a compound serves as a key indicator of its therapeutic value (Lephart, 2016). Antioxidant activity is associated with multiple mechanisms, including the decomposition of peroxides, prevention of chain initiation, binding of transition metal ions and inhibition of hydrogen abstraction. Phenolics, tannins and flavonoids, which contribute to its antioxidant properties by neutralizing free radicals and singlet oxygen (Okur, 2022). To evaluate its antioxidant potential, the reducing power assay, an in vitro model, was employed to assess the efficacy of the herbal extract.

Reducing power ability

The presence of reductones is associated with the reducing properties. Which exert antioxidant action by donating hydrogen atom which results in antioxidant action (Nafiu *et al.*, 2015). In studies, reaction of reductones with peroxide precursors are also reported. Results were tabularized in Table 7. We are observed that a dose-response relationship is found in the reducing power activity; the activity increased as the concentrations increased for each individual of this plant extract. The results revealed a reducing power ability of 79.81 $\pm$ 0.22% at the highest concentration of 2000 $\mu\text{g/ml}$ and additionally, the IC₅₀ values

were found to be concentration-dependent, with a value of 92.17 $\mu\text{g/ml}$ for reducing power ability. The results of this study suggest that extract may serve as a potential natural antioxidant, helping to prevent or slow down ageing and oxidative stress-related degenerative diseases.

FTIR characterization

The functional groups in the dried powder of methanolic extract of *Carica papaya* were identified using FTIR analysis (Gurung *et al.*, 2009) and displayed in Fig 2. The peak at 2397.3 (amino-related component), 2282.6 (free amino acid with hydrohalides), 1457.12, (aromatics), 1312.47 (nitro compounds), 1296 (aliphatic amines), 1045.3-1037.6 (phosphorus compound), 990.38-961.45 (alkanes) and 938.3 (alkanes).

CONCLUSION

The extraction of TPC from *Carica papaya* seeds plays a significant role in determining their pharmacological properties, particularly antioxidant activity. This was confirmed through the reducing power ability assay, which demonstrated an effective free radical-scavenging ability. The high concentration of phenolic compounds was closely associated with strong antioxidant potential. Overall, *Carica papaya* emerges as a promising natural source of antioxidants with potential applications in health supplements for managing oxidative stress and related diseases. These findings highlight the need for further research into their pharmacological properties, which could lead to novel therapeutic approaches for various disorders.

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Author contributions

Tanishka and Nandini: Wrote the whole manuscript, Sandeep Sirohi and Jaidev : Optimization process, Avinash Singh: Manuscript Evaluation, DV Surya Prakash: FTIR analysis and Manuscript Evaluation.

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Conflict of interest

The authors declare no conflict of interest.

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