International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(7): 557-561
www.biochemjournal.com
Received: 15-04-2024
Accepted: 20-05-2024

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Comparison of extraction technique on recovery of phenolic compounds obtained from papaya leaves (*Carica papaya* L. leaves)

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DOI: https://doi.org/10.33545/26174693.2024.v8.i7g.1525

Abstract
This study was conducted to understand the different extraction methods *viz* conventional, microwave-assisted extraction and ultrasound-assisted extraction by extracting the phytochemicals from the leaves of *Carica papaya* L. & the influence of process parameters *viz*, temperature, ultrasound power and microwave power. Experiments were conducted using the single-factor experiment design wherein one factor was varied at a time keeping other factors constant. Variables selected for the experiment were Temperature (40, 50, 60 °C) & Time (30 min.) in case of conventional extraction; Power (150, 175, 200), Time (30 min) in ultrasound-assisted extraction and Power (400, 500, 600 W), Time (30 seconds) in microwave-assisted extraction. *Carica papaya* L. The analysis showed the TPC ranged from 25.28 mg GAE/g to 41.60 mg GAE/g, TFC ranged from 50.70 mg QE/g to 13.60 mg QE/g & 15.27% to 25.62%. The highest Total phenolic content, Total Flavonoid Content & Antioxidant activity was observed in treatment 6 employing ultrasound assisted extraction with power 200 watts. It is concluded from the experiment to extract phytochemicals from papaya leaf ultrasound can be employed.

Keywords: Extraction, *Carica papaya* L. leaves, ultrasound assisted extraction, and microwave assisted extraction

Introduction

There are several different antioxidant compounds that are present in fruits and vegetables such as tocopherols, phenolics, carotenoids, and anthocyanin's (Jakubowski and Bartosz, 1997) [11]. About 20% of all plants have been the subject of pharmaceutical research, which has good effects on the human health, including the treatment of terminal and detrimental illnesses (Naczk and Shahidi, 2006) [16]. A wide variety of bioactive chemicals can be produced by plants. Fruits and vegetables include high amounts of phytochemicals that offers protection against damage from free radicals (Suffredini *et al.*, 2004) [21]. Because they function as natural antioxidants, plants that contain advantageous phytochemicals may help the body meet its requirements (Suffredini *et al.*, 2004) [21]. Numerous studies have demonstrated the high antioxidant content of several plants. For example, phenolic chemicals like flavonoids, tannins, and lignins that are present in plants, as well as vitamins A, C, and E, all function as antioxidants (Suffredini *et al.*, 2004) [21]. Because they function as natural antioxidants, plants that contain advantageous phytochemicals may help the body meet its requirements (Boots *et al.*, 2008) [4]. Numerous studies have demonstrated the high antioxidant content of several plants. For example, phenolic chemicals like flavonoids, tannins, and lignins that are found in plants, as well as vitamins A, C, and E, all function as antioxidants (Suffredini *et al.*, 2004) [21]. By postponing or preventing oxidation brought on by reactive oxygen species (ROS), antioxidants regulate and lessen oxidative damage to food, so extending its shelf life and improving its quality (Ames *et al.*, 1993) [2]. A number of phenolics, ascorbic acid, and beta carotene have been shown to have potent anti-inflammatory, anti-aging, and cancer-preventive properties (Duthie *et al.*, 1996) [10]. Papaya (Carica papaya) cultivation in India is widespread, especially in states like Karnataka, Andhra Pradesh, Gujarat, and Maharashtra. It is from Caricaceae family and is known for its fast growth and high yield of fruit Papayas are prized for their medical qualities as well as their nutritional worth. One such property is the enzyme papain, which helps in digestion and has uses in the cosmetic and pharmaceutical industries (Prakash, O. and Gupta, R. K., 2009) [17]. Tropical climates are ideal for cultivation since it requires well-drained soil, warm temperatures, and sufficient moisture (Morton, J. F.,1987) [15]. Papaya leaf extract's potential health advantages and therapeutic qualities have drawn a lot of interest. Flavonoids, alkaloids, and phenolic acids are among the many bioactive chemicals that give it its anti-inflammatory, immune-modulating, and antioxidant qualities (Baliga *et al.*, 2012) [3]. Because papaya leaf extract contains antioxidant and antibacterial qualities, it has showed promise as an edible covering to extend fruit shelf life. It is an edible coating that creates a barrier to prevent moisture loss and microbial growth on the fruit's surface, so extending its freshness and postponing spoiling. Studies have shown that coatings containing papaya leaf extract can successfully stop bacteria and fungus that cause spoiling from growing on fruits like mangoes and strawberries (Rojas-Graü *et al.*, 2007, Aloui *et al.*, 2014) [18, 1]. Furthermore, the antioxidant components in the extract aid in lowering oxidative stress and preserving fruit quality while it is being stored (Chiumarelli., 2015) [8]. Using papaya leaf extract as an edible coating offers a sustainable and all-natural way to extend the shelf life of fruit, possibly negating the need for artificial preservatives. The aim of this study is to investigate the process parameters and extraction techniques that impact the extraction of polyphenols using conventional methods as well as non-conventional techniques like ultrasound- and microwave-assisted extraction, which may one day be used to investigate *Carica papaya* L. leaves as a viable and alternative source of shelf life.

Material and Methods

The present study has been undertaken to study extraction of phenolic compounds from *Carica papaya* L. leaves. The study aimed to investigate the extraction of phytochemicals from *Carica papaya* L. leaves using conventional method, ultrasound-assisted and microwave assisted extraction for phenolic compounds. The following procedures were used for the experimental work in order to meet the study's objectives. To achieve the objectives of the study, experimental work will be carried in the following way.

Raw Materials and Chemicals

The *Carica papaya* L. leaves were procured from the pollination center, Department of Entomology, College of Agriculture. For experimental studies, the leaves were cleaned and dried using try dryer at temperature of 60 °C. The dried leaves were crushed to obtain finely ground powder. All reagents and chemicals used in the experiments were of analytical grade. After drying the *Carica papaya* L. leaves were pulverized in hammer mill to the size of 1- 2 mm. The dried powder was stored in glass jars till further processing.

Extraction of *Carica papaya* L. leaves

For extraction, properly mixed *Carica papaya* L. Leaves powder and solvent mix at different liquid-solid ratios were subjected to ultra-sonication, Microwave and Conventional extraction by heating as explained in Table 1 & 2 with different power and temperature range for the extraction of *Carica papaya* L. Leaves extract. Subsequently the solutions were passed through muslin cloth followed by centrifugation and filtration through filter paper. The supernatant was collected and stored at 4°C for further analysis.

Table 1: Experiments for Extraction of *Carica papaya* L. leaves

Method	Levels	Reference
Conventional Extraction	Temperature (40, 50, 60 °C) Time (30 min) Liquid-Solid ratio (100 ml/g)	Sayeed and Thakur, 2020
Ultrasound-Assisted Extraction	Power (150,175,200 W) Time (30 min) Liquid-Solid ratio (100 ml/g)	
Microwave-Assisted Extraction	Power (400,500,600W) Time (30s) Liquid-Solid ratio (100 ml/g)	Cardoso-Ugarte, 2014.

Table 2: Completely Randomized Design (CRD) design of Different Extraction Methods

Method	Treatment No.	Levels
Microwave-Assisted Extraction	T_1	400 W
	T_2	500 W
	T_3	600 W
Ultrasound-Assisted Extraction	T_4	150 W
	T_5	175 W
	T_6	200 W
Conventional Extraction	T_7	40 °C
	T_8	50 °C
	T_9	60 °C

Total phenolic content (TPC)

Total phenolic content was measured using the Folin-Ciocalteu method with little modification (Singleton and Rossi, 1965) [20]. Briefly, a 20 µL extract sample solution was mixed with 80 µL of Folin-Ciocalteu reagent (10%). After 4 min, 100 µL of 7.5% (w/v) Na_2CO_3 was added to each sample and allowed to stand for 2 h at room temperature. Then the absorbance was measured at 765 nm using a UV- Vis spectrophotometer. The estimation of TPC was calculated by a calibration curve obtained with gallic acid. Results were expressed as gallic acid equivalents (mg GAE/g dw).

Total flavonoid contents (TFC)

Total flavonoid contents were determined by the spectrophotometric method (Dewanto *et al.*, 2002) [9]. Sample extract or standard solution (250 µL), deionized water (1.25 ml) and 5% $NaNO_2$ solution (75 µL) was mixed successively and reacted for 6 min then 10% $AlCl_3.6H_2O$ solution (150 µL) was added to the mixture. After 5 min of incubation at room temperature, 1 M NaOH solution (0.5 ml) was added and then the final volume was made up to 3.0 ml with deionized water. Quercetin was used as the standard. The absorbance will be read against a blank at 510 nm. The results were expressed in terms of milligrams of quercetin equivalents (mg QE/g).

Antioxidant activity

The antioxidant properties was analysed using DPPH (2,2-diphenyl-1-picrylhydrazyl). DPPH radical-scavenging activity of sample extracts were determined according to the method reported by Brand-Williams *et al*. (1995) [5]. The reaction mixture consisting of 1.5 ml of DPPH working solution (4.73 mg of DPPH in 100 ml of ethanol) and 300 µL sample extract was shaken and incubated for 40 min in the dark at room temperature. The absorbance was measured at 515 nm against a blank, using a UV-vis spectrophotometer (Scientific instruments industries, Make-Lasany). DPPH free radical-scavenging ability was calculated using the following formula:

$$\text{Scavenging activity (\%)} = \frac{(\text{Absorbanceofcontrol} - \text{Absorbanceofsample})}{\text{Absorbanceofcontrol}} \text{x } 100$$

Results and Discussions

Effect of extraction method on TPC, TFC & Antioxidant Activity

Table 3 shows the variation of total phenolic content (TPC), total flavonoids content (TFC) & Antioxidant Activity among different methods of extraction used. Overall, the results suggest that among all the extraction procedures used, ultrasound-assisted extraction showed the highest total phenolic content (TPC), total flavonoids content (TFC) & Antioxidant Activity in papaya leaf extracts.

Table 3: Highest TPC, TFC & Antioxidant Activity across different methods & Treatments.

Method	TPC (mg GAE/g).	TFC (mg QE/g)	Antioxidant Activity (%)
Conventional Method	30.227	7.152	15.270
Microwave Assisted method	37.381	11.179	20.137
Ultrasound Assisted Method	41.605	13.669	25.620

The augmentation of extraction employing ultrasound has been linked to the transmission of ultrasonic pressure waves and the consequent cavitation events. Extractant mass transfer is enhanced by high shear forces (Jian-Bing *et al*., 2006) [12]. Cavitation bubble implosion causes macro-turbulence, high-speed particle-to-particle collisions, and disturbance in the biomass's micro-porous particles, which quickens internal and eddy diffusion. Additionally, a fast-moving stream of liquid passes through the surface cavity due to cavitation near the liquid-solid contact. Cavitation on the product surface results in micro-jet impediment, which impairs particle disintegration, erosion, and surface peeling. By exposing fresh surfaces, this action increases mass transfer even more.

Influence of temperature on TPC, TFC & antioxidant activity in conventional extraction.

The result in Table 4 revealed the amount of TPC, TFC & antioxidant activity observed at different temperatures in conventional method. All the three variables shows increasing trend with increase in temperature at constant time period.

Table 4: TPC, TFC & antioxidant activity of extracts at different temperatures.

Temperature	Total phenolic content	Total flavonoid Content	Antioxidant Activity
40 °C	25.2833	5.70533	13.1453
50 °C	27.2907	6.77033	14.4093
60 °C	30.2273	7.152	15.2703

Mechanisms were employed by (Maillard and Berset., 1995) [14] to elucidate the behaviour of polyphenols at elevated temperatures. First, the insoluble phenolic compounds may be released when the lignin bonds to phenolic acids are disrupted. It has been demonstrated that there are twice as many bound phenolic acids as free phenolic compounds (measured following the hydrolysis of plant tissue). Second, high temperatures have the potential to break down lignin itself, producing additional phenolic acids. This could explain why phenolic yield increases with temperature in PLE extractions. The breakage of the lignin–phenolic acid linkages or the lignin itself breaking down and producing more phenolic acid could be the cause of the increase in TPC seen at high temperatures in extractions.

Effect of Microwave extraction power on TPC, TFC & antioxidant activity

The result in Table 5 shows the amount of TPC, TFC & antioxidant activity observed at different microwave power. All the three variables shows increasing trend with increase in microwave power at constant time period.

Table 5: TPC, TFC & antioxidant activity of extracts at different MW power.

Microwave power	Total phenolic content	Total flavonoid Content	Antioxidant Activity
400 watts	30.5	7.25	15.36
500 watts	33.13	9.22	19.11
600 watts	37.38	11.17	20.12

According to Yansheng *et al.* (2011) [25], microwave treatment increases yield owing to quicker heating rates during microwave treatment in comparison to traditional heating and damage to vegetable tissue from microwave fields. In MAE, microwaves heat the sample's interior, increasing internal pressure and rupturing the cell wall, which releases bioactive chemicals (Vinatoru et.al., 2017) [13]. The temperature rose from 60 to 210 C as the microwave power increased from 200 to 600 W, which accounts for the higher TPC values at 600 W. Rising temperatures facilitate the extraction of bioactive chemicals from sample matrices and increase solute solubility by reducing solvent viscosity and surface tension (Mandal *et al*., 2007) [23] as same was found in over case.

Effect of Ultrasonic Power on TPC, TFC & antioxidant activity

The values for TPC, TFC and antioxidant activity in the extracts increased as ultrasound power is increased as shown in table 6. It is generally known that raising the ultrasonic power applied to the liquid medium enhances extraction performance by improving cavitation and sonochemical effects. However, at higher power values encourage agitation over cavitation in the liquid medium, reducing cavitation and resulting in a reduced yield (Chemat F, *et al*., 2017) [7]. Similar results were seen by Tabio-García *et al*., 2021 [22], in their study on the optimisation of ultrasound-assisted extraction of betalains and polyphenols from Amaranthus hypochondriacus var. Nutrisol.

Table 6: TPC, TFC & antioxidant activity of extracts at different US power.

Microwave power	Total phenolic content	Total flavonoid Content	Antioxidant Activity
150	35.29	8.23	19.62
175	38.33	12.44	21.90
200	41.60	13.66	25.61

Table 7: Comparisons of means using hsd test

Treatment No.	Total phenolic content	Total flavonoid Content	Antioxidant Activity
1	30.508^{f}	7.258^{f}	15.612^{e}
2	33.135^{d}	9.228^{d}	19.114^{d}
3	37.381^{c}	11.179^{c}	20.317^{c}
4	35.293^{d}	8.24^{e}	19.626^{cd}
5	38.331^{b}	12.447^{b}	21.905^{b}
6	41.605^{a}	13.669^{a}	25.62^{a}
7	25.283^{h}	5.705^{g}	13.145^{f}
8	27.291^{g}	6.77^{f}	14.409^{e}
9	30.227^{f}	7.152^{f}	15.27^{e}

Note: The means with different Letters as superscripts are significant (p<0.05).The means with same letters or having common letter(s) are not significantly different.

Conclusion

This study was conducted to understand the different extraction methods *viz* conventional, microwave-assisted extraction and ultrasound-assisted extraction by extracting the TPC, TFC and antioxidant activity from Papaya leaves (*Carica papaya* L. leaves) and the influence of process parameters *viz*, extraction temperature, ultrasound power and microwave power. Experiments were conducted using the single-factor experiment design wherein one factor was varied at a time keeping other factors constant. Variables selected for the experiment were Temperature in case of conventional extraction; Power in ultrasound-assisted extraction microwave-assisted extraction. The analysis showed the TPC ranged from 25.28 mg GAE/g to 41.60 mg GAE/g, TFC ranged from 50.70 mg QE/g to 13.60 mg QE/g & 15.27% to 25.62%. The highest TPC, TFC & Antioxidant activity was seen in treatment 6 as shown by table 7 employing ultrasound assisted extraction with power 200 watts. It is concluded from the experiment to extract phytochemicals from papaya leaf ultrasound can be employed.

References

1. Aloui H, Khwaldia K, Licciardello F, Hamdi M. Physiological and biochemical changes in pomegranate fruit stored under modified atmosphere packaging. Journal of the Science of Food and Agriculture. 2014;94(5):888-895.
2. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proceedings of the National Academy of Sciences of the United States of America. 1993;90(17):7915-7922.
3. Baliga MS, Meera S, Vaishnav L, Palatty PL. Scientific validation of the ethnomedicinal properties of the Ayurvedic drug Triphala: A review. Chinese Journal of Integrative Medicine. 2012;18(12):946-954.
4. Boots AW, Haenen GR, Bast A. Health effects of quercetin: From antioxidant to nutraceutical. European Journal of Pharmacology. 2008;585(2, 3):325-337.
5. Brand-Williams W, Cuvelier ME, Berset CLWT. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology. 1995;28(1):25-30.
6. Cardoso-Ugarte GA, Sosa-Morales ME, Ballard T, Liceaga A, San Martín-González MF. Microwave-assisted extraction of betalains from red beet (*Beta vulgaris*). LWT - Food Science and Technology. 2014;59(1):276-282.
7. Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano-Tixier AS, Abert-Vian M. Ultrasound-assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols, and applications. A review. Ultrasonics Sonochemistry. 2017;34:540-560.
8. Chiumarelli M, Hubinger MD, Ferrari CC. Shelf-life extension of fresh-cut pineapple (*Ananas comosus* L. Merril) by application of edible coating enriched with antioxidant or antimicrobial agents. Postharvest Biology and Technology. 2015;101:154-163.
9. Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry. 2002;50(10):3010-3014.
10. Duthie SJ, Ma A, Ross MA, Collins AR. Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. Cancer Research. 1996;56(6):1291-1295.
11. Jakubowski W, Bartosz G. Estimation of oxidative stress in Saccharomyces cerevisiae with fluorescent probes. International Journal of Biochemistry & Cell Biology. 1997;29(11):1297-1301.
12. Jian-Bing J, Xiang-hong L, Mei-qiang C, Zhi-chao X. Improvement of leaching process of geniposide with ultrasound. Ultrasonics Sonochemistry. 2006;13(5):455-462.
13. Vinatoru M, Mason TJ, Calinescu I. Ultrasonically assisted extraction (UAE) and microwave-assisted extraction (MAE) of functional compounds from plant materials. Trends in Analytical Chemistry. 2017;97:159-178.
14. Maillard MN, Berset C. Evolution of antioxidant activity during kilning: Role of insoluble bound phenolic acids of barley and malt. Journal of Agricultural and Food Chemistry. 1995;43(7):1789-1793.
15. Morton JF. Papaya. In: Fruits of Warm Climates. Miami, FL: Julia F. Morton; c1987. p. 97-107.
16. Naczk M, Shahidi F. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. Journal of Pharmaceutical and Biomedical Analysis. 2006;41(5):1523-1542.
17. Prakash O, Gupta RK. Therapeutic uses of Carica papaya. In: Natural Products and Medicinal Plants Research. Houston, TX: Studium Press LLC; c2009. p. 328-337.

18. Rojas-Graü MA, Tapia MS, Rodríguez FJ, Carmona AJ, Martín-Belloso O. Alginate and gellan-based edible coatings as carriers of antibrowning agents applied on fresh-cut Fuji apples. Food Hydrocolloids. 2007;21(1):118-127.
19. Sayeed R, Thakur M, Gani A. *Celosia cristata* Linn. flowers as a new source of nutraceuticals: A study on nutritional composition, chemical characterization and in vitro antioxidant capacity. Heliyon. 2020;6(12).
20. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965;16(3):144-158.
21. Suffredini IB, Sader HS, Gonçalves AG, Reis AO, Gales AC, Varella AD, Younes RN. Screening of antibacterial extracts from plants native to the Brazilian Amazon Rain Forest and Atlantic Forest. Brazilian Journal of Medical and Biological Research. 2004;37(3):379-384.
22. Tabio-García D, Paraguay-Delgado F, Sánchez-Madrigal MÁ, Quintero-Ramos A, Espinoza-Hicks JC, Meléndez-Pizarro CO, *et al*. Optimization of the ultrasound-assisted extraction of betalains and polyphenols from *Amaranthus hypochondriacus* var. Nutrisol. Ultrasonics Sonochemistry. 2021;77:105680.
23. Mandal V, Mohan Y, Hemalatha S. Microwave assisted extraction—an innovative and promising extraction tool for medicinal plant research. Pharmacognosy Reviews. 2007;1(1):7-18.
24. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions. 2006;160(1):1-40.
25. Yansheng C, Zhida Z, Changping L, Quingshan L, Paifang Y, Welz-Bierman U. Microwave-assisted extraction of lactones from *Ligusticum chuanxiong* Hort. using protic ionic liquids. Green Chemistry. 2011;13:666-670.