Moumita Saha et al /J. Pharm. Sci. & Res. Vol. 15(1), 2023, 978-984

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Papaya Seed Waste: A Potential Source of Therapeutic Diet

Moumita Saha[2*], Shirsha Mukherjee[1], Pushpita Mukherjee[2], Sudeshna Sengupta[2], Malavika Bhattacharya[2], Anirban Ghosh[3], Sirshendu Chatterjee[2]

[1]Department of Microbiology, St. Xavier's College (Autonomous), Kolkata- 700016, West Bengal, India
[2]Department of Biotechnology, Techno India University, EM-4, Salt Lake, Sector- V, Kolkata- 700091, West Bengal, India
[3]Department of Zoology, Netaji Subhas Open University, West Bengal

* saha58187@gmail.com

Abstract

Recently, there has been an increasing study on seeds so as to use them as potential sources of therapeutics because they contain many bioactive secondary metabolites. Our experimental research focused mostly on comparing quantified phyto-nutrient estimations, assessing the antioxidant and antibacterial properties of raw and ripe *Carica papaya* seed. It has been highlighted that the raw seed extract showed highest amount of total polyphenol (80.55±1.95 mg GAE/gm. of DW), total flavonoid (29.91±0.47 mg QE/gm. of DW), tannin (16.08±0.27 mg TAE/ gm. of DW), total soluble protein (4.10±0.52 mg BSAE/gm. of DW) as well as total vitamin B1 (43.13±0.19 mg THE/gm. of DW) and vitamin C (19.35±0.10 mg AAE/ml. of FW); whereas ripe seed extract showed maximum carbohydrate content i.e. 26.22±3.99 mg GE/gm. of DW and lipid content too i.e. 12.44±6.84%, The *in vitro* free radical scavenging activity was maximum showed by raw seeds extract i.e. 71.55±0.21%; whereas *in vitro* antimicrobial activity, the aqueous extract shows no zone of inhibition when compared with ethanolic extract. The overall result acknowledged the fact the wasted seeds could be used to treat disorders related to oxidative stresses and in a more cost-effective way can be a potential therapeutic diet source.

Keywords: *Carica papaya* seeds, phytonutrients, antioxidants, antimicrobial.

INTRODUCTION

The greatest risk to human health is from infectious diseases, causing death of nearly 50,000 people every day [1]. A further complication of the situation is created by the rapid development of multidrug resistance microbes to synthetically manufactured antimicrobials [2]. Due to the emergence of resistance, research institutes, and pharmaceutical companies, have been carrying out research to find out new sources of antibiotics [3].

Using of local plants as a substitute for synthetic drugs for curing diseases is widespread throughout world due to their pharmacological properties [4]. These plants provide the means of being an effective and inexpensive alternative medicine [5].

The plant kingdom provides a never-ending supply of bioactive compounds that are essential for the therapy of many ailments [6]. Plants harbour extensive sources of antibacterial molecules. Various medicinal plant extracts are used in the treatment of different contagious disorders due to their potential antibacterial activity [7]. A wide range of secondary metabolites are abundant in plants, exhibiting antioxidant and antibacterial properties in vitro, such as tannins, terpenoids, alkaloids, and flavonoids [8]. Out of the 422,127 species of plants reported worldwide, approximately 35,000 to 70,000 species are used as medicinal plants [9]. The health promoting adoptions of plants vary from employing and application of different parts via use of various ways of extracts and decoctions [10].

Carica papaya L (family Caricaceae) is a quick flourishing, deciduous, single-stemmed small tree, not so tall, with large leaves [11]. Several species of this family have been used as therapeutic agents for various diseases [12]. Papaya is a rich source of potential natural antioxidants in the form vitamins as well as other nutrients such as minerals and dietary fibre [13]. Each and every parts of the papaya tree, being its roots, leaves, flowers, and fruits or seeds have excellent medicinal properties. According to some previous studies, papaya seeds that are discarded as waste have some major health benefits [14]. Papayas feature edible black seeds with a strong, pungent flavor. They can occasionally be pulverized and used in place of black pepper. Papaya seeds can be used to treat roundworms (*Ascaris lumbricoides*) infections, indigestion, diarrhoea, skin ailments, and colds and they can also be used as a source of fatty acids [15]. The papaya seed extract is used for the treatment of bleeding piles as well as liver and spleen enlargement. They are also useful in controlling hypertension and hypercholesterolemia [16]. The anti-hyperglycemic effect of unripe mature seeds has also been reported [17]. The health benefits of papaya seeds are because of the presence of different bioactive compounds, such as polyphenolics and carotenoids, alkaloids, terpenoids and tannins present in them that confer antimicrobial activities to the seeds [18, 19]. Seed extracts of papaya are shown to exhibit antibacterial activity against a number of gram positive and gram negative bacteria [20]. Such plant-based antimicrobials are successful in treating infectious diseases while also eliminating the majority of the negative side effects associated with synthetically made antimicrobials [21, 22].

Depending on the above scientific detections, our present research study includes the phytonutrient screening of raw and ripe papaya seed extracts (aqueous and 100% ethanolic) by spectrophotometric assays and studying their antimicrobial sensitivity via disc diffusion assay methods. The overall workflow of this experiment is represented through a flowchart in Figure 1.

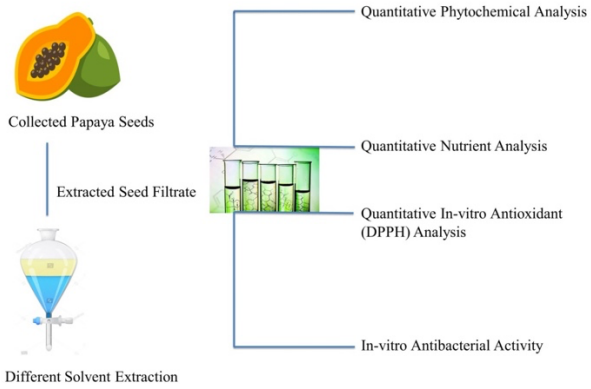

Figure 1: The Overall Workflow

MATERIALS AND METHODS

Chemicals

For the experiment, the chemicals used had an analytical grade from Merck Life Science Mumbai. Folin-ciocalteu, aluminium chloride and ascorbic acid were obtained. SD Fine- Chem Limited in Mumbai supplied Gallic acid and sodium hydroxide. From SRL Pvt. Ltd Quercetin, sodium nitrite, sodium carbonate, and tannic acid were purchased. DPPH was collected from Sisco Research Laboratories Pvt. Ltd., Maharashtra. Besides these, Luria Bertani agar, Luria broth, Anthrone reagent, and Bovine Serum Albumin (BSA) were bought from Himedia, Mumbai. Starch as indicator from SRL. Iodine solution (KI + KIO3) and the laboratory grade Potassium dichromate.

Collection of the Seed Samples

Both the Raw and Ripe *Carica papaya* (Figure 2) were collected from a local vegetable-fruit of Salt Lake, West Bengal, India, in the month of February 2022. Following their collection, each papaya was divided into pieces, and the seeds were removed.. After taking out the seeds they were cleaned and washed to get them free of dirt. They were dried under the sun for 1 week at least.

Figure 2: The Raw and Ripe Papaya Seeds

Preparation of Seed Sample

After complete drying of water content from the seeds, they were subjected to mechanical grinding until a coarse powder was processed. Solvent extraction using the powdery form was done with double distilled water (aqueous) and 100% ethanol respectively [23].

Technique for Solvent Extraction

For the preparation of seed extracts, to 50 ml of solvent (ethanol and distilled water), 1gm of each seed powder (raw and ripe seed) was added. Following this, overnight extraction was carried out at room temperature by keeping the conical flasks on a shaker. Filtration of the extracts was carried out using Whatman No.1 filter paper and kept at 4˚c for future biochemical analyses [23].

Determination of Extractive Value

The value of extractive yield was determined using the standard method, slightly modified, using 100% ethanol and double-distilled water, respectively. [24].

Quantitative Phytochemical Analysis

Quantification of Total Polyphenol Concentration

A small modification to the Folin-Ciocalteau method's basic protocol [25] was used for the quantitative determination of the total polyphenolic compounds, which was carried out in triplicate. At 765nm, the absorbance was measured. Gallic acid standard was used to produce the calibration curve. The total amount of polyphenols was stated as mg gallic caid equivalents/g of dry material.

Quantification of Total Flavonoid Concentration

By slightly modifying the conventional methodology of the aluminum chloride colorimetric method [26], the total flavonoid content was measured in triplicate. At 510 nm, absorbance was measured. Quercetin was used as the reference substance to create the calibration curve. The amount of flavonoids was represented as mg of quercetin equivalents/g of dry material.

Quantification of Total Tannin Concentration

With little alterations to the standard Folin-Ciocalteu method [27], quantitative estimation of total tannin content was done. At 700 nm, absorbance was measured. Tannic acid was used as the reference substance to create the calibration curve. The amount of tannin was represented as mg of tannic acid equivalent/g of dry material.

Quantitative Nutritional Analysis

Quantification of Total Carbohydrate Concentration

Quantification was done via the Anthrone method. Glucose was used to prepare the standard curve of the experiment. The absorbance was measured at 610 nm. The expression of the result was mg Glucose equivalents/g of dry material [28].

Quantification of Total Protein Concentration

Quantification was done via the Bradford assay. Bovine serum albumin (BSA) was used to prepare the standard curve of the experiment. The absorbance was taken at 595 nm. The expression of the result was mg BSA equivalents/g of dry material [29].

Quantification of Total Lipid Concentration

Quantification was done via the Folch method using a solvent mixture comprising methanol: chloroform in the ratio of 1:2. The weight of lipid was measured as mg lipid per 100g of dry material [30].

Quantification of Vitamin B1 Concentration

Quantitative determination of thiamine content was done following standard method [31] with little suggestions.

Absorbance was taken at 360 nm. The results were expressed in the form of mg Thiamine Equivalent/g fresh weight.

Quantification of Vitamin C by Iodometric Titration Method

Estimation of vitamin c content was done by Iodometric titration method. The samples were taken with a standard iodine solution as an oxidising agent with starch as an indicator and with samples and control (analytical grade Ascorbic acid) [32].

***In-vitro* antioxidant assay (DPPH radical scavenging assay)**

The stability of the 2, 2-diphenyl-2-picrylhydrazyl free radical scavenging activity of the extracts was measured using the standard approach to assess the presence of antioxidant capabilities in natural products [33]. At 517 nm, absorbance was detected. By employing ascorbic acid as the standard, the calibration curve was modified. Triplicates of this experiment are run.

The following formula was used to determine the inhibiton percentages:

% inhibition = (Control OD- Sample OD)/ Control OD *100

***In-vitro* Antimicrobial Property**

For the purpose of determining in-vitro antibacterial activity, disc diffusion techniques were used. Gram-positive bacteria *S. aureus* and *E. coli*, which were acquired from the laboratory of the Microbiology Department at Calcutta University, were utilized in this investigation. For this test, sterile Analytical grade water was used to create the sample extracts, which were then filtered through 0.2-m Whatman Filter paper. Every microorganism was sub-cultured in a volume of 100 µl in 5 ml of sterile Luria broth, which was then incubated for 24 hours at 37 °C. 20 µl of test bacteria were smeared and seeded onto sterile LB Agar plates that had been preheated during the log phase of newly sub cultured tubes. With the help of sterile forceps, sterile paper discs were applied to the top of inoculated agar plates. The sample extracts were then pipetted out in 20 µl aliquots onto the paper discs affixed to the agar surface. The plates were given a few minutes to dry before being incubated for 24 hours at 37°C. The area of the inhibition zone (in mm.) created by the extracts surrounding the disc was used to measure the antibacterial activity. Every test was run in triplicate. [34-36].

Statistical Analysis of Data

The results of all quantitative experiments were performed in sets of triplicate and were represented as average ± standard deviation. Statistical analyses like mean, standard curve, standard deviations were done using the software Microsoft Excel. At P value less than 0.05, statistical significance was acknowledged.

RESULTS

Extractive Value (%)

The present course of study has shown that the extractive value of ripe papaya seed aqueous extract *was* highest, i.e. 13±1%, whereas ethanolic extract of same ripe seed shows 6±1.73%, which is the lowest. Percentage Measurements of extractive value is represented in Table 1.

Table 1: Table Showing Extractive Value (%)

Type of Seed	Aqueous Extract (%)	Ethanolic Extract (%)
Ripe papaya seeds	13±1	7.33±0.57
Raw papaya seeds	8.67±1.54	13±1

Total Polyphenol Concentration

Quantification was done using the Gallic acid standard curve of the experiment (R^2=0.9984). It is observed that the total polyphenol content (Figure3A) is more in raw seeds (80.55±1.95 mg GAE/g of dry weight) than in ripe seeds (70.73±3.41 mg GAE/g of dry weight) in the case of ethanolic extracts. Given the case of aqueous extract, the polyphenol content is more in raw seeds (25.20±0.10 mg GAE/g of dry weight) than in ripe seeds (20.28±0.07 mg GAE/g of dry weight).

Total Flavonoid Concentration

Quantification was done using the Quercetin standard curve of the experiment (R^2=0.9964). It is observed that the total flavonoid content (Figure 3B) is more in raw seeds (29.92±0.48 mg QE/g of dry weight) than in ripe seeds (18.78±2.38 mg QE/g of dry weight) in the case of ethanolic extracts. Given the case of aqueous extract, the flavonoid content is more in raw seeds (15.30±0.80 mg QE/g of dry weight) than in ripe seeds (14.64±0.90 mg QE/g of dry weight).

Total Tannin Concentration

Quantification was done using the Tannic acid standard curve of the experiment (R^2=0.9946). It is observed that the total tannin content (Figure 3C) is more in raw seeds (16.08±0.27mg TAE/g of dry weight) than in ripe seeds (9.68±1.36mg AE/g of dry weight) in the case of aqueous extracts. Given the case of ethanolic extract, the tannin content is more in raw seeds (11.58±0.24mg TAE/g of dry weight) than in ripe seeds (6.64±0.31mg TAE/g of dry weight).

Total Carbohydrate Concentration

Quantification was done using the Glucose standard curve of the experiment (R^2=0.995). It is observed that the total carbohydrate content(Figure 4A) is more in raw ethanolic seed extract (18.29±0.38mg GE/g of dry weight) as compared to ripe ethanolic seed extract (15.21±2.38 mg GE/g of dry weight). Also, the carbohydrate content is more in ripe aqueous seed extract (26.22±3.99 mg GE/g of dry weight) than in raw aqueous seed extract (24.22±0.56 mg GE/g of dry weight).

Total Protein Concentration

Quantification was done using the BSA standard curve of the experiment (R^2=0.9947). It is observed that the total protein content (Figure 4B) is more in raw seed extracts (4.10±0.52 mg BSA/g of fresh weight) as compared to ripe extracts (2.06±0.12 mg BSA/g of fresh weight).

Total Lipid Concentration

Quantification was done using the Folch method. It is observed that the total lipid content (Figure 4C) is more in ripe seed extracts (12.44±6.84%) as compared to raw seed extracts (3.11±2.03%).

Total Vitamin B1 (Thiamine) Content

According to the result, it was observed that the amount of total Vitamin B1 content (Figure 4D) in Raw papaya seed was significantly higher (43.13±0.19 mg THE/g dry weight) than the ripe papaya seed (33.13 ±0.31 mg THE/g dry weight).

Total Vitamin C (Ascorbic acid) Content

The results of this assay were depicted in Figure 4E .The amount ranged from 13.17±0.15 mg AAE/ml fresh weight (ripe papaya seed) to 19.35±0.10 mg AAE/ ml fresh weight (raw papaya seed).

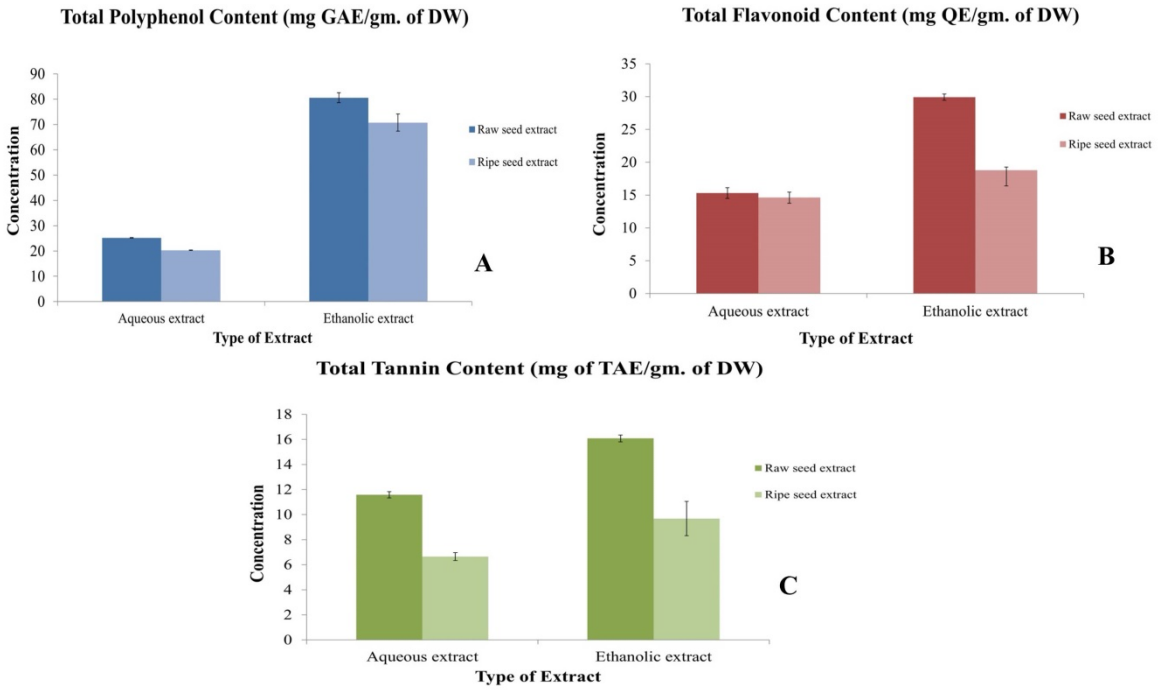

Figure 3: Comparative Result of Different Phytochemical Constituents [A: Total Polyphenol Content; B: Total Flavonoid Content; C: Total Tannin Content]

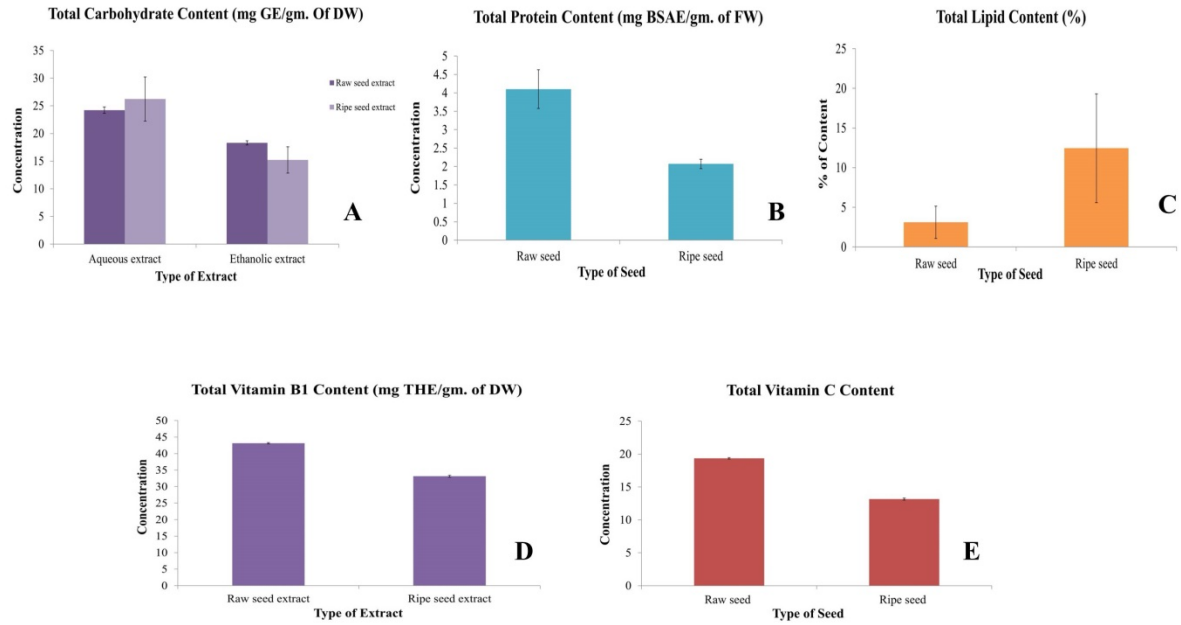

Figure 4: Comparative Result of Different Nutritional Components [A: Total Carbohydrate Content; B: Protein Content; C: Total Lipid Content; D: Total Vitamin B1 Content; E: Total Vitamin C (Ascorbic acid) Content

Results of *In-vitro* Antioxidant Assay

The inhibition percentage as calculated using the Ascorbic Acid standard curve (R^2=0.996) was less for aqueous seed extracts as compared to ethanolic extracts(Figure 5); in raw seeds ethanolic extract (71.55±0.21%) than in ripe seeds(68.25±0.33%) in the case of aqueous extracts. Given the case of aqueous extract, the % inhibition is more in raw seeds (58.22±4.80%) than in ripe seeds (33.33±2.76%).

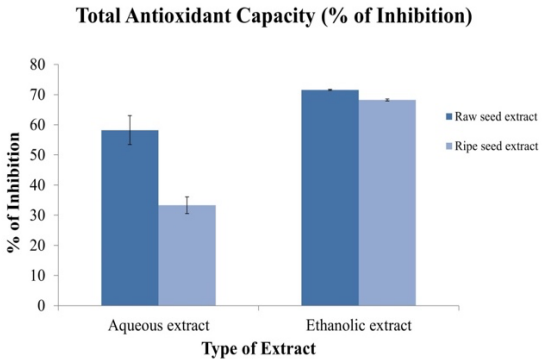

Figure 5: Comparative Result of Antioxidant Capacity (% of Inhibition)

Results of Antimicrobial Activity

Only the ethanolic extracts of raw and ripe papaya seeds exhibited zones of inhibition (Figure 6) observed against the two bacterial strains. No zone of inhibition was observed in the case of the aqueous extracts; additionally, the raw seed extract (ethanolic) shows a higher antimicrobial property as compared to the ripe seed extract (ethanolic) which is clearly represented via Table 2.

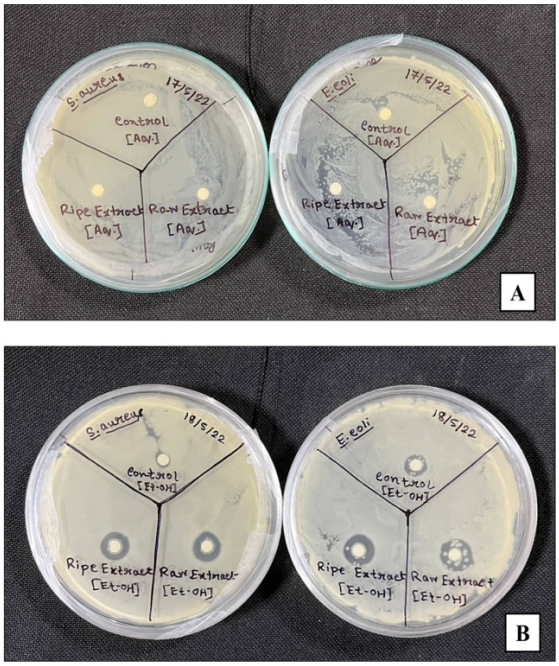

Figure 6: [A] Agar Plates Showing Zone of Inhibition against Raw and Ripe Papaya Seed Aqueous Extract With Respect To Control (Double Distilled Water), [B] Agar Plates Showing Zone of Inhibition against Raw and Ripe Papaya Seed Ethanolic Extract With Respect To Control (100% Ethanol)

Table 2: Comparison of Antimicrobial Activity of Ethanolic Seed Extract by Measuring Zone of Inhibition (mm.) with Respect to Control Solvent (100% ethanol)

Organism	Zone of inhibition (in mm) = Mean±S.D.		
	Control	Raw seed extract	Ripe seed extract
S. aureus	5.6±0.6	12.3±0.3	11.3±0
E. coli	9.4±0.3	14.1±0.3	10.3±0.4

DISCUSSIONS

The health promising effect of plants is mainly due to the presence of bioactive phytochemicals such as flavonoids, polyphenols, tannins, alkaloids, and many other secondary metabolites that exhibit a large number of antioxidant properties [37]. Such phytochemicals have been seen to exhibit medicinal properties and a little amount of cytotoxicity in-vitro but additional research should be carried out to evaluate their efficacy and mode of action [38]. Following the preparation of seed extracts and subjecting those to different quantitative assays, it is seen that the phytochemical and nutritional content is more in raw seeds than in ripe seeds. Also, raw seeds exhibited a higher antioxidant activity due to the presence of more phytochemicals. The variation between the presences of phytochemicals in the two seed variants is due to the maturity timing, exposure to pests, and presence of environmental factors such as moisture, pH, rainfall, temperature and pollution [39].

The pharmaceutical and nutraceutical industries may be able to benefit significantly from the action of phenolic compounds against oxidative stress-related mechanisms. Plant secondary metabolites with strong antioxidant, antibacterial, and chelating properties fall under the group of flavonoids [40, 41]. The structure of hydroxyl groups and their processes of substitution affect the antioxidant properties of flavonoids. The flavonoids classes' anti-oxidative activities result from a variety of reaction mechanisms, including the scavenging of free radicals, the chelation of metal ions (such as iron and copper), and the inhibition of enzymes that produce free radicals [42-44]. All potential ROS, which can harm cells, can be inhibited or controlled depending on their particular structure. Previous research has found that flavonoids can protect against a number of ailments. Tannins are not found in the leaves of many plants, but rather in the stem and bark. The antioxidant, antibacterial, and anti-cancer properties of the tannins were outstanding. These polymeric plant compounds are astringent and unpleasant; they can darken leather and can sediment proteins, amino acids, alkaloids, and nitrogenous chemicals. The tannin-protein combination has enduring antibacterial and antioxidant effects [45-47].

In this comparative study of seeds we estimated two types of vitamins, which are Vitamin C and Vitamin B1. Vitamin C is one of the key nutrients taking part in many important reactions of the human body, such as enhancing immune functions [46]. Vitamin C, the chemical name L-Ascorbic acid is a six-carbon hydrophilic or water-soluble organic molecule. The molecular formula of L-ascorbic acid is C6H8O6 and the molar mass is 176.13 g/mol. It is an antioxidant whose structure is similar to glucose [48].

The amount of vitamin c is higher in ripe papaya seed than raw papaya seed and vitamin B1, a vital nutrient for human health; thiamine is also required for treating a number of conditions linked to oxidative stress. Vitamin riboflavin is needed for oxidative phosphorylation as well as other physiological processes [49].

The results obtained from Kirby-Bauer's disc diffusion assay indicate that the ethanolic extracts have a higher antimicrobial activity as compared to the aqueous extracts. The presence of phytochemicals in the seed extracts is likely the reason behind the antimicrobial property. Polyphenols that have been quantified more in ethanolic extracts are mainly responsible for the nucleic-acid synthesis of both Gram-positive and Gram-negative Bacteria. For this reason, wider zones of inhibition are seen for ethanolic extracts [50, 51].

Papaya seeds contain a triterpenoid aldehyde compound which is a potent antibacterial agent. This compound can disrupt the cell membrane of bacteria thereby forming pores on the cell surface [52]. The presence of flavonoids aids in protein denaturation and disrupts cell membrane thereby causing cell death [52]. Each bioactive compound exhibits some mechanisms that are antibacterial [53].

CONCLUSION

From the results of the experiment, it can be concluded that the papaya seed extracts (ethanolic) possess antimicrobial activities against both *S.aureus* and *E.coli*. Although the phytochemical and nutritional content of both the raw and ripe seeds varied, both were successful in inhibiting the growth of bacteria. In future, further studies can be carried out to isolate the different phytochemicals and other compounds that could be used to prepare hydro-alcoholic solutions of potential therapeutics more cost-effectively.

Thus, the study explains that the seeds that are considered "Waste" and discarded by us can be used for herbal drug development exhibiting health promising effects, thereby transforming them into "Wealth."

Acknowledgement

The authors are extremely thankful to the Vice chancellor of Techno India University, West Bengal, for providing all the necessary lab equipment required for research work.

Abbreviations

GAE= Gallic Acid Equivalent
QE= Quercetin Equivalent
TAE= Tannic Acid Equivalent
GE=Glucose Equivalent
BSAE= Bovine Serum Albumin Equivalent
THE= Thiamine Hydrochloride Equivalent

Conflict of Interest

No conflict of interest is declared by the author.

REFERENCES

1. Ahmad I and Beg A. (2001). Antimicrobial and phytochemicals studies on 45 Indian medicinal plants against multidrug resistant human pathogens Ethnopharm. 74(87), 113-23. DOI: 10.1016/s0378-8741(00)00335-4
2. Adekunle A.S., Adekunle O.C. (2009). Preliminary assessment of antimicrobial properties of aqueous extract of plants against infectious diseases. Research article Biology and Medicine. 1(3), 20-24.
3. Latha S.P., Kannabiran K. (2006). Antimicrobial activity and phytochemicals of Solanumtrinobatum Linn. African Journal of Biotechnology. 5(23), 2402-2404.
4. Bibi the B., Jisha V.K., Salitha C.V., Mohan S. Valsa A.K. (2002). Antibacterial activity of different plant extracts. Indian journal of microbiology. 42, 361-363.
5. Pretorius C and Walt E. (2001). Purification and identification of active compound of Carpobrotusedulis L. J. Ethnopharn.76, 87–91
6. Ahmad I., Beg A.Z. (2001). Antimicrobial and phytochemical studies on 45 Indian Medicinal plants against multi- drug-resistant human pathogens. Journal of Ethnopharmacology.74, 87-91.
7. Renisheya JJ, Malar T, Johnson M, Mary UM, Arthy A. (2011). Antibacterial activities of ethanolic extracts of selected medicinal plants against human pathogens. Asian Pac J Trop Biomed. 576-578.
8. Bushra I, Fozia Abdul W, Ali R, UllahHussain, Iqbal Hamid, Almas M, Ahmad A. (2012). Antimicrobial activity of Malvaneglecta and Nasturtium microphyllum. Int J Res Ayurveda Pharm. 3, 808-810.
9. Bibi, Y., Nisa, S., Chaudhary, F. and Zia, M. (2011). Antibacterial activity of some selected medicinal plants of Pakistan. BMC Complem Altern Med. 11, 892-897.
10. Ukaegbu-Obi K.M., Ifediora A.C., Ifediora H.N. Chukwu B. (2016). In Vitro Combined Antibacterial Effect of Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale*) On Some Pathogenic Organisms. Analele Universităţii din Oradea, Fascicula Biologie Tom. XXIII. 1, 32-36.
11. Singh O, Ali M. (2011). Phytochemical and antifungal profiles of the seeds of Carica papaya L. Indian J Pharm Sci. 73(4), 447-51. DOI: 10.4103/0250-474X.95648.
12. Alabi, OA., Haruna, MT., Anokwuru, CP., Jegede, T., Abia, H., Okegbe, V. and Esan, E. (2012). Comparative studies on antimicrobial properties of extracts of fresh and dried leaves of *Carica papaya* (L) on clinical bacterial and fungal isolates. Pelagia Research Library. 3 (5), 3107-3114.
13. Aravind. G, Debjit B, Duraivel. S, Harish. G. (20130. Traditional and Medicinal Uses of Carica papaya. Journal of Medicinal Plants Studies. 1(1), 7-15.
14. Mulyono Lienny M. (2013). Antibacterial activity of papaya (*Carica papaya* L.) seed ethanol extract against *Escherichia coli* and *Staphylococcus aureus.* Jurnal Ilmiah Mahasiswa Universitas Surabaya. 2(2), 1-9.
15. Sukadana I M, Santi S R and Juliarti N K. (2008). Antibacterial activity of triterpenoid group compound from papaya seed (*Carica papaya* L.). J. Kim. 2(1), 15–8.
16. Gill LS. editor. (1992). *Carica papaya* L. In: Ethnomedicinal uses of plants in Nigeria. Benin City: Uniben Press. 57-8.
17. Olagunja JA, Ogunlana CO, Gbile Z. (1995). Preliminary studies on the hypoglycemic activity of ethanolic extract of unripe mature fruits of *Carica papaya*. Nig J BiochemMolBiol. 10, 21-3.
18. Deshpande SN. (2013). Preliminary phytochemical analysis and in vitro investigation of antibacterial activity of *Acacia nilotica* against clinical isolates. Journal of Pharmacognosy and phytochemistry. 1(5), 23-27.
19. Rodrigues, L. G. G., Mazzutti, S., Vitali, L., Micke, G. A., & Ferreira, S. R. S. (2019). Recovery of bioactive phenolic compounds from papaya seeds agroindustrial residue using subcritical water extraction. Biocatalysis and Agricultural Biotechnology. 22, 101367.
20. Tang CS, Syed MM, Hamilton RA. (1972). Benzyl isothiocyanate in the Caricaceae. Phytochemistry.11, 2531-3.
21. Al-Sahlany, S. T. (2017). Production of biodegradable film from soy protein and essential oil of lemon peel and use it as cheese preservative. Basrah Journal of Agricultural Sciences. 30(2), 27-35.
22. Iwu, M.W. Duncan, A.R. Okunji, C.O. (1999). New antimicrobials of plant origin. (Janick, J. (ed.), Perspectives on New Crops and New uses). ASHS Press, Alexandria.
23. Saha M, Chowdhury S R, Mitra D, Ghosh C, Ghosh P, Chatterjee S. (2021). Preliminary phytochemical screening with the evaluation of antioxidant and antimicrobial efficacy *Citrus limon* and *Citrus aurantifolia* leaf hydro- alcoholic extract: A comparative study. 6(4), 98-105.

24. Khandelwal KR. (2002). Practical Pharmacognosy, Technique and Experiments. Nirali Prakashan, Ninth Edition. 23, 10-23.11 & 25.1-25.6.
25. Singleton VL, Orthofer R, LamuelaRaventos RM. (1999). Analysis of total phenols and other oxidation substrates and antioxidants using Folin-Ciocalteau reagent. Methods in Enzymology. 299, 152-78.
26. Zhishen J, Mengcheng T, Jianming W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 64(4), 555-559.
27. Broadhurst RB, Jones WT. (1978). Analysis of condensed tannins using acidified vanillin. Journal of the Science of Food and Agriculture. 29(9), 7 88-794
28. Ludwig TG, Goldberg JV. (1956). The anthrone method for the determination of carbohydrates in foods and oral rinsing. J Dent Res. 35(1), 90-4. DOI: 10.1177/00220345560350012301.
29. Kruger, Nicholas. (2002). The Bradford Method for Protein Quantitation. 10.1385/1-59259-169-8:15.
30. Eggers, Lars & Schwudke, Dominik. (2016). Liquid Extraction: Folch. In book: Encyclopedia of Lipidomics. pp.1-6. 10.1007/978-94-007-7864-1_89-1.
31. Poornima GN, Ravishankar RV. (2009). Evaluation of Phytonutrients and Vitamin Contents in a Wild yam, *Dioscorea belophylla* (Prain) Haines. African Journal of Biotechnology. 8(6), 971-973.
32. Kashyap G. and Gautam DM. (2012). Analysis of Vitamin C in Commercial and Naturals Substances by Iodometric Titration found in Nimar and Malwa region. Journal of Scientific Research in Pharmacy. 1(2), 77-78.
33. Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. (2010). Antioxidant activity In vitro of Selenium-contained protein from the Se- enriched *Bifidobacterium animalis* 01. Anaerobe. 16, 380-386.
34. Ghosh P, Biswas S, Biswas M, Dutta A, Sil S, Chatterjee S. (2019). Morphological, ethno biological and phytopharmacological attributes of *Tridax procumbens* Linn. (Asteraceae): A review. International Journal of Scientific Research in Biological Sciences. 6 (2), 182-191.
35. Ghosh P, Biswas M, Biswas S, Dutta A, Hazra L, Nag, SK, Sil S, Chatterjee S. (2019). Phytochemical screening, antioxidant and antimicrobial activity of leaves of *Cleome rutidosperma* DC. (Cleomaceae). JPSR. 11(5), 1790-1795.
36. Zheng W, Wang SY. (2001). Antioxidant activity and phenolic compounds in selected herbs, J Agric Food Chem. 49(11), 5165-5170.
37. Ghosh P, Biswas S, Dutta A, Biswas M, Das S, Das C, Ghosh C, Chatterjee S. (2019). Evaluation of phytochemical constituents and antioxidant property of leaf acetone extracts of five herbaceous medicinal weeds. J Pharm Sci & Res. 11(8), 2806–2813.
38. Rajurkar NS, Hande SM. (2011). Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. Indian J Pharm Sci. 73(2), 146–151.
39. Dhanapal V, Samuel TB, Muddukrishniah K, Vijayan S. (2018). Screening of *Euphorbia Hirta* extracts for antioxidant activity. Indian Journal of Medical Research and Pharmaceutical Sciences. 5(6), 1–15.
40. Dey P, Dutta S, Chaudhuri TK. (2014). Phytochemical analysis of the leaves of Clerodendrum viscossum Vent. Int J Pharm Pharm Sci. 6(2), 254–258.
41. Chandha S, Dave R. (2009). In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. African J Micro Res. 3(13), 981–996
42. Fukumoto LR, Mazza G. (2000). Assessing antioxidant and prooxidant activity of phenolic compounds. J Agric Food Chem. 48(8), 3597–3604.
43. Kasolo JN, Bimenya GS, Ojok L, Ochieng J and Ogwal-okeng J W. (2010). Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. J Med Plant Res. 4(9), 753–757.
44. Kuspradini H, Wulandari I, Putri AS, et al. (2019). Phytochemical, antioxidant and antimicrobial properties of Litsea angulata extracts. [version 2; peer review: 3approved]. F1000Res. 7, 1839
45. Basma AA, Zakaria Z, Latha LY, Sasidharan S. (2011). Antioxidant activity and phytochemical screening of the methanol extracts of Euphorbia hirta L. Asian Pac J Trop Med. 4(5), 386–390
46. Subramanian SP, Bhuwaneshwari S, Prasath GS. (2011). Antidiabetic and antioxidant potentials of Euphorbia hirta leaves extract studied in streptozotocin-induced experimental diabetes in rats. Gen Physiol Biophys. 30(3), 278–285.
47. López-Pastor JA, Martínez-Sánchez A, Aznar-Poveda J, García-Sánchez AJ, García-Haro J, Aguayo E. (2020). Quick and Cost-Effective Estimation of Vitamin C in Multifruit Juices using Voltammetric Methods. Sensors (Basel). 20(3), 676.
48. Caritá AC, Fonseca-Santos B, Shultz JD, Michniak-Kohn B, Chorilli M, Leonardi GR. (2020). Vitamin C: One compound, several uses. Advances for delivery, efficiency and stability. Nanomedicine. 24, 102117.
49. Biswas S, Ghosh P, Dutta A, Biswas M, Chatterjee S. (2021). Comparative Analysis of Nutritional Constituents, Antioxidant and Antimicrobial Activities of Some Common Vegetable Wastes. Current Research in Nutrition and Food Science Journal. 9(1), 62-74.
50. Cowan MM. (1999). Plant products as antimicrobial agents. ClinMicrobiol Rev. 12(4), 564–582.
51. Martiasih M, Sidharta B B R and Atmodjo P K. (2014). Antibacterial activity of extract papaya seeds (*Carica papaya* L.) against *Escherichia coli* and *Streptococcus pyogenes*. J. Penelit. 5–7.
52. Sabir A. (2005). Antibacterial activity of Trigona sp. propolis flavonoid against Streptococcus mutans (in vitro). J. Kedokt Gigi. 38(3), 135–41. https://doi.org/10.20473/j.djmkg.v38.i3.p135-141/
53. Fitriani A, Aryani A, Yusuf H and Permatasari Y. (2012). The Exploration of Ketosynthase Gene on Endophytic Bacterial Root of *Vetiveria zizanioides* L. Int. J. Basic Appl. Sci. 13(4), 112–9.