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Estimation of Polyphenol and Antioxidant Content from Papaya (*Carica papaya*) and Mango (*Mangifera indica*) Seed, Peel and Leaves

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ABSTRACT: Papaya (*Carica papaya*) and mango (*Mangifera indica*) are two tropical fruits which are widely known for their rich nutritional properties and various health benefits. Papaya belongs to family Caricaceae which has its origin in South Mexico. Mango belongs to the family Anacardiaceae and were originated from India and southern China. As the pulp of these fruits is known to be consumed rapidly while their seeds, peels and leaves are often thrown away as waste. This study involves the estimation of total polyphenol and anti-oxidant activity of the by-products of papaya and mango fruits. The anti-oxidant activity by DPPH method of % inhibition of papaya seed=180 peel=125 leaves=64 is higher compared to mango by-products. In FRAP, ABTS, Radical Cation method of mango samples shown higher levels than papaya. Vitamin c content of mango seeds exhibits high as 15.6mg/100g compared to papaya seed, papaya peel and leaves has higher levels of vitamin c compared to mango peel and leaves. The total polyphenol activity (GAE/g) of mango seed=118.4, peel=55.31, leaves=19.04 exhibits higher value compared to papaya. Papaya seed and peel has more beta carotene content compared to mango peel has more beta carotene content than papaya. Seeds of papaya and mango has 2.05% and 2.15% pectin, mango peel has more pectin content than papaya 12.45%>8.94% and mango peel has shown slightly more pectin content than papaya as 4.25>3.58. Papaya peel have medium number of abundances of tannin content, seeds and leaves have less in number whereas mango have equal amounts of abundance of tannin content. This study mainly features the importance of the bioactive compounds found in the by-products (seed, peel, leaves) of papaya and mango and their health benefits.

KEYWORDS: Antioxidant activity, Beta carotene, Mango, Papaya, Vitamin C.

1. INTRODUCTION

Carica Papaya or Papaya is acquiring a new significance due to its high vulnerability to pests and environmental factors. Biotechnological modifications of papaya are crucial for developing resistant varieties (Geetika et al., 2018). C. papaya L., a member of the Caricaceae family, is also known as papaya, pawpaw, and kates. This perennial tree emerges from the regions of central America and southern Mexico (Yap et al., 2021). Papaya contains abundance of essential nutrients such as vitamin A, vitamin C, vitamin E, polyphenols carotenoids and enzymes include papain and chymopapain. It also contains wide range of therapeutic properties which exhibits strong antioxidant effects, anti-diabetic properties, chemoprotective abilities and antimicrobial activities which fight against bacteria, fungi, and parasites. Moreover, papaya exhibits positive immunomodulatory effects and shown to strengthen the body's defense mechanism by enhancing a shift towards cell mediated (th-1) immunity (Heena et al., 2019). However, papaya leaves have come out as valuable in having health- boosting elements and bioactive compounds.

In India, Ayurvedic practitioners recommend the boiled leaves of papaya for alleviating malarial and dengue fevers. Papaya leaf extract is believed to be effective in increasing platelet count as well as red and white blood cells in patients recovering from viral fever (Dharmarathna et al., 2013).

Papaya tissues have many bioactive compounds carpaine, BITC, benzyl glucosinolates, latex, and papain. Apart from the study about these compounds, the antioxidant properties and health benefits of phenolic compounds and carotenoids in papaya have not been totally investigated. By analyzing the mechanisms of these compounds papaya's nutritional and therapeutic properties arf essential for the disease prevention and treatment. Advanced tools from omics sciences are required to acquire about the functioning of phenolic compounds and carotenoids in papaya and about their health benefits (Ovando-Martínez et al.,2020).

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Mangifera Indica or Mango, known to as the "king of fruits," has its significant importance not only for taste but also for its nutritional properties. Mangoes are known to have originated from South Asia. However nowadays they are also cultivated across tropical and sub-tropical regions. Mangoes belong to the genus *Mangifera*, which includes approximately 30 species of tropical fruit-bearing trees in the family Anacardiaceae. Different parts of the mango tree exhibit various medicinal properties which is stated by the ayurvedic medicine (Shah et al.,2010). The by-product of the mango fruit (seed, peel, leaves) is rich in vitamins such as vitamin C and vitamin A, minerals and dietary fiber. On the far side of the edible pulp, the peels, seeds, and leaves of mangoes are rich in various bioactive compounds, including polyphenols, flavonoids, and carotenoids. These components have been correlated with diverse health benefits, particularly due to their antioxidant ability. Mango leaves (MLs) act as an abundant supply of minerals which includes nitrogen, potassium, phosphorus, iron, sodium, calcium, and magnesium, in addition to vitamins such as vitamin-A, B, E, and C. A important bio-macromolecule found in mango leaves is protein (Kumar et al., 2021).

Mangoes (*Mangifera indica* L.) are not only a major tropical fruit universally but also contains root sources of beneficial by-products which have been produced during processing. These by-products (peels, seeds, and residue) have high number of bioactive compounds. Mango peels and residue contain dietary fibre, carotenoids, and phenolic compounds, which are proven to improve cardiovascular health, helps in managing type 2 diabetes, diminish metabolic syndrome, and reduce cancer risk. Mango seeds are loaded with vegetable oils, proteins, and antioxidants with antibiotic potential (Wall-Medrano et al., 2020).

Mango peels are generous in polyphenols, carotenoids, and also vitamins which determines higher antioxidant content. The peels have also been shown to exhibit antimicrobial and anti-inflammatory properties, and their rich fiber content helps in treating gastro intestinal disorders and digestive health. Mango peel contains high cellulose content (30%) and lignin (16%) (Reddy et al.,2011; Banerjee et al.,2018).



Fig 1: Papaya peel, seeds and leaves.



Fig 2: Mango peel, seeds and leaves.

2. MATERIALS AND METHODS

2.1 Collection of Papaya and Mango (Peel, Leaf and Seed):

The peel, leaves and seed of Papaya and Mango was collected from the Super market in Hyderabad and agricultural field in Telangana. The peel was selected from a fully ripe papaya and mango fruits which has intact and undamaged peels. Healthy and mature leaves was collected from various branches of selected papaya and mango tree which were free from any signs of pests and diseases. Then the seeds were removed from the ripe papaya ad mango fruit using gloves.

2.2 Solvent Extraction (Methanol extraction):

100 grams of the material (papaya and mango-peel, seed and leaves) were weighed and placed separately into three different glass containers Add 100ml of organic solvent (methanol) to each container, ensuring that the solvent covers the plant material completely. After 24 hours, it was filtered using muslin cloth and centrifuged at 5000 rpm for 15 minutes. After extraction, the samples were

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centrifuged (4000 rpm/10 min) and filtered. Then the solid residue is separated from the methanol extract by the filtration process. Collect the (filtrate) methanol extract in a clean glass container. The solvent extracts are collected and used to estimate total antioxidant and polyphenol contents from the papaya and mango plant extracts (peel, seed and leaves) (Sanjukta kar et al.,2023).

2.3 Determination of Vitamin C:

About 100 g of the papaya and mango sample (peel, seed and leaves extract) was thoroughly extracted with ethanol. Then the extract was concentrated into a residue. After that pipette out 10ml of each of the plant extract into a separate beaker. Add 1-2 drops of glacial acetic acid into each of the beaker containing papaya and mango peel, seed and leaf extracts for the stabilization of vitamin c content. The DCPIP (2,6 dichlorophenol indophenol) solution was prepared to 200 ml and filtered and kept aside. Add few drops of the DCPIP mixture into each of the beakers containing plant extracts until pink colour appears. Titrate it by adding 10 ml of ascorbic acid solution (dissolve 100 mg liq. Ascorbic acid in 50 ml of 20 glacial acetic acid and add 100 ml distilled water) drop by drop until it turns to pink colour. Note the endpoint of the titre values when the pink colour remains.

2.4 Determination of Antioxidants by DPPH Method:

0.1014 mM of fresh DPPH in methanol was prepared. Trolox was used as a standard; dilutions were made to have a final volume of 200 µl. The different extracts or Trolox solutions (200 µl) were mixed with 3.8 ml of DPPH. After 30 minutes of incubation in the dark at room temperature we read the absorbance at 517 nm. Next, we made adequate dilution to determine the IC50. Results were expressed as µg TrE/g ± Standard deviations. DPPH scavenging activity was determined by calculating the percentage of inhibition:

%DPPH radical scavenging effect = $A0 - A1/A0 \times 100$ (Gaye et al., 2019).

2.5 Determination of Total Antioxidant Activity by FRAP Method:

0.1 mL of sample extract and 0.1 mL FeCl3 (3 mM in 5 mM citric acid) were mixed well in a 1.5 mL Eppendorf tube and incubated for 30 min in a water bath at 37 °C. The mixture was then added to 0.9 mL of 1 mM TPTZ in 50 mM HCl and vortexed. After 1 min, the absorbance is read at 620 nm using a Spectro quant Pharo 100 spectrophotometer (Merck, United States). A standard curve of Trolox is prepared following such procedures and results are expressed as µmol Trolox equivalent antioxidant capacity/100 g dry matter. (Lim et al., 2019).

2.6 ABTS ASSAY:

The ABTS radical cation was produced through the interaction of 250 μ M ABTS and 40 μ M K₂S₂O₈. Following the addition of 990 μ L of the ABTS solution to 10 mL of the fruit extract, the absorbance at 734 nm was measured. The percentage decrease in absorbance was determined and plotted against the concentration of the extracts, using Trolox as the standard reference (Özgen et al., 2006). The following formula was used to calculate the percentage of reduction power: Percentage of reduction power = [(A blank – A sample) / A blank] × 100 where A is the absorbance. (Addai et al., 2013).

2.7 Total Polyphenolic Activity:

After undergoing several dilutions, a test sample volume of 200 μ L was combined with 150 μ L of Folin-Ciocalteu reagent, 600 μ L of 20% Na2CO3 solution, and 2.32 mL of distilled water. Following a 1-hour incubation in darkness at room temperature, the absorbance was measured at 760 nm using a Perkin-Elmer UV/Visible spectrophotometer Lambda 365. Gallic acid (GA) served as the standard for calibration. The results, expressed as mg GAE/g ± Standard deviations, were obtained through meticulous analysis. The assay was due according to Mohdaly et al. with some modifications. (Gaye et al., 2019).

2.9 Determination for Tannins:

(0.5g) of extract was boiled in 20 ml of distilled water in a test tube and then filtered. A few drops of 0.1% FeCl3 was added and observed. A brownish green or a blue-black coloration indicate the presence of tannins. (Yanah et al., 2021).

2.10 Analysis of Beta Carotene:

Beta-carotene was extracted from fruit pulp or micellar portions post-simulated gastrointestinal digestion using an acetone-ethanol mixture (1:1), followed by petroleum ether. This process was repeated to ensure thorough extraction. The extract was saponified with 30% methanolic potassium hydroxide at room temperature for 3 hours. After removing the alkali through washing, the solvent was evaporated using a rotary evaporator. The residue was redissolved in petroleum ether and stored under refrigeration. Before

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analysis, petroleum ether was evaporated with nitrogen gas, and the residue was dissolved in the mobile solution. Beta-carotene was determined using reverse-phase HPLC (Shimadzu LC 10 AVP) with a UV-visible detector on a C18 column, using a mobile phase of 65% acetonitrile, 15% methylene chloride, and 20% methanol with 1.3 mmol/L ammonium acetate. Detection occurred at 450 nm, with peak identities confirmed by retention time and standard spectra. Precautions were taken to minimize light and air exposure, using nitrogen to replace air before flask stoppering and conducting experiments under yellow lighting. (Supriya Veda et al.,2007).

2.11 Pectin Content:

Pectin extractions were conducted following the method outlined by Nguyen and Savage, with certain modifications. Dried peel powder from each cultivar at various maturity stages was combined with a 1.5% aqueous solution of citric acid (Merck Sharp & Dohme Corp., Kenilworth, NJ, USA) at a ratio of 1:40 (w/v). The mixture was stirred continuously for 20 minutes and subsequently filtered through four layers of cheesecloth to separate the supernatant from the insoluble fraction. Pectin was then precipitated by adding absolute ethanol (98% purity) at a ratio of 1:2 (w/w) into the supernatant, followed by overnight incubation at room temperature. The precipitated pectin was washed three times with 75%, 85%, and 98% (v/v) ethanol to eliminate soluble impurities. The resulting pellet was then subjected to freeze-drying using the Free Zone 2.5 L Benchtop Freeze Dry System (Laconica, Kansas City, MO, USA) until a constant weight was achieved. (Nguyen et al., 2019).

3. RESULTS AND DISCUSSION

As mentioned in the (Table 1) the total antioxidant activity by the method of DPPH of papaya seeds is shown more as 180 compared to mango, peel of papaya shown more range as 125 than mango, leaves of papaya is higher than mango. By the method of FRAP (Fe^{2+}/g) seeds of mango shown as 118.5 which is more than papaya seed (105.5), Mango peel shown as 245.5 which is more than papaya and mango leaves with 426.7 is shown more than papaya leaves. By ABTS method (GAE/g) seeds of mango (144.5) shown slightly more than papaya seeds (123.7), peel of mango is in the range of 230.5 more than papaya, leaves of papaya is higher than mango. By radical cation inhibition method (mg/100g) mango by-products are higher than papaya per 100g of the dried sample. Mango seeds shown high levels (15.6) of vitamin c content compared to papaya whereas peel and leaves of mango shown low levels of vitamin c content than papaya.

ASSAY TYPE		SEEDS		PEEL		LEAVES	
		Papaya	Mango	Papaya	Mango	Papaya	Mango
DPPH inhibition/ppm)	(%	180	120	125	85	64	42
FRAP (Fe ²⁺ /g)		105.5	118.5	215.2	245.5	350.5	426.7
ABTS (GAE/g)		123.7	144.5	204.6	230.5	285.5	338.2
RadicalCInhibition (mg/100)	Cation g)	10.5	11.8	19.3	25.4	31.6	36.2
Vitamin C (mg/100)g)	9.8	15.6	62.8	23	30.2	25

Table 1: Antioxidant	activity of Seed	Peel. Leaves of F	apava and Mango
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In a study by Ang et al (2012), the DPPH, FRAP, ABTS assays of papaya peel showed as 19.70 µg TE/mL and papaya seed was in the range of 16.05 µg TE/mL which is comparably lower than the current study. In a study conducted by Yap et al., (2020) on papaya leaves, DPPH and ABTS activities shown as 215 and 571 which are said to be higher compared to above results. In a study, papaya seed waste by Saha et al (2023) Raw seed extract of Carica papaya showed vitamin c content as 19.35mg/100g which is higher than the current values. In a study, evaluation of nutritional components of *Carica papaya* L. at different stages of ripening conducted by Chukwuka et al., (2013) vitamin c content at ripens stage notes as 65.70mg/100g which is slightly higher than the

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current values of papaya peels. In a study by Fowomola et al (2010) Vitamin c content of mango seed is 0.56mg/100g which is lowest than the current values.

S.	PARAMETERS	SEEDS		PEEL		LEAVES	
No		Papaya	Mango	Papaya	Mango	Papaya	Mango
1.	Total Polyphenols (GAE/g)	7.31	118.4	13.05	55.31	15.30	19.04
2.	Beta carotene (IU)	280.72	84.25	493.51	98.63	2010.34	2345.62
3.	Pectin (%)	2.05	2.15	8.94	12.45	3.58	4.25
4.	Tannins (Abundance)	+	++	++	++	+	++

Table 2: Phytochemical analysis of Seed, Peel, Leaves of Papaya and Mango

Note- Abundance means: +++More in number, ++ Medium in number, +Less in number

As mentioned in the above the total polyphenol activity by the method of Folin-Ciocalteu method is higher in mango samples (seed, peel, leaves) and less in papaya. Beta carotene (IU) is more in mango samples compared to papaya. Pectin content (%) of seeds of papaya and mango are slightly similar, peel of mango is higher as 12.45% than papaya and leaves of mango with 4025% and lower in papaya with 3.58%. Tannin content is more in papaya peel compared to seed and leaves whereas in mango seed, peel and leaves it is observed as medium in abundance.

In a study of antioxidant potential of papaya seed and peel by Ang et al (2012), the total polyphenol content of papaya peel has 15.18 GAE/g which is higher compared to current study and papaya seed has 6.75 GAE/g which is lower than the above TPC values. In a study by Rojas et al (2020) dried mango peels have 72.61mg/g of pectin content whereas in a current study 12.45% of pectin is present which is considered lowest. Ranganath et al., (2018) analyzed the carotenoid compositions of different-colored mango peel at different phases of ripening, and identified highest β -carotene concentration (13.01 µg/g FW) in one of the mango varieties (yellow-colored Arka Anmol), which is shown as lowest compared with the current study.

CONCLUSION

This study highlights the estimation of total antioxidant activity, phytochemicals like polyphenols, tannins, pectin, beta carotene and nutrients like vitamin c of dried samples of papaya (*Carica papaya*) and mango (*Mangifera Indica*) by-products like seed, peel and leaves. Tannins are abundantly present in papaya peel and mango by-products which helps in long-term inflammation, wound healing and protects from carcinogens. Papaya leaves are known to have associated in treating dengue fever. These leaves in the form of juice of the leaf extract is consumed orally which is found to have increased platelet count and the speedy recovery. Using the mango seed powder gastro intestinal problems like diarrhea, dysentery and gums can be cured. Overall, the study signifies the importance of the by-products (seed, peel, leaves) of papaya and mango which are often considered as the waste products.

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