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Research Article

Comparison of Phytochemical Chemical Constituents of Papaya Fruit and Leaves

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ABSTRACT
Papaya *(Carica papaya Linn.)* is commonly known for its food and nutritional values throughout the world. The medicinal properties of papaya fruit and other parts of the plant are also well known in traditional systems of medicine. During the last few decades considerable progress has been achieved regarding the biological activity and medicinal application of papaya and now it is considered as valuable nutraceutical fruit plant. In the present paper we investigate the nutritional value of different parts of papaya fruit and leaves, mineral composition, and quantification of phytochemical constituents such as polyphenols, tannins, alkaloids, saponins and flavanoids using the conventional methods.
Keywords: Pawpaw, Carica papaya, Phytochemicals,Triterpenoids, Total polyphenols

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INTRODUCTION
Papaya, *Carica papaya Linnaeus*, (pawpaw), belongs to the family of Caricaceae. It is a tropical plant originally from America and is a large perennial herb with a rapid growth rate. The plants are usually short-lived, but can produce fruits for more than 20years in some cases. The papaya has a rather complicated means of reproduction. The plants are male, hermaphrodite, or female (1). The male trees are uncommon, but sometimes occur when the planters collect their own seeds. Hermaphrodite trees (flowers with male and female parts) are the commercial standard, producing a pear shaped fruit. These plants are self pollinated (2).Usually these trees are grown for fruits and latex. The fruits possess great taste as well as health benefits. Papaya enjoys a rich tradition for using in day to day life both as foods and medicines. It is known as “ Tree of Life” by the people of Mayan civilization and they workship these trees. *Carica papaya* plants produce natural compounds (annonaceous acetogenins) in leaf bark and twig tissues that possess both highly anti-tumour and pesticidal properties. The leaves,seeds and the latex of the papaya are used to cure digestive problems. Papaya contains the protein digesting enzyme papain which stimulates natural digestion. It was suggested that a potentially lucrative industry based simply on production of plant biomass could develop for production of anti-cancer drugs, (pending Food and Drug Agency approval) and natural (botanical) pesticides (3). The high level of natural self-defence compounds in the tree makes it highly resistant to insect and disease infestation (4). They observed the leaf extract increases the platelet count. The latex, ripe fruits, unripe fruits, seeds, seeds juice, root, leaves, flower and stem bark of *C. papaya* are used as antimicrobial, anthelmentic, antimalarial, antifungal, anti-amoebic, hepatoprotective, male and female antifertility, immunomodulatory and against histminergic(5). Researchers found that juice obtained from the tender leaves of papaya cures

Dengue fever and scientific report shows as activity for the same (6). This study focused on the evaluation of phytochemical constituents in the fruit and leaves of papaya.

MATERIALS

Collection of plant materials

Fresh, unripe and ripe fruits and mature as well as tender leaves of Carica papaya were obtained from a farmhouse near Angamaly, Cochin, Kerala. Different parts of the fruits like rind, flesh and seeds were separated and used for extraction.

Chemicals

Acetic acid, ammonium hydroxide, sodium carbonate, aluminium chloride, sodium hydroxide, copper sulphate reagent, anthrone reagent, nitric acid , methanol, petroleum ether, acetone, hexane (all the chemicals and solvents were purchased from E Merck Limited ,Mumbai, India). Gallic acid, quercetin, tannic acid, bovine serum albumin and D-glucose, were purchased from Sigma Aldrich Ltd (St Louis, USA).

METHODS

Standardised methods were adopted for the estimations of the phytochemical quantification, nutritional values and mineral composition of Carica papaya leaves (mature and tender) as well as flesh, rind and seeds of ripe and unripe fruits.

Preparation of Plant Extracts

Crude plant extracts were extracted by Soxhlet extraction method (7). About 200 gms of plant material was uniformly packed into a thimble and extracted with 750 ml of methanol as a solvent. The process of extraction continues for 6 hours till the solvent in siphon tube of an extractor became colourless. The solvent extract was filtered and dried under vacccum at 80^{o}C to get the dried extract and refrigerated at 4 ^{o}C. The process was repeated using other plant parts and the crude extracts were used for phytochemical analysis.

PHYTOCHEMICAL ANALYSIS

Phytochemical quantification for alkaloids, tannins, saponins, flavanoides and total polyphenols were carried out according to standard methods.

Determination of Alkaloids

Alkaloid content was determined by harborne method(8). 1g dried extract was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added , covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue obtained was the alkaloid, which was dried and weighed. The experiment was repeated to get concordant values.

Determination of Total Phenolics

The amount of total phenolics in the crude extracts of papaya samples were determined with the Folin-Ciocalteu reagent method(9). 2 ml of Folin-Ciocalteu reagent and 10 ml of sodium carbonate (Na_2CO_3, 30% w/v) were added to 2 ml of the plant extract solution (1 mg/ml). The resulting mixture was incubated in hot water for 5 min. The absorbance of the sample was measured at 700 nm using a UV-visible spectrophotometer (Chemito Spectroscan UV2600). The total phenolics were expressed as mg/g of gallic acid equivalents (GAE). The linear standard graph of gallic acid (GA) was prepared by plotting the absorbance of different concentrations of GA (0-10 mg/ml) prepared in methanol along the X-axis and absorbance along the Y-axis.

Determination of Total Flavonoids

This method is based on the formation of the flavonoids - aluminium complex which has an absorption maximum at 510nm (10). 100µl of the sample in methanol (10 mg/ml) was added to 300µl of distilled water followed by 30µl 5% sodium nitrite ($NaNO_2$) . After keeping 5 min at 25°C, 30µl of 10% aluminium chloride (Al_2Cl_3)was added and kept for 5 min. The reaction mixture was treated with 200µl of 1 mM sodium hydroxide (NaOH). Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. Blank samples were prepared from 100 µl of the plant extracts and 300µl of distilled water followed by

30µl 5% $NaNO_2$, and then diluted to 1ml with water. The linear standard graph of quercetin was prepared by plotting the absorbance of different concentrations of quercetin (0-5 mg/ml) prepared in methanol along the X-axis and absorbance along the Y-axis. The estimations were carried out in triplicates . The results were expressed as mg quercetin (QE)/g extract.

Determination of Tannin

Tannin content was determined by the Folins-Denis colorimetric method(11). 1g crude methanol extract of papaya sample was dispersed in 50 ml of distilled water and shaked well. The mixture was allowed to stand for 30 min at 28^0C and filtered through whatman No. 42. 2 ml of the above filtrate was added to a 50 ml volumetric flask. Similarly 2 ml standard tannin solution (tannic acid) and 2 ml of distilled water were put in separate volumetric flasks to serve as standard and blank. Then 2ml of Folin-Ciocalteu reagent and 2.5 ml of saturated sodium carbonate ($Na_2CO_{3)}$ solution were added to each flask. This solution was made upto 50 ml with distilled water and allowed to incubate at 28^0C for 30 min. Their respective absorbance was measured using UV-visible spectrophotometer at 760 nm using the reagent blank to calibrate the instrument at zero.

Determination of Saponin Content

The saponin content was estimated by gravimetric method(12). Weighed out 1g of dried methanol extract of the papaya sample into a 250 ml of RB flask and refluxed with 90% meOH thrice for half an hour. The combined extracts were filtered and de-solventised, then it was treated with 25 ml of petroleum ether (60 –80^0) for half an hour. Cooled and the solvent was removed by decantation.The soft extract was dissolved in 25 ml of 90% v/v methanol. The sample was filtered and concentrated to 5 ml. 25 ml of acetone was added drop by drop with constant stirring, in order to precipitate the saponins. The precipitate was filtered through a pre weighed sintered crucible and dried at a constant weight at 105^0.

Determination of Fat Content

The petroleum ether soluble extractives(13) were prepared by refluxing 2 g of dried extracts of the papaya sample for 1 hour using 50ml of petroleum ether at 60^{o}C. Repeated the process for three times till the extraction was complete. All the petroleum ether washes were collected and concentrated to dryness under vaccum and weighed. The experiment was repeated using other tissue samples. The fat content was estimated by the equation:

$$\text{Total fat content(\%)} = \frac{\text{weight of the petroleum ether extract obtained x 100}}{\text{weight of the plant extract taken}}$$

Determination of Protein

Protein concentration in the samples was determined by Lowry's method (14). 500 mg of dried extracts of papaya samples were dissolved in 0.1N NaOH. Different dilutions of bovine serum albumin (BSA) solutions were prepared by mixing stock BSA solution (1 mg/ ml) with water (0.05 to 1 mg/ ml). Pipetted out 0.2 ml standard solution from each other and 0.2 ml of sample solution to different test tubes and added 2.5 ml of alkaline copper sulphate reagent (analytical reagent). The solutions were mixed well and incubated at room temperature for 15 mins. Then 0.25 ml of Folin Ciocalteau solution (reagent solution) was added to each tube and incubated for 30 min. The absorbance was measured at 660 nm. Protein concentration of sample was determined from the calibration curve by plotting the absorbance of different concentrations of BSA along the X-axis and absorbance along the Y-axis.

Determination of Carbohydrate

The carbohydrate content was determined using anthrone method (15). 50 mg of dried extract of papaya sample was dissolved in 100 ml water. Different dilutions of D-glucose (1 mg/ml) were prepared. Pipetted out 0.2 ml of the sample solution into a test tube and made upto 1 ml using distilled water. Then added 4 ml anthrone reagent and incubated in hot water at 60^o C for 10 minutes. Cooled and absorbance was measured at 620 nm and carbohydrate concentration of sample was determined from the calibration curve by plotting the absorbance of different concentrations of standard along the X-axis and absorbance along the Y-axis.

Mineral Composition

The composition of minerals represents the quantity of organic and inorganic metals present in the samples. The minerals composition determined were sodium, calcium iron, phosphorus, potassium, zinc, copper, magnesium, manganese, cobalt, cadmium, and lead. 0.1 gm of the samples [dried extract of papaya leaf (mature and tender) , rind, seed and flesh of ripe and unripe papaya fruit] were digested with 100ml of 2% nitric acid (HNO_3) in a microwave digestion system for 1hr and cooled it for half an hour and filtered into a 100 ml standard flask. It was made up to the mark with deionized water. 5ml of the sample was injected and the minerals were determined using ICPMS (X-series thermofisher).

RESULTS AND DISCUSSION

Recently papaya leaf juice is being used by patients affected by dengue fever. It was an exciting fact that even doctors advised people to drink papaya leaf juice as a herbal remedy to recover from this type of fever in which the patients platelet level became extremely low. This made us to study the phytochemical profile of different parts of papaya.

Different grades and different parts of fruits and leaves were extracted with methanol and the percentage yield obtained was shown in Table 1. The nutritional values obtained from the phytochemical studies of leaves, ripe and unripe fruits were described in Table 2. All the values obtained are the average of three repeatable analysis. Table 1 shows that the flesh of ripe papaya has the highest yield and the unripe rind has the lowest. Table 2 shows that all the samples were having moisture greater than 80%. Also from the Table 2, it is clear that the flesh of ripe papaya is rich in carbohydrate content and the rind of unripe papaya has the lowest. All the parts of papaya has the protein percentage ranging 1-3 percentage except the flesh of unripe papaya which is less than 0.2 percentage. Fat content in all the samples were ranging 0.08-1.5 percentage.

Sr no	Raw material	Percentage yield (g/100 g raw material)
1	Mature leaf	5.23
2	Immature leaf	3.59
3	Ripe flesh	12.67
4	Unripe flesh	3.86
5	Ripe seed	4.69
6	Unripe seed	4.85
7	Ripe rind	6.38
8	Unripe rind	3.39

Table 1: Percentage yield of methanolic extract of papaya leaves and fruit

The result of the mineral composition is shown in Table 3 clearly indicated that *Carica papaya* leaves and fruits are rich source of mineral elements. The percentage of potassium, magnesium and sodium is high in fruits and leaves (Table 3). The percentage of pottassium is exceptionally high in papaya leaves.

This result becomes so important when the presence of minerals like Ca, Mg, Na, K, Fe and Mn in the *Carica papaya* leaves indicates the usefulness of the leaves in the coagulation of blood, the proper functioning of the heart and nervous system and the normal contraction of muscles. Magnesium, assist in the assimilation of phosphorus. Lack of magnesium can be responsible for tetany, tuberculosis, diabetes, cancer and all nervous diseases (16). Maybe this is the reason why pawpaw leaves especially the green ones are being employed in the treatment of the above listed diseases (17). Potassium is necessary for muscular weakness which is associated with malaria, and also slows down sclerosis of the vascular system. It contributes to the fight against bacteria and cleanses the digestive system. Sodium takes part in the metabolism of water, promotes digestion, assimilation, osmosis, cleanses the digestive system, combats stomach acidity and alkalize the blood (16). This is the reason behind the brown pawpaw leaf being used as a cleanser in herbal remedy (17). The presence of Iron signifies that the leaves can be used against anaemia, tuberculosis and disorder of growth (16). Manganese, according to Claude and Paule, (16) is necessary for the functioning of the pituitary gland, the pineal gland and the brain. It promotes hepato-renal function, combat anaemia and it is also essential for growth.

Phytochemical analysis were done to find out the percentage of polyphenols, alkaloids, tannins ,flavanoids and saponins in *carica papaya* leaves (mature and immature) and fruit (ripe and unripe). In the case of leaves, the results shows (Table 4) that the mature leaves have higher percentage of flavanoids than the immature leaves. But, the percentage of tannins, alkaloids and saponins are almost same in both mature and immature leaves (Table 4). Details of phytochemical contents in different parts of ripe and unripe fruit is shown in Table 5. Flesh and seeds of unripe fruit of papaya has less percentage of polyphenols (0.06%) whereas rind has higher percentage (0.13%). But the flesh, seed and rind of unripe papaya has total polyphenol percentage ranging 0.1 -0.2 percentage. Also different parts of ripe fruit has higher percentage of tannins than in the unripe fruit. But , the percentage of flavanoides are high in different parts of unripe papaya than in the ripe one. Interestingly, percentage of saponins is higher in the flesh and rind of ripe fruit .But the seeds of both ripe and unripe fruit has almost the same percentage of saponins (Table 5) . The presence of saponins supports the fact that pawpaw leaf and fruit has cytotoxic effects such as permealization of the intestine as saponins are cytotoxic, (18) . It also gives the bitter taste to the leaves. Seeds and rind of papaya fruit is rich in phytochemical constituents than flesh .

Alkaloid content is higher in flesh and seed of ripe papaya fruit and leaves which is shown in Table 5. Also seeds of ripe and unripe fruit has same percentage of alkaloids. Alkaloids are the most efficient therapeutically significant plant substance. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties (19). The presence of alkaloids in the leaves shows that these plants can be effective anti-malaria, since alkaloids consist of quinine, which is an anti-malarial agent (20).

Sr No	Parameter	Fresh Ripe fruit (g/100 g raw material)			Fresh Unripe fruit (g/100 g raw material)			Fresh Leaves (g/100 g raw material)
		Seed	Flesh	Rind	Seed	Flesh	Rind	
1	Moisture	83	80	82.5	88.31	89.86	92.16	88
2	Carbohydrate	1.42	7.6	1.65	0.28	0.55	0.22	2.14
3	Protein	1.32	2.9	2.43	1.83	0.16	2.13	1.12
4	Fat	1.42	1.45	1.36	1.02	1.1	0.08	0.87

Table 2: Nutritional Value of different parts of papaya fruit (g/100 g sample)

Sl. No		Leaf (mg/100 g raw material)	Fruit (g/100 g raw material)
1	Li	ND*	ND*
2	Be	ND*	ND*
3	Na	32	0.047
4	Mg	270	0.27
5	K	9660	6.12
6	Ca	10	0.26
7	Ti	0.4	0.0012
8	Mn	3	0.0004
9	Fe	1.6	0.0010
10	Cu	0.6	0.0058
11	Zn	5.3	0.0037

Table 3: Mineral composition of unripe fruit and leaves of Carica papaya

Sr No		Mature Leaf (g/100 g)	Immature leaf (g/100 g)
1	Polyphenols(GAE)	0.47	0.26
2	Tannis (TAE)	0.18	0.2
3	Flavanoids (QE)	0.73	0.48
4	Alkaloids	0.22	0.27
5	Saponins	0.55	0.56

Table 4: Phytochemical contents in papaya leaves (g/100g) *GAE- gallic acid equivalent, *TAE – Tannic acid equivalent, *QE – Quercetin equivalent

Sr No	Parameter	Ripe fruit (g/ 100 g raw material)			Unripe fruit (g/ 100g raw material)		
		Flesh	Seed	Rind	Flesh	Seed	Rind
1	Polyphenols (GAE)	0.1	0.14	0.2	0.06	0.06	0.13
2	Tannins (TAE)	0.67	0.18	0.34	0.09	0.13	0.18
3	Flavanoids (QE)	0.10	0.59	0.27	0.23	0.68	0.55
4	Saponins	1.07	0.68	1.48	0.38	0.60	0.51
5	Alkaloids	0.69	0.40	0.47	0.002	0.10	0.18

Table 5: Phytochemical Contents In Papaya Ripe And Unripe Fruit (g /100 g raw material) *GAE- gallic acid equivalent, *TAE – Tannic acid equivalent, *QE – Quercetin equivalent

CONCLUSION

This study has shown the phytochemicals, nutritional values and mineral composition of mature and immature *Carica papaya* leaves and ripe and unripe fruit. This partly shows the use of this plant in herbal medicine, as a rich source of phytochemicals, coupled with the presence of the essential minerals. Papaya leaves can be seen as a potential source of useful food and drug items. The presence of alkaloids in them explains the reason why it is being effectively used as an anti-malaria agent(17).

According to the book 'Nature cure for cancer' there are many reports that cancer sufferers have been healed by drinking papaya leaf concentrate(21). According to Dr.Jerry McLaughlin of the University of Purdue(22), he has found a chemical component in the papaya tree that is "one million times stronger than the strongest anti-cancer medicine"(23). Scientific studies are to be focussed in this area for the preventive and corrective activity on inflammatory cells

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