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Impact of pre-treatment and drying method on the bioactive compounds in papaya leaf

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Abstract
The fruits, leaves, seeds, and latex of the herbaceous plant Carica papaya are all used as medicines. The biological activity and medical uses of papaya have advanced significantly over the past few decades, and the fruit is increasingly valued for its nutraceutical properties. The leaves of papaya contain several active components and presence of these compounds makes papaya leaf an abundant source of health benefits. Due to the local availability and low cost of the leaf and understanding the therapeutic potential, people have started consuming papaya leaves by several thermal processes water boiled with papaya leaf, papaya leaf juice etc. Hence the study was carried out to find out the impact on bioactive compounds after pre- treatment and drying method in papaya leaves. In the present study the papaya leaves were dried using different pre-treatments like plain tray drying, Immersing in the hot water before drying, steaming before drying and shade drying. The various bioactive compounds analyzed are polyphenols, carotenoids, flavanoids, tannins, alkaloids, saponins and total antioxidants. The results indicates that hot water immersed tray dried leaf powder had the highest content of polyphenols-135.42 mg/g, carotenoids-0.092 mg/g and tannins-82.95 mg/g. In case of flavanoids- 67.24 µg/mg, saponins-33.11% and total antioxidants-211.78 µg/mg steamed-tray dried leaf powder showed a positive effect. It was observed that the alkaloid-15.12% content was highest in plain dried leaf powder. Shade dried leaf powder possessed lowest values in all the bioactive compounds. In conclusion, it's been observed that pre-treatment and different drying methods have positive effect on the bioactive compounds of papaya leaves.

Keywords: Red lady, thermal treatments, sun drying, bioactive compounds, positive effect

Introduction
Papaya is a green tree with a delicate stem that resembles like a palm. It is originally from the tropics of the Americas, the Carica papaya is currently grown in tropical and warm, semi-tropical regions all over the world (Nwamarah *et. al*, 2019) [29]. The papaya (*Carica papaya* L.) is a significant fruit crop that is widely grown and consumed, both for its tasty flavour and its many medicinal benefits (Lim, 2012) [24]. Additionally, it is grown in the Antilles, tropical Africa, India, Sri Lanka, and other Asian nations. This species is typical of tropical and subtropical locations, needs temperatures between 21 and 33 °C, and cannot survive in cold weather (less than 15 °C) (Chan & Paull, 2008)[9]. Among the 57 nations that are located in tropical and subtropical climates, India is one of the top producers of papaya. India has produced a variety of papayas, including the Arka Surya, Arka Prabhath, Pusa Majesty, Pusa Delecious, Pusa Dwarf, Pusa Nanha, Coorg Honey Dew, Solo, Red Lady, Ranchi, CO_2, CO_5, CO_7, CO_6, and Barwani. The flesh hue, skin tone, and size of each of these kinds varies (Gorane, *et al.*, 2018) [15].

Papaya is a year-round fruit that is a nutritional poweplant (Herbst, 2001) [16]. The papaya's leaves are spirally arranged in a terminal cluster, simple, and on petioles that are 30 to 70 cm long. The leaves are 60 to 90 cm in diameter, alternately arranged, dark green to yellow-green, bright, and visibly marked by off-white nerves embedded and reticulated veins; the underside surface is pale green-yellow and opaque, with visibly prominent vascular structures. Each leaf has a lifespan of 4 to 6 months (Afzan *et al.*, 2012) [1]. In addition to being low in calories and rich in various vitamins and minerals, papaya leaves also contain an enzyme that can be used to tenderise meat and alleviate indigestion (Herbst, 2001) [16]. Carpaine, pseudocarpaine, and the dehydrocarpaine I and II are only a few of the alkaloids found in papaya leaves that have significant medical and industrial applications (Ranasinghe *et al.*, 2012) [34]. There are numerous phenolic compounds found in papaya leaves, including protocatechuic acid, p-coumaric acid, caffeic acid, chlorogenic acid, 5,7-dimethoxycoumarin, kaempferol, and quercetin.

The leaves also contain active substances like papain, chymopapain cysteine, ascorbic acid, flavonoids, and cynogenic glucosides, which boost the blood's overall antioxidant capacity (Julianti *et al.*, 2014) [20]. Alkaloids, saponin, tannin, flavonoids, anthraquinones (free and bound), phlobatannin, cardiac glycosides, terpenoids, and proanthocyanidin are among the phytochemical substances which id identified in papaya leaves (Nugroho *et al.*, 2017) [28]. The processing loss must be minimized since papaya leaves contain a variety of bioactive chemicals that are quickly influenced by the procedures used to prepare them. Because it depends on the kinds of chemical compounds present and the kinds of plants, it is impossible to forecast how a particular drying technique would affect the preservation of raw quality (Lin *et al.*, 2012) [28] hence the study was conducted to find out the difference in bioactive compounds due to different pre- treatment and drying method in papaya leaves.

Materials and Methods

Selection and Collection of leaves

Red leady papaya variety was selected for the present study. The leaves were collected from plants which were not sprayed with any chemicals and the collected leaves were thoroughly washed with running tap water followed by rinsing in deionised water to obtain clean leaves (Fadzilah *et al.*, 2020) [11]. The leaves which are used in the present study were obtained from College of Agriculture, Vellayani.

Drying of leaves

The leaves were dried on four treatments as depicted in the Table 1.

Table 1: Drying treatments

SL. No.	Treatments	Methods
1.	D1	Drying in tray drier at 55-60°C for 6 hours.
2.	D2	Steaming at 90 °C for 3 minutes followed by drying in tray drier at 55-60 °C for 6 hours
3.	D3	Immersing in hot water at 85 °C for 2 minutes followed by drying in tray drier 55 [-60 °C for 6 hours
4.	D4	Shade drying for 3 days

(Giao *et al.*, 2009) [14]

The leaves were dried until 7% of moisture was attained.

Bioactive Compounds in dried papaya leaf powder

For extraction of sample, 25 g of papaya leaf powder from each drying methods were taken and dissolved in 250 ml of ethanol and left it for 24 hours. The suspension was filtered with filter paper through a Whatman No. 41 the filtrate was concentrated in a rotary evaporator at 45 °C under reduced pressure. The extracted sample was used for the quantitative estimation of bioactive compounds including polyphenols, carotenoids, flavanoids, tannins, alkaloids, saponins and total antioxidants using standard method with slight modifications.

1. Polyphenols: The total amount of polyphenol content was measured using the Folin-Ciocalteu technique. One ml of the sample (250 l) was made up by adding 1.25 ml of the Folin-Ciocalteu reagent (0.2 N diluted in MeOH). A reagent blank was created using MeOH in place of the sample. A 75 g/L sodium carbonate solution was added to 1 ml of the mixture after 5 min of room temperature incubation. Samples were incubated for two hours at room temperature, and the absorbance at 765 nm was measured. Total phenol concentration was determined by conducting each test three times, and it was represented as g of gallic acid equivalents (GAE) per 100 g of extract. (Ondo *et al.*, 2013) [32].

2. Carotenoids: Weigh 1.25 g of the sample and homogenise it with acetone to extract the carotenoids. Subsequent additions of 12.5 ml of acetone were made to create a paste, which was then transferred into a sintered funnel (5 m) connected to a 250 ml Buchner flask and filtered under vacuum to determine the total amount of carotenoids. Three times through this process were carried out, or until the material was colourless. The resulting extract was transferred to a separatory funnel with a capacity of 500 ml and 20 ml of petroleum ether. To avoid the formation of an emulsion, the acetone was eliminated by gradually adding ultrapure water (Milli-Q-Millipore). There was no need for the aqueous phase. The process was carried out four times until no more solvent was left behind. The extract was then poured into a 50 ml volumetric flask containing 1.25g of anhydrous sodium sulphate through a funnel. Petroleum ether served as the volume's filler, and samples were read at 450 nm (Carvalho *et al.*, 2012) [6]. The following formula was used to get the total amount of carotenoids:

$$\text{Carotenoids contents } (\mu g/g) = \frac{A \times V(ml) \times 104}{A1cm\% \times P\ (g)}$$

Where A = Absorbance, V = Total extract volume (10 ml), P = Sample weight (1.25 g)

A1 cm1% = 2592 (β-carotene Extinction Coefficient in petroleum ether).

3. Flavanoids: The Aluminium chloride colorimetric assay was used to determine the total flavanoids concentration. In a 10 ml volumetric flask, 1 mg of the extract and 4 ml of distilled water were combined to create the reaction mixture. After five minutes, 0.3 ml of 10% Aluminium chloride was added to the flask along with 0.30 ml of 0.5% sodium nitrite. 2 ml of 1M sodium hydroxide was treated for 5 minutes before being diluted with distilled water to make 10 ml. In the same way as previously described, a set of reference standard solutions of quercetin (20, 40, 60, 80, and 100 g/ml) were made. Using a UV/visible spectrophotometer, the absorbance of the test and standard solutions was measured against the reagent blank at 510 nm. (Lee *et al.*, 2012) [23]. The amount of flavanoids in total was calculated as g of QE per milligrams of extract.

4. Tannins: The Folin-Ciocalteu method was used to determine the sample's tannin content. The measurement of the blue colour produced when tannin-like substances reduce phosphotungsto molybdic acid in an alkaline media is the basis for colorimetric tannin estimation. Tannic acid standard solution (20-100 g) and one mg/1 ml of extract were combined to make 7.5 ml of distilled water. Following that, 1mL of a 35% sodium carbonate solution and 0.5mL of the Folin-Ciocalteu reagent were added. With distilled water, the volume was increased to 10 ml, and the absorbance was determined at 700 nm. (Indira, 2016) [17].

5. Alkaloids: 1 mg of the plant extract (1 mg of DMSO) was diluted in 1 ml of 2N HCl before being filtered. This solution

was transferred to a separating funnel, and then 5 ml of phosphate buffer and 5 ml of bromocresol green solution were added. The mixture was vigorously agitated with 1, 2, 3, and 4 ml chloroform before being collected in a 10 ml volumetric flask and chloroform was added to dilute it to that volume. In the same way as previously described, a series of reference standard solutions of atropine (20, 40, 60, 80, and 100 g) were made. Using a UV/visible spectrophotometer, the absorbance of the test and standard solutions was measured against the reagent blank at 470 nm. (Fazel *et al.*, 2008) [12].

6. Saponins: The total saponins content was analyzed by the vanillin-sulpuric acid assay. 72% (v/v) sulphuric acid was put in a shaking water bath for 15 min at 60 °C and 2.5ml was taken and it was incubated with 1mg/ml of plant sample extracts, standards or reagent blank with 0.25 ml of 0.8% (w/v) vanillin in ethanol. Diosgenin was used as the standard and the reagent blank made up with the solvent used for extracting the plant samples (extraction solvent). The absorbance of the standards and extracts is measured at 544 nm using a UV-VIS spectrophotometer after chilling in water at room temperature for 5 minutes (Chen *et al.*, 2010) [10].

7. Total antioxidants: The phospho-molybdenum technique, as described by (Prieto *et al.*, 1999) [33], 3 ml of the reagent solution (0.6 M sulfuric acid, 28 mm sodium phosphate, and 4 mm ammonium molybdate) were mixed with 1 mg/ml of the extract. Ascorbic acid was employed as the standard, and 3ml of the reagent solution and 1ml of the standard at various concentrations were taken. The reaction solution-filled tubes underwent a 90-minute incubation period at 95 °C. After cooling to room temperature, the solution's absorbance at 695 nm was measured using a UV-VIS spectrophotometer against a blank. The quantity of grammes of ascorbic acid equivalent is used to indicate the total antioxidant activity. The calibration curve was created by combining methanol with ascorbic acid.

Statistical Analysis

The main tools achieved to calculate the significant difference in the treatment means were one way analyses of variance (ANOVA).

Result and Discussion

A quantitative study was done to find out the bioactive compounds in red lady papaya leaves and the results (Table 2) revealed that the leaves contain several bioactive compounds like polyphenols, carotenoids, Flavanoids, tannins, alkaloids, saponins and total antioxidants.

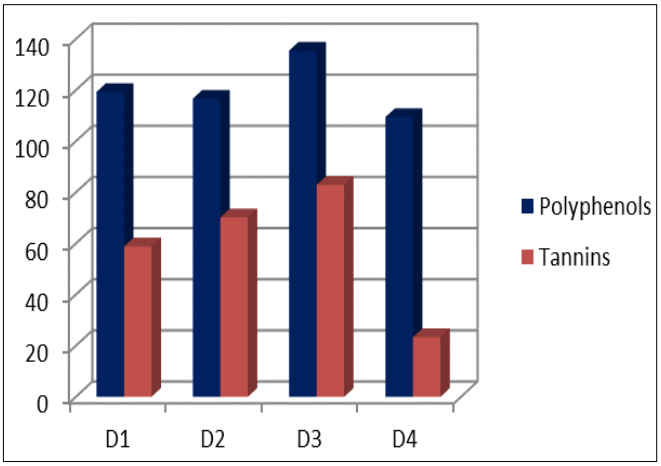
Table 2: Bioactive compounds of papaya leaf powder in different treatments

Treatments	D1	D2	D3	D4	SE(m)	CD
Polyphenols	119.25^{b}	116.62^{c}	135.42^{a}	109.42^{d}	0.081	0.249
Carotenoids	0.091 ^{ab}	0.071^{c}	0.092 ^{a}	0.089 ^{b}	0.001	0.003
Flavanoids	44.56 ^{b}	67.24 ^{a}	33.59 ^{c}	8.46 ^{d}	0.013	0.040
Tannins	58.83 ^{c}	70.20 ^{b}	82.95 ^{a}	23.34 ^{d}	0.011	0.033
Alkaloids	15.12 ^{a}	14.66 ^{b}	12.66 ^{c}	11.21^{d}	0.009	0.028
Saponins	22.82^{c}	33.11^{a}	28.31^{b}	17.60^{d}	0.009	0.029
Total antioxidants	190.52 ^{b}	211.78^{a}	175.36^{c}	77.15^{d}	0.009	0.028

In case of polyphenols among all the treatment D3 (hot water immersed tray dried leaf powder) has the highest value 135.42 mg/g and D4 (Shade Dried Leaf Powder) 109.42 mg/g has the lowest value (Figure 1). Akila *et al.* (2018) [5] have reported that hot water blanched guava leaf has high amount 109.5 mg GAE/g) total phenolic content than other treatments. As a result of heat treatment the tannins in the leaves degenerated into simple phenolics compounds that could be the reason for the substantially higher phenol content of dry papaya leaves than the fresh (Olumide *et al.*, 2022) [31]. According to Maiani *et al.* (2009) [26] heat processing can alter the structure of food matrices which can result in the increase of extractability and bioavailability of carotenoids. Ferracane *et al* (2008) [13] also reported that when using hydrothermal processes like blanching, carotenoids can be extracted more efficiently because the cellulose structure is broken down and non-covalent protein-carotenoids interactions are thermally disrupted, which is concorded with the result of present study in which carotenoids content (figure 2) was highest in D3 (0.092 mg/g) followed by D1 (0.091 mg/g). Agamou *et al.* (2015) [2] in her studies was shown that fresh moringa leaves have a moderate amount of carotenoids and the values showed an increase with heat treatment like blanching. Highest flavanoids content was noted in D2 (steamed- tray dried leaf powder) 67.24 µg/mg and lowest was noted in D4 (shade dried leaf powder) 8.46 µg/mg (figure 3). According to Agati*et al.* (2002) [3] Sun-drying caused water to evaporate more quickly, which stopped the metabolism of flavanoids and reduced the amount of flavanoids present. Total flavanoids content in leaf samples is mostly influenced by water and sunlight. Furthermore, flavanoids are heat-sensitive (Chaaban *et al.*, 2017) [7] this may be the reason for the over-all low flavanoids content of the papaya leaves especially in the case of shade dried papaya leaf powder. Similar studies conducted by Akila *et al.* (2018) [5] and Jia *et al.* (1999) [19] highest flavanoids content was seen in fresh leaves than the leaves which undergo thermal treatments.

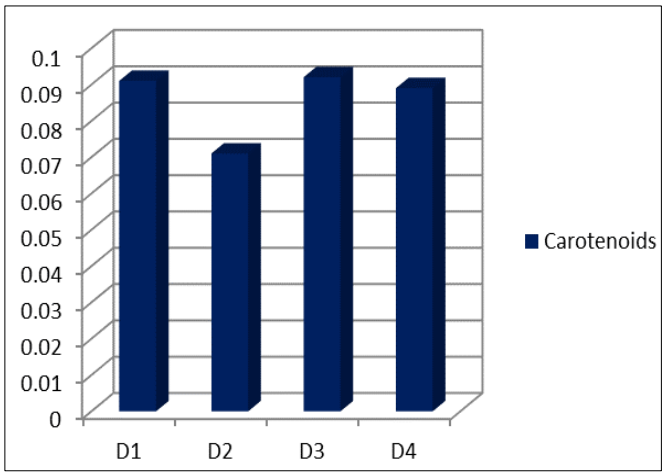
Among all the treatments D3 (hot water immersed tray dried leaf powder) has the highest amount of tannin content (82.95 mg/g) and it was significantly different ($p \leq 0.05$) from other treatments. Tannin was seen more in D2 & D3 which were pre-treated. Pre- treatments may help in reduction of oxidation rates of bioactive compounds and this may be the reason for the higher concentration of tannin content in treatments like D2 and D3. The result are supported by the study conducted by Irondi *et al.* (2013) [18] in papaya seed in which low tannin concentration was seen in oven dried and sun dried seeds than other treatments. Alkaloids are secondary metabolites that were found in papaya leaf extract and have the biological feature of being poisonous to foreign species' cells (Nobori*et al.*, 1994) [27]. In the present study the alkaloid content(figure 4) was observed highest to lowest from D1 (Plain dried leaf powder) > D2 (steamed- tray dried leaf powder) > D3 (hot water immersed tray dried leaf powder) > D4 (shade dried leaf powder) with the values 15.12% > 14.66% > 12.66 > 11.21% respectively. Their widely recognised therapeutic values may be due to the reported high alkaloid content. (Nwamarah *et al.*, 2019) [29]. In case of saponins D2 (steamed- tray dried leaf powder) has highest content i.e. 33.11% and the lowest content was observed in D4 (shade dried leaf powder) 17.60% (figure 4). The results of the study was concorded with a study conducted [By Kha *et al.* (2021) [21] with noni fruit powder it was observed that the saponins content was significantly affected by drying temperature and pre-treatments have significant effect on

retaining the saponins content. According to Ahmed & Langthasa (2022) [4] fresh drumstick leaves has highest amount of saponins and in case of drying, samples which had undergone pre-treatments showed higher retention of saponins content than the other treatments. Because saponins are cytotoxic, their presence in pawpaw leaf supports the notion that it has cytotoxic effects like permeating the intestine. (Okwu & Okwu, 2004) [30]. Similar result was seen in case of total antioxidants also, D2 (steamed- tray dried leaf powder) had the highest value (211.78 µg/mg) and lowest value (77.15 µg/mg) was recorded in D4 (shade dried leaf powder) which is represented in figure 3.The results of the present study was on par with the study conducted by Akila *et al.* (2018) [5] in guava leaves that were steam blanched, guava leaf herbal tea combination had higher antioxidant activity than guava leaf herbal tea that hadn't been blanched. Chan *et al.* (2012) [8] concluded that drying boosts the antioxidant property in the leaves of laurel clockvine (T. Laurifolia) and white mulberry (M. Alba). According to Kunyanga *et al.*(2012) [22] after blanching Moringa oleifera leaves for five minutes in boiling water, a rise in DPPH radical scavenging activity was seen.



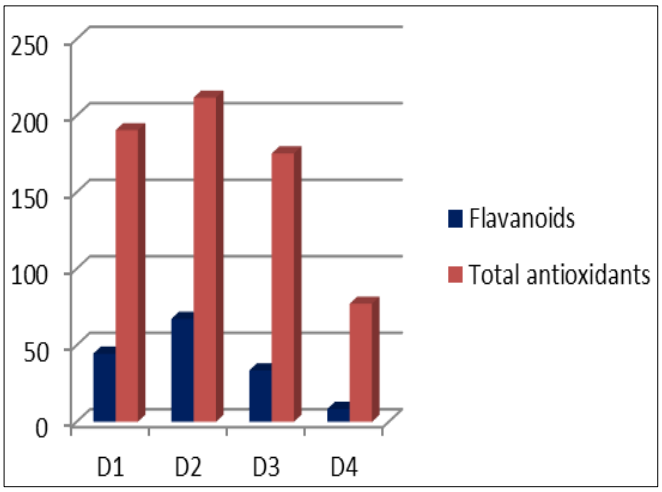
D1- Plain dried leaf powder, D2- steamed- tray dried leaf powder, D3- hot water immersed tray dried leaf powder and D4- shade dried leaf powder

Fig 1: Polyphenols and tannin content of papaya leaf powder in different treatments



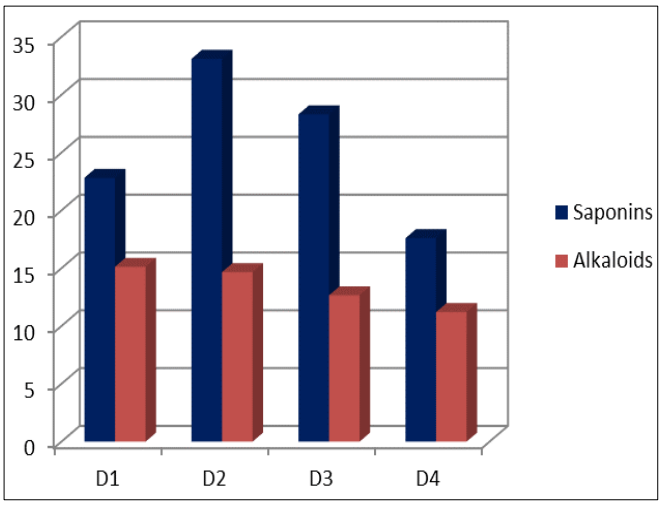
D1- Plain dried leaf powder, D2- steamed- tray dried leaf powder, D3 - hot water immersed tray dried leaf powder and D4- shade dried leaf powder.

Fig 2: Carotenoids content of papaya leaf powder in different treatments



D1- Plain dried leaf powder, D2- steamed- tray dried leaf powder, D3- hot water immersed tray dried leaf powder and D4- shade dried leaf powder

Fig 3: Flavanoids and total antioxidant content of papaya leaf powder in different treatments



D1- Plain dried leaf powder, D2- steamed- tray dried leaf powder, D3- hot water immersed tray dried leaf powder and D4- shade dried leaf powder.

Fig 4: Saponins and alkaloid content of papaya leaf powder in different treatments

Conclusion

The study concluded that pre-treatment and various drying techniques have significant effect on the bioactive compounds of papaya leaves. Immersing in the hot water before drying has a positive impact on the bioactive compounds like, polyphenols, carotenoids and tannin. In case of flavanoids, saponins and total antioxidants steaming before drying showed positive effect and simple tray drying was discovered to be the most suitable for alkaloids. Among all the treatments shade drying had lowest impact on all bioactive compounds. From the obtained results of the study, it was evident that appropriate pre-treatments and drying methods should be selected to obtain maximum amount of bioactive compounds in papaya leaves.

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Interest of conflict

The authors declare no conflicts of interest with regard to the publication of this paper

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