IMPACT OF DIFFERENT SOLvents ON PHYTOCHEMICAL PROFILING AND ANTIOXIDANT ACTIVITY OF CARICA PAPAYA LEAF FROM NORTHWESTERN HIMALAYAN REGION

Kanika Dulta, Kiran Thakur, Parveen Chauhan Avinash Sharma and P.K. Chauhan*

Faculty of Applied Sciences and Biotechnology, Shoolini University, Solan-173229, (H.P) India.

Abstract

Carica papaya an eminent medicinal plant used in the Asian and West countries to go through with a diverse group of diseases. This study was conducted to investigate the total phenolic, flavonoid and antioxidant potential of Carica papaya leaves with four different solvents i.e. aqueous, chloroform, ethanol and methanol. Phenolic and flavonoid content was found to be high in ethanol extract i.e. 73 mg GAE/g and 54 mg QE/g respectively. Ethanol extract of Carica papaya is also high in its antioxidant potential using DPPH and ABTS assay i.e. IC$_{50}$ value 56.34 and 68.5 μg/ml. Ethanol was found to be the best solvent of choice for extracting natural compounds to obtain maximum medicinal benefits and could be used against various infectious diseases for medicinal formulation.

Key words: Carica papaya, Antioxidants, Phytochemicals, Total phenolic and flavonoid content.

Introduction

Medicinal plants are being used from ancient times in the Ayurvedic, Unani and other traditional medicine methods for treating the various diseases. These effects of plants are due to the presence of phytochemicals, such as phenolic acids, flavonoids, tannins, hydroxycinnamic acid and phenolic diterpenes, which are among the best known for their medicinal properties (Prakash et al., 2017). WHO estimates that 80% of the world’s population relies on medicinal plants for primary health care (Mothana et al., 2008; WHO, 2002; Gupta et al., 2010; Ngoci et al., 2011; Prakash and Sandhu, 2012). Drugs produced from unmodified natural or semi-synthetic products derived from natural sources constituted 78% of new drugs approved by the Food and Drug Administration (FDA) of the United States (Suffredini et al., 2006; Ngoci et al., 2011). Plants are a valuable source of a wide variety of secondary metabolites used as pharmaceutical goods, agrochemicals, flavorings and additives (Alonso-Amelot, 2018). Due to the presence of different phytonutrients (Nostro et al., 2000; Prakash et al., 2016), medicinal plants are useful for the healing and cure of various human diseases.

Carica papaya belongs to family Caricaceae, commonly known as Papaya, Paw Paw, Kates, and Papaw. It is found in most tropical and subtropical countries of the world. Carica papaya is short-lived, single-stemmed/unbranched, hollow, perennial herbaceous with a varying height ranging from 10 to 30 ft. The papaya nuts, bark and leaves are used as medicinal items to treat various diseases such as warts, cancer cell growth, corns, constipation, amenorrhea, general debility, dyspepsia, sinuses, eczema, diabetes, malaria, cutaneous tubercles, glandular tumor, blood pressure, diabetes, malaria, expel worms and stimulate reproductive organs, syphilis, and gonorrhea (Aravind et al., 2013). The literature suggests that fruit and leaf extracts from the Carica papaya are used to treat various diseases such as warts, cancer cell growth, corns, constipation, amenorrhea, general debility, dyspepsia, sinuses, eczema, diabetes, malaria, cutaneous tubercles, glandular tumor, blood pressure, diabetes, malaria, expel worms and stimulate reproductive organs, syphilis, and gonorrhea (Aravind et al., 2013). The literature suggests that fruit and leaf extracts from the Carica papaya are used to treat dengue fever. It is also stated that the Carica papaya leaf extract works to combat RBC sickling (Imaga et al., 2009).

Therefore, the study aimed to determine the effects of aqueous and other organic solvents on the extraction yield, phytochemical and antioxidant potential of papaya
leaves. This will help in determining its medicinal value, which may be useful in increasing the database for the medicinal plant or could be used as an antioxidant in food, pharmaceutical and medicinal preparations.

**Materials and Methods**

**Collection of plant material**

Leaves of *Carica papaya* were collected in March and April from the Solan district of Himachal Pradesh, India.

**Morphological evaluation**

In the morphological evaluation, various organoleptic characters such as color, odour, taste, size & shape were determined (Kokate *et al.*, 2005).

**Preparation of extract**

**Extraction**

Four different solvents were used for the extraction of bioactive compounds from the leaves of *Carica papaya* including aqueous, chloroform, ethanol and methanol. *Carica papaya* leaves were rinsed with distilled water. Afterward, the samples were dried under shade in the laboratory and then homogenized into a fine powder using a mortar and pestle. Powdered material was extracted with all the solvents. Then kept at the shaker for three days. Using Whatman filter paper No.1 (42 μm), extracts were filtered and the filtrate was processed and used for further study (Turner, 2006).

**Extraction yield**

The extraction yield was determined from \( \frac{(W1/W2)}{100} \), where W1 is the extract weight after solvent evaporation, and W2 is the plant sample dry weight.

**Qualitative phytochemical analysis**

Biochemical tests were done to check the presence of different phytochemicals such as alkaloids, flavonoids, saponins, steroids and tannins in *Carica papaya* leaves extract by following standard methods (Harborne, 1973; Sofowora, 1993; Roopashree *et al.*, 2008; Das *et al.*, 2010; Harborne, 2012).

**Detection of alkaloids**

**Mayer’s test**

Extracts were dissolved in dilute hydrochloric acid and washed individually. The filtrate was treated with Mayer (Potassium Mercuric Iodide) reagent 2-3 drops. The development of a yellow color precipitate has indicated the presence of alkaloids.

**Detection of carbohydrates**

**Benedict’s test**

Extracts were treated and heated gently with 2-3 drops of Benedict’s reagent. Precipitate color yellow/green/red showed the presence of reduced sugars.

**Detection of flavonoids**

**Lead acetate test**

Extracts of lead acetate were treated with a few drops of the solution. Precipitating the formation of yellow color indicated the presence of flavonoids.

**Detection of tannins or phenols**

**Ferric chloride test**

Extracts were treated with a solution of 3-4 drops of ferric chloride. The appearance of bluish or greenish-black coloration indicates the presence of pyrogallol or catechol tannins. The formation of a bluish-black color indicated the presence of phenols.

**Detection of amino acid**

**Millon’s test**

3-4 drops of Millon’s reagent were added to the extracts and heated. The presence of amino acids revealed the existence of white precipitate that changed to brick red on heating.

**Total phenolic content**

Total phenolic content was measured using the Folin-Ciocalteu test following the updated procedure described by Singleton and Rossi, (1965). 5 mg of every extract was dissolved in 5 ml of methanol for the preparation of regular extract solutions. About 1 ml of Folin-Ciocalteu reagent was taken and diluted to 10 ml with distilled water. The working standards of each extract were prepared by mixing 1 ml standard solution with 9 ml distilled water. Added 1 ml of diluted Folin-Ciocalteu reagent to each test tube and allowed for 6 minutes to stand. Through adding distilled water and incubating at room temperature for 90 min, 10 ml of 7% sodium carbonate solution was then added to the reaction mixtures in each test tube and further diluted to 25 ml. The absorbance of the sample was measured with a 760 nm UV spectrophotometer. A calibration curve of the gallic acid (0 to 100 mg/ml) was drawn to determine the total phenolic content. The total phenolic content was measured as the equivalent milligrams of gallic acid (mg GAE / g) per gram of dry sample.

**Total flavonoid content**

Total flavonoid content was determined by the aluminum chloride colorimetric assay according to the method described by Meda *et al.*, (2005). Approximately 5 mg of each of the extracts is dissolved in 5 ml methanol. 1 ml was combined with 9 ml distilled water from each
of these normal solutions and eventually with 1 ml NaNO₂ (5%). The mixtures were allowed to stand 6 min for a continued reaction. Then 2 ml of 10% aluminum chloride solution was applied to each and allowed to stand for 5 minutes. Then the mixtures were added with 2 ml of sodium hydroxide (1 M) in series. Eventually, a UV spectrophotometer was used to measure the absorbance of the mixture at 510 nm. Drawn for determining the normal quercetin curve of the total flavonoid material (0 to 100 mg/ml). The total content of flavonoids was measured as milligrams of equivalent quercetine (mg QE/g) per gram of dry sample.

**In-vitro antioxidant activity**

**DPPH radical scavenging activity**

The method defined by Barros et al., (2007) calculated the radical scavenging behavior of the extract DPPH. DPPH solution was prepared by dissolving 20 mg DPPH in 100 ml (stock solution) methanol. 3 ml was taken from this solution, and its absorbance at 515 nm (control solution) was set to 0.75. To prevent free radicals, the DPPH stock solution was coated with aluminum foil and kept in the dark for 24 hours. 5 mg of each extract was dissolved in 5 ml methanol for the preparation of stock solutions. Different dilutions (25, 50, 75 and 100 μg/ml) were prepared from stock solutions through serial dilution. Approximately 2 ml of each dilution was mixed with a solution of 2 ml DPPH and incubated for 15 min in darkness. Ascorbic acid has been used as a typical antioxidant compound in all the assays for comparative analysis. The percentage inhibition of DPPH free radical by extracts was calculated using the following formula:

\[
% \text{ Inhibition} = \left( \frac{A_c - A_s}{A_c} \right) \times 100
\]

Where \(A_c\) is the absorbance of the control and \(A_s\) is the absorbance of the extract/standard. The free radical scavenging activity of samples was expressed as \(IC_{50}\) value, which represented the effective concentration of extract/standard required to scavenge 50% of DPPH radicals.

**ABTS free radical scavenging assay**

The 2, 2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) free radicals scavenging assay was used to determine the antioxidant potential of extracts (Re et al., 1999). Solutions in 100 ml of methanol were prepared for ABTS (7 mM) and potassium persulphate (2.45 mM). These two solutions were thoroughly mixed for the formation of free radicals and kept in the dark overnight. Around 3 ml of this stock solution was taken and its absorbance at 745 nm was set to 0.76 (control solution). Approximately 300 μl of the test sample was mixed with 3 ml of ABTS solution and incubated at 25°C for 15 min.

The absorbance of the mixture was measured using a spectrophotometer with a double beam of 745 nm. The same procedure was followed for the preparation of various ascorbic acid dilution (positive control). The data was collected in triplicates, and the formula used to measure the percentage of ABTS free radicals scavenging activity:

\[
% \text{ Inhibition} = \left( \frac{A_c - A_s}{A_c} \right) \times 100
\]

**FTIR analysis**

Fourier transforms infrared (FTIR) spectroscopy is a technique used in the chemical investigation of substances. It is based on the measure of the vibration of a molecule by IR radiations at a specific range of wavelengths within chemical functional groups and generates a biochemical spectrum of the sample. FTIR spectra were recorded for the sample in the middle IR region (4000-4000 cm⁻¹) using an avatar-330 FTIR type instrument (David and Mauer, 2010; Ekpenyong, 2012).

**Statistical analysis**

Total phenolic, total flavonoid content and values of \(IC_{50}\) were calculated using linear regression analysis. In triplicates, each sample was evaluated independently and the results were expressed as mean value ± standard deviation (n=3).

**Results and Discussion**

**Morphological features**

Morphological features of the *Carica papaya* plant showed that leaves were 25-75 cm in diameter, the surface was fine, greenish or purplish-green, palmate, deeply 7-lobed, glabrous, prominently veined; lobes deeply and broadly toothed, with characteristic odour and bitter in taste (Table 1).

**Table 1:** Morphological characteristics of *Carica papaya* leaves.

<table>
<thead>
<tr>
<th>Features</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Greenish or purple green</td>
</tr>
<tr>
<td>Odour</td>
<td>Unpleasant</td>
</tr>
<tr>
<td>Shape</td>
<td>Palmately lobed, veined</td>
</tr>
<tr>
<td>Size</td>
<td>25-75cm in width</td>
</tr>
<tr>
<td>Texture</td>
<td>Fine</td>
</tr>
<tr>
<td>Taste</td>
<td>Bitter</td>
</tr>
</tbody>
</table>

**Extraction yield**

The extracts from the dried leaves of the plant were made by using different solvents. Aqueous and other organic solvents were studied for their effects on the extraction yield of *Carica papaya*. Results showed a significant difference in the extraction yield using different solvents (Fig. 2). Among solvents tested, aqueous extract
resulted in the highest extraction yield (14.98%), followed by methanol (11.88%), ethanol (9.33%) and chloroform (7.98%). The weight and percentage compositions are shown in (Table 2).

Asghar et al., (2016) reported that the extraction yield of papaya leaf extract was obtained in the following descending order; water (28%), methanol (15.90%), ethanol (11.47%), ethyl acetate (18.89%), dichloromethane (13.85%), n-butanol (08.25%) and n-hexane (08.56%). Solvent polarity, the structure of the extracted products, and method of extraction greatly affect the antioxidant and antibacterial activities of the plant extracts.

### Phytochemical profiling

Phytochemical screening tests revealed the presence of alkaloids, carbohydrates, flavonoids, phenols, saponins, and proteins. Similar findings were also reported by (Ayyola et al., 2010; Sherwani et al., 2013; Akhila et al., 2015; Baskaran et al., 2012).

### Total phenolic and flavonoid content

The total phenolic content from the standard gallic acid curve was calculated using the equation: \( y = 0.741x + 0.1326 \), while the total flavonoid content was calculated using the standard quercetin curve using the equation: \( y = 0.965x + 0.0362 \). The extracts of different organic solvents of *Carica papaya* had a prominent yield. The most extractable solvents of the highest phenolic contents were ethanol and methanol (Fig. 3). The highest phenolic compound was achieved by ethanol extract (73.7 ± 2.77) followed by methanol (45.0 ± 2.04) and aqueous extract (36.3 ± 2.38). Whereas lowest phenolic content was obtained with chloroform extract (15.0 ± 2.08 mg GAE/g dry leave powder). The total flavonoid content was highest in ethanol extract (54.9 ± 2.38) followed by methanol (24.4 ± 3.10), aqueous (16.5 ± 3.11) and chloroform extract (11.9 ± 2.24) (Fig. 4).

Asghar et al. (2016) reported that the highest phenolic content was achieved with ethanol extract (65.12 mg GAE/g dry leave powder) followed by methanol extract (54.28 mg GAE/g dry leaf powder). Dichloromethane extract (1.2 mg GAE/g dry root powder) provided the lower phenolic content. The highest flavonoid content was obtained by extracting ethanol (21.88 mg QE/g dry powder), followed by extracting methanol. The lowest content was in the dichloromethane extract (0.13 mg QE/g dry powder), followed by extracts of n-hexane and n-butanol.

Vuong et al., (2013) reported that the total phenolic content of *Carica papaya* fruit extracts with methanol and ethanol extract was found to be 15.03 and 9.43 mg GAE/g dry powder, respectively. Those contents were found to be lower than those obtained in this report. The total phenolic content of the ethanol solvent release extract showed 63.59 mg GAE/g crude powder which is in good agreement with the present study (73.7 mg GAE/g dry leave powder).

Mandal et al., (2015) also estimated the total phenolic content of papaya leaf and it was found to be 57.6 ± 4.69 mg GAE/g dry extract. On the other hand, methanol extract produced the highest flavonoid content, *i.e.* an extract of 0.34 ± 1.34 mg QE/g.

### Table 2: The total yield of different extracts.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Amount of dried plants used (g)</th>
<th>Yield (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>5g</td>
<td>7.98%</td>
</tr>
<tr>
<td>Chloroform</td>
<td>5g</td>
<td>14.98%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>5g</td>
<td>9.33%</td>
</tr>
<tr>
<td>Methanol</td>
<td>5g</td>
<td>11.88%</td>
</tr>
</tbody>
</table>
Table 3: Total phenolic content of different extracts of Carica papaya leaves.

<table>
<thead>
<tr>
<th>Phenolic content</th>
<th>(mg GAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>15.5 ± 2.08</td>
</tr>
<tr>
<td>Chloroform</td>
<td>36.3 ± 3.41</td>
</tr>
<tr>
<td>Ethanol</td>
<td>56.9 ± 2.38</td>
</tr>
<tr>
<td>Methanol</td>
<td>45.0 ± 2.04</td>
</tr>
</tbody>
</table>

Fig. 3: Quantification of total phenolic content in different extracts of Carica papaya.

Table 4: Total flavonoid content of different extracts of Carica papaya leaves.

<table>
<thead>
<tr>
<th>Flavonoid content</th>
<th>(mg QE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>11.9 ± 2.24</td>
</tr>
<tr>
<td>Chloroform</td>
<td>16.5 ± 3.41</td>
</tr>
<tr>
<td>Ethanol</td>
<td>56.9 ± 2.38</td>
</tr>
<tr>
<td>Methanol</td>
<td>24.4 ± 3.10</td>
</tr>
</tbody>
</table>

Fig. 4: Quantification of total flavonoid content in different extracts of Carica papaya.

Furthermore, Maisarah et al., (2013) recorded the highest phenolic and flavonoid content of young papaya leaves, i.e. 424.89 ± 0.22 mg GAE/100 g dry weight, and 333.14 ± 1.03 mg RE/100 g dry weight, respectively. The variance in solvent polarities will explain those variations.

In vitro antioxidant activity

In the case of the DPPH assay, the percent free radical scavenging potential in ethanol extract was most potent with an IC$_{50}$ value of 56.34 µg/ml. The IC$_{50}$ values of other extracts; such as methanol, chloroform and aqueous were 86.48, 134.5 and 187.4 µg/ml respectively (Fig. 5). Ascorbic acid was used as a standard and its IC$_{50}$ value was 33.0 µg/ml (Table 5). In the case of ABTS scavenging assay again ethanol extract was more potent with an IC$_{50}$ value of 68.5 µg/ml. (Fig. 6) The IC$_{50}$ values of other extracts; such as methanol, chloroform and aqueous were 72.5, 102.2 and 162.8 µg/ml respectively (Table 6). Ascorbic acid was used as a standard and its IC$_{50}$ value was 28.2 µg/ml.

Fidrianny et al., (2016) reported that the lowest IC$_{50}$ value of DPPH scavenging activity was found to be 0.84 µg/ml which was given by ethanol leaf extract of Calina papaya. The ethanol leaf extract of Bangkok papaya showed the lowest IC$_{50}$ of ABTS scavenging activity i.e 1.79 µg/ml.

Asghar et al., (2016) recorded that the highest potential for free DPPH radical scavenging was found with Carica papaya ethanol leaf extract (75.05%) followed by pulp extract (68.07%) with the same solvent. Carica papaya bark and root extract also showed promising potential for radical scavenging of DPPH; particularly for extracts of ethanol and methanol, where bark extract superseded the pulp’s scavenging ability. Bark’s highest potential for free DPPH radical scavenging could be attributed to the promising amount of phenolic and flavonoid content in their extracts.

Mandal et al., (2015) examined Carica papaya DPPH scavenging capacity for aqueous, methanol, and
Setyawati et al., (2016) reported that the FTIR spectrum was 3463,958 cm\(^{-1}\), respectively associated with N-H stretching, O-H groups, H-bonded alcohols, phenols, and carboxylate acid. We observed peaks of 2927,351 cm\(^{-1}\), related to aldehyde, alkane C-H stretching and ketones, 1737,584 cm\(^{-1}\) associated with alkane C-H stretching, 1642,675 cm\(^{-1}\) associated with alkene C=C stretching, C=N stretching, N-H primary amine stretching, and amide stretching. Meanwhile, the peak was observed at 1574,113 cm\(^{-1}\), with C-H stretching alkanes, C=C stretching alkenes, C=N stretching primary and secondary amines, and amide. 1384,386 cm\(^{-1}\), joined to classes of O-H, H-bonded alcohol, phenolic and carboxylate acids.

**Table 7: FTIR peak of Carica papaya leaf extract.**

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Bond</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3574</td>
<td>O-H</td>
<td>Alcohol</td>
</tr>
<tr>
<td>2242</td>
<td>CN</td>
<td>Nitrile</td>
</tr>
<tr>
<td>2166</td>
<td>S-CN</td>
<td>Thiocyanate</td>
</tr>
<tr>
<td>2011</td>
<td>N=C=S</td>
<td>Isothiocyanate</td>
</tr>
<tr>
<td>1600</td>
<td>C-H</td>
<td>Aromatic</td>
</tr>
<tr>
<td>985</td>
<td>C=C</td>
<td>Alkenes</td>
</tr>
</tbody>
</table>

**Conclusion**

This study was carried out to test the medicinal profile of the Carica papaya leaves by extracting secondary metabolites with aqueous and organic solvents. It can be adjudicated that total phenolic and flavonoid contents were high in ethanol extract. The study proved that the ethanol leaf extract of Carica papaya was also comparably high in antioxidant activity. From the results, it was concluded that this plant could be useful in the future for drug development for treating or preventing diseases.

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Impact of different solvents on phytochemical profiling and antioxidant activity of Carica Papaya leaf

References


