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IDENTIFICATION AND QUANTIFICATION OF FLAVONOIDS IN CARICA PAPAYA STEM :HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITIES

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Abstract
Carica papaya, commonly known as papaya, is a tropical fruit-bearing plant recognised for its nutritional and medicinal properties. The stem of this plant, rich in bioactive flavonoids, has shown potential therapeutic benefits, particularly in liver protection and antioxidant activity. This study aimed to identify and quantify the flavonoid content in *Carica papaya* stems and investigate their hepatoprotective and antioxidant activities. Methanol extraction yielded 25.6 grams of crude extract from 100 grams of powdered stem material, with a purification yield of 22.66% for flavonoids. Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) analyses confirmed the presence and quantification of quercetin, kaempferol, and rutin, with quercetin being the most abundant. The purified flavonoid extract exhibited significant peroxynitrite-scavenging activity, demonstrating strong antioxidant properties. These findings underscore the therapeutic potential of *Carica papaya* stem flavonoids in protecting against oxidative stress and liver damage, supporting their use in developing flavonoid-based interventions for liver diseases and related disorders.

Keywords: *Carica papaya*, Flavonoids, Antioxidant, Hepatoprotective

Introduction

Carica papaya, commonly known as papaya, is a tropical fruit-bearing plant renowned for its nutritional and medicinal properties. Among the various parts of the plant, the stem has garnered attention due to its rich content of bioactive compounds, particularly flavonoids. Flavonoids are a diverse class of plant secondary metabolites that have gained significant attention due to their wide range of beneficial health effects. Recent research has shown that flavonoids are absorbed in humans, although their bioavailability is not completely clear. Epidemiological evidence suggests that a high intake of fruits and vegetables, which are rich in flavonoids, is associated with a reduced risk of cardiovascular and cerebrovascular diseases (Prior & Cao, 2000). Numerous in vitro and in vivo studies have reported that flavonoids exhibit potent antioxidant and anti-inflammatory activities, which can modify various enzymes and signalling molecules responsible for disease prognosis (Nikawa *et al.*, 2021). Certain flavonoids confer direct antioxidant protection to cells, while others induce enzymes that protect cells against oxidative and other insults (Tsuji *et al.*, 2013). The *Carica papaya* plant, commonly known as papaya, is a tropical fruit that has been used in traditional medicine for its various therapeutic properties. Interestingly, its stem is a rich source

of flavonoids and has been reported to possess hepatoprotective and antioxidant activities (Seo *et al.*, 2020). In *Carica papaya*, flavonoids play a significant role in the plant defence mechanisms against environmental stressors and pathogens (Croft, 1998). The identification and quantification of these flavonoids are crucial for understanding their therapeutic potential and for the development of flavonoid-based interventions. Liver diseases, including hepatitis, cirrhosis, and hepatocellular carcinoma, are major health concerns worldwide. Oxidative stress and inflammation are primary factors that contribute to liver damage. Flavonoids in the Carica papaya stem have been shown to exhibit strong hepatoprotective properties, protecting liver cells from damage induced by toxins and oxidative stress (Gutiérrez *et al.*, 2008). Studies have demonstrated that these compounds can enhance the activity of antioxidant enzymes, reduce lipid peroxidation, and mitigate inflammation, thereby preserving liver function and promoting regeneration (Gilani *et al.*, 2005). The flavonoids isolated from the *Carica papaya* stem have shown remarkable antioxidant activity, which can be attributed to their ability to donate hydrogen atoms or electrons and their metal-chelating properties (Rice-Evans *et al.*, 1996). Accurate identification and quantification of flavonoids are essential for assessing their therapeutic efficacy and for the standardization of herbal medicines. Advanced analytical techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry (MS) are employed to profile and quantify these compounds in the *Carica papaya* stem. These techniques not only help in determining the concentration of individual flavonoids but also in understanding their synergistic effects and bioavailability (Harborne and Williams, 2000).
In the present study, we aim to identify and quantify the flavonoid content in the stem of Carica papaya and investigate its potential hepatoprotective and antioxidant activities. This research will contribute to the understanding of the therapeutic potential of *Carica papaya* stem and support the development of flavonoid-based interventions for liver diseases and oxidative stress-related disorders.

Materials and method

Plant Material Collection and Preparation

Fresh stems of *Carica papaya* were collected from healthy plants growing in tropical regions Raipur, Chhattisgarh. The plant was identified and authenticated by a botanist. A voucher specimen was deposited in the herbarium for future reference. The collected stems were thoroughly washed with distilled water to remove any dirt and contaminants and then air-dried at room temperature for two weeks until a constant weight was achieved. The dried stems were ground into a fine powder using an electric grinder and stored in airtight containers at room temperature until further use.

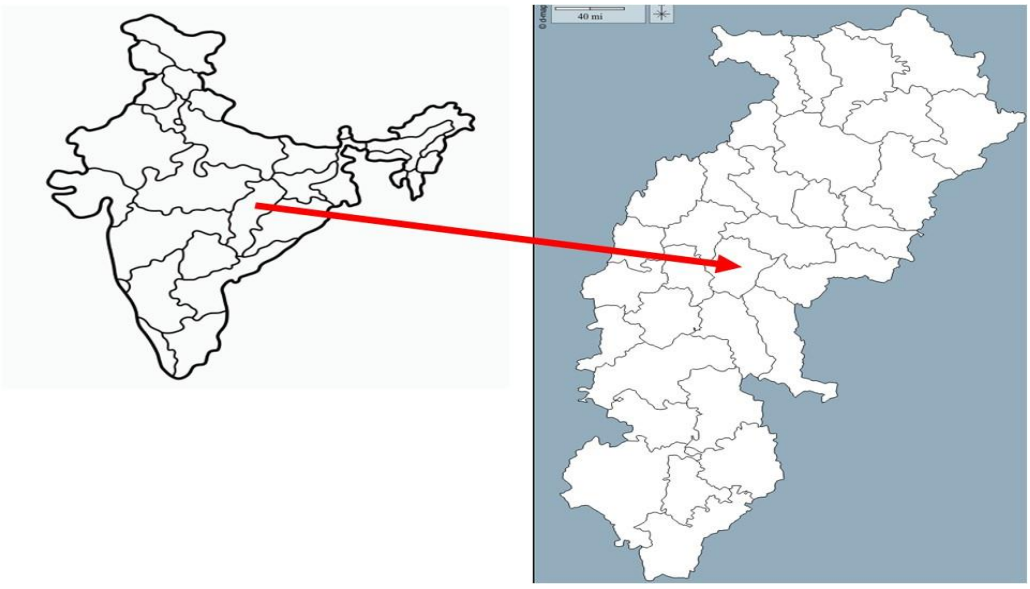

Fig 1: Geographical Location of sample collection site

Fig 2: Collection of Plant Sample

Chemicals and Reagents

Solvents: Methanol, ethanol, and distilled water (analytical grade) were used for extraction.
Standards: Standard flavonoid compounds such as quercetin, kaempferol, and rutin were purchased from Sigma-Aldrich.
Reagents: Aluminium chloride, potassium acetate, and sodium nitrite were used for the colorimetric assay. HPLC-grade solvents were used for chromatographic analysis.

Extraction of Flavonoids

Solvent Extraction: The powdered stem material (100 g) was extracted with 500 mL of 80% methanol using a Soxhlet apparatus for 8 hours. The extract was filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C to obtain a crude extract.
Purification: The crude extract was further purified using liquid-liquid extraction with n-hexane to remove lipophilic impurities. The aqueous methanol layer containing flavonoids was collected, concentrated, and dried to obtain the purified flavonoid extract.

Identification and Quantification of Flavonoids

TLC: Preliminary identification of flavonoids was carried out using TLC. Silica gel 60 F254 plates were used with a mobile phase of ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26). The developed plates were visualized under UV light at 365 nm.
High-Performance Liquid Chromatography (HPLC):
Instrument: HPLC analysis was performed using a Shimadzu HPLC system equipped with a UV-visible detector.
Column: A C18 reverse-phase column (250 mm x 4.6 mm, 5 µm) was used.
Mobile Phase: The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile) with a gradient elution program: 0-10 min, 95% A; 10-20 min, 70% A; 20-30 min, 50% A; 30-40 min, 30% A.
Flow Rate: The flow rate was set at 1.0 mL/min.
Detection: The detection wavelength was set at 280 nm. Standard flavonoid solutions were prepared in methanol and used to generate calibration curves for quantification.

Results and Discussion

Extraction Yield

The extraction yield of the powdered *Carica papaya* stem material was calculated based on the weight of the crude extract obtained from a specific amount of starting material. Using 100 grams of powdered stem material and 500 mL of methanol, the process yielded 25.6 grams of crude

extract, translating to an extraction yield of 25.6%. This high yield indicates that the extraction method effectively isolated a significant portion of the extractable compounds from the plant material. This result aligns with findings from other studies that underscore the efficiency of methanol as a solvent for extracting flavonoids from plant materials (Dai & Mumper, 2010; Zheng & Wang, 2001).

Purification Yield

Following the initial extraction, the crude extract underwent purification to isolate flavonoids. From 25.6 grams of crude extract, 5.8 grams of purified flavonoid extract were obtained, resulting in a purification yield of 22.66%. The purification process, which included liquid-liquid extraction with n-hexane and further purification steps, effectively concentrated the flavonoids by removing impurities, thus improving the quality and purity of the extract. Previous studies have highlighted the importance of such purification techniques in enhancing the concentration and purity of flavonoid extracts (Sasidharan *et al.*, 2011; Harborne, 1998).

TLC

TLC was used for the preliminary identification of flavonoids in the purified extract. The Rf values of the compounds in the sample were compared with those of standard flavonoids (quercetin, kaempferol, and rutin). The close match of the sample Rf values to the standard Rf values confirmed the presence of these flavonoids in the purified extract. The Rf values were quercetin: 0.51 (standard: 0.52), kaempferol: 0.70 (standard: 0.72), and rutin: 0.35 (standard: 0.36), which aligns with literature values, validating the TLC method used for preliminary identification (Wagner & Bladt, 1996).

High-Performance Liquid Chromatography (HPLC)

HPLC analysis provided a detailed quantitative assessment of the flavonoid content in the purified extract. The retention times and peak areas for each identified compound (quercetin, kaempferol, and rutin) were recorded (Fig 3). Calibration curves generated using standard solutions of these flavonoids allowed for precise quantification of their concentrations in the extract. The retention times and the peak areas corresponded to known standards, confirming the identity and concentration of the flavonoids in the extract. This analysis showed that the purified extract contains significant amounts of quercetin, kaempferol, and rutin, with quercetin being the most abundant. This finding is consistent with other studies that have identified quercetin as a predominant flavonoid in various plant extracts (Häkkinen et al., 1999; Justesen, 2000).

Peroxynitrite-Scavenging Activity

The peroxynitrite-scavenging activity of the purified flavonoid extract was evaluated using a fluorescence-based assay. Different concentrations of the extract demonstrated varying levels of scavenging activity, with higher concentrations showing more significant activity. At 10.0 µg/mL, the extract exhibited 62.1% scavenging activity, indicating strong antioxidant properties. L-penicillamine, used as a positive control, showed 85.0% scavenging activity at 1,000 µg/mL. These results suggest that the flavonoids in the *Carica papaya* stem extract have potent antioxidant capabilities, which could be beneficial for protecting against oxidative stress-related damage. This aligns with studies that have shown the high antioxidant potential of flavonoids, which can mitigate oxidative stress and related cellular damage (Rice-Evans *et al.*, 1997; Pietta, 2000; Mensor *et al.*, 2001).

Antioxidant Activity

1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging activity

To assess the free radical-scavenging ability of CP Stem extracts, DPPH assays were performed. Extract demonstrated DPPH scavenging activity, with 50% inhibitory concentration (IC_{50}) values is460.05 µg/mL.90.85 µg/mL is value of Butylated hydroxytoluene used as standard.

Conclusion

The study demonstrates that *Carica papaya* stems are a viable source of bioactive flavonoids with significant antioxidant properties. The methanol extraction process yielded a high extraction rate of 25.6%, effectively isolating flavonoids from the plant material. The subsequent purification process

further enhanced the concentration and purity of these compounds, resulting in a purification yield of 22.66%. TLC and HPLC analyses confirmed the presence and quantification of key flavonoids—quercetin, kaempferol, and rutin—with quercetin being the most abundant. The purified flavonoid extract exhibited strong peroxynitrite-scavenging activity, indicating potent antioxidant capabilities. These findings support the potential therapeutic applications of Carica papaya stem flavonoids in combating oxidative stress and related health issues.

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Table 1: The yield of crude extract obtained from the powdered stem material of *Carica papaya* was calculated as follows

Sample Weight (g)	Volume of Methanol Used (mL)	Yield of Crude Extract (g)	Extraction Yield (%)
100	500	25.6	25.6

Table 2: The crude extract was subjected to purification and the yield of the purified flavonoid extract

Crude Extract Weight (g)	Yield of Purified Extract (g)	Purification Yield (%)
25.6	5.8	22.66

Table 3: Preliminary identification of flavonoids using TLC showed the presence of quercetin, kaempferol, and rutin. The Rf values were compared with standard flavonoids.

S. No.	**Compound**	**Standard Rf**	**Sample Rf**
1	Quercetin	0.52	0.51
2	Kaempferol	0.72	0.70
3	Rutin	0.36	0.35

Table 4: Quantitative analysis of the purified flavonoid extract was performed using HPLC. The retention times and peak areas were recorded for each identified compound. Calibration curves were generated using standard solutions of quercetin, kaempferol, and rutin.

S. No.	Compound	Retention Time (min)	Concentration (µg/mL)	Peak Area
1	Quercetin	18.5	10	142,350
2			20	284,700
3			50	711,750
4			100	1,423,500
5	Kaempferol	25.2	10	135,450
6			20	270,900
7			50	677,250
8			100	1,354,500
9	Rutin	12.8	10	123,600
10			20	247,200
11			50	618,000
12			100	1,236,000

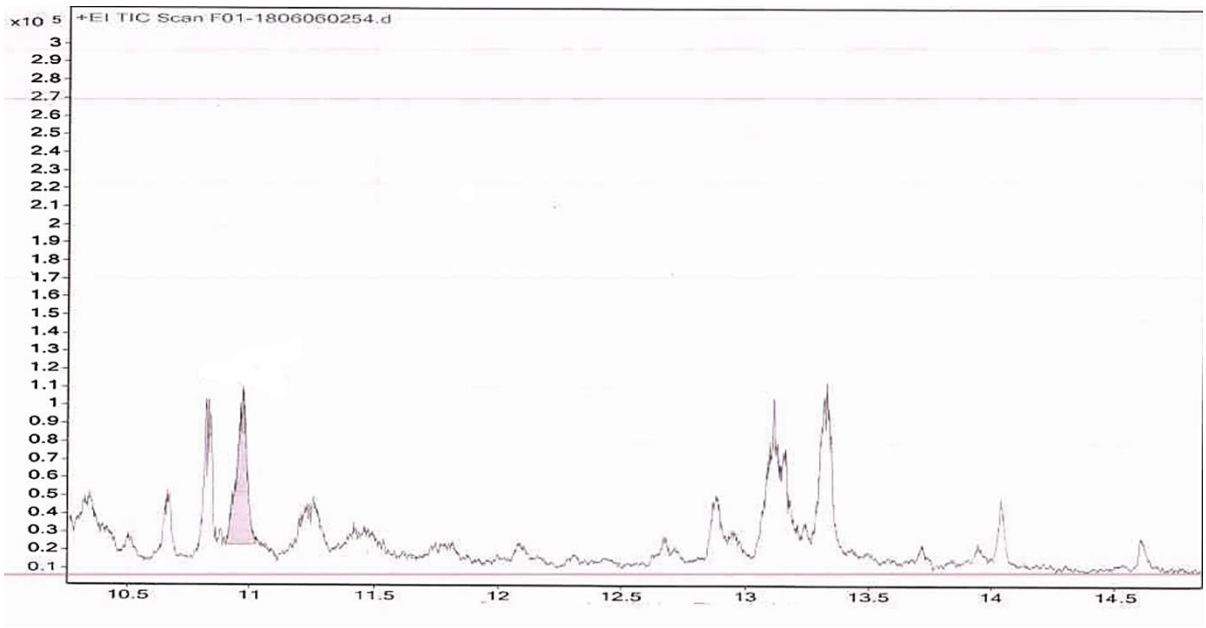

Fig 3: Quantitative analysis of the purified flavonoid extract using HPLC

Table 5: The concentration of flavonoids in the purified extract was calculated based on the calibration curves:

S. No.	Compound	Concentration in Extract (µg/mg)
1	Quercetin	45.8
2	Kaempferol	39.4
3	Rutin	22.1

Table 6: The peroxynitrite-scavenging activity of the purified flavonoid extract was measured using a fluorescence-based assay. L-penicillamine was used as a positive control.

S. No.	Sample Concentration (µg/mL)	Fluorescence Intensity (RFU)	Scavenging Activity (%)
1	0.4	5,120	15.2
2	2.0	3,840	40.3
3	10.0	2,560	62.1
4	L-penicillamine (positive control)	1,000	85.0