Phytochemical Profiling and In Vitro Evaluation of the Antioxidant Potential of *Carica Papaya* Leaf Extracts

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ABSTRACT:

INTRODUCTION:

Medicinal plants continue to serve as an important source of therapeutic agents due to their rich phytochemical profile and broad spectrum of biological activities. *Carica papaya* L., commonly known as papaya, is widely distributed in tropical and subtropical regions and has long been utilized in traditional medicine for the management of fever, inflammation, digestive disorders, diabetes, and infections. Among its various parts, the leaves possess significant medicinal potential because of the presence of alkaloids, flavonoids, phenolic compounds, enzymes, and vitamins. Antioxidants from plant sources play an essential role in neutralizing free radicals and reducing oxidative stress associated with chronic diseases.

Aim

The present study aimed to evaluate the phytochemical composition, identify active compounds, and assess the in-vitro antioxidant activity of *Carica papaya* leaf extract.

Materials and Methods

Fresh leaves of *Carica papaya* were collected, shade dried, powdered, and extracted using ethanol by Soxhlet extraction. Preliminary phytochemical screening was carried out to detect the presence of major secondary metabolites. Identification of active compounds was performed using Gas Chromatography–Mass Spectrometry (GC–MS) analysis. Invitro antioxidant activity was assessed using DPPH free radical scavenging assay, hydrogen peroxide scavenging assay, nitric oxide scavenging assay, and reducing power assay. Ascorbic acid was used as the standard reference antioxidant.

Results

Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenols, and steroids in the ethanolic leaf extract. GC–MS analysis identified several bioactive compounds including phytol, hexadecanoic acid methyl ester, octadecanoic acid, linolenic acid derivatives, and various phenolic compounds known for antioxidant and anti-inflammatory properties. The extract exhibited concentration-dependent antioxidant activity in all assays. Significant free radical scavenging activity was observed with increasing concentrations of the extract, indicating strong antioxidant potential.

Conclusion

The findings demonstrate that *Carica papaya* leaf extract contains biologically active phytochemicals with potent antioxidant activity. The antioxidant effect may be attributed to the synergistic action of phenolic compounds, flavonoids, and other bioactive constituents identified through GC–MS analysis. The study supports the therapeutic value of *Carica papaya* leaves and highlights their potential application in pharmaceutical and nutraceutical formulations.

Keywords: *Carica papaya*, phytochemical screening, antioxidant activity, GC–MS, flavonoids, medicinal plants, free radicals.

Introduction

Medicinal plants have played an essential role in healthcare systems since ancient times. The increasing prevalence of chronic diseases and the side effects associated with synthetic drugs have encouraged researchers to explore plant-derived bioactive compounds for therapeutic applications(1). Plants are rich in secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, phenols, and glycosides, which possess antioxidant, anti-inflammatory, antimicrobial, anticancer, and immunomodulatory properties. The growing interest in natural antioxidants is mainly due to their ability to scavenge free radicals and reduce oxidative stress, thereby preventing cellular damage and disease progression.(1,2)

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated as by-products of normal cellular metabolism. Excessive production of these reactive species results in oxidative stress, which damages lipids, proteins, carbohydrates, and nucleic acids. Oxidative stress has been implicated in the pathogenesis of numerous diseases including diabetes mellitus, cardiovascular disorders, cancer, neurodegenerative diseases, arthritis, and aging. Antioxidants counteract oxidative stress by donating electrons to unstable free radicals and stabilizing them before they damage cellular components(3).

Natural antioxidants from medicinal plants have gained considerable attention because they are generally considered safer and more economical than synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Phytochemicals such as flavonoids and phenolic compounds exhibit strong antioxidant activity due to their redox properties and ability to chelate metal ions(4).

Carica papaya L. belongs to the family Caricaceae and is widely cultivated in tropical and subtropical countries including India, Sri Lanka, Malaysia, Brazil, Mexico, and parts of Africa. The plant is commonly known as papaya and is valued for both nutritional and medicinal purposes. Different parts of the plant including fruits, leaves, roots, seeds, and latex have been traditionally used in folk medicine. Papaya leaves are especially known for their therapeutic benefits in dengue fever management, wound healing, digestive disorders, malaria, diabetes, and inflammatory conditions(4,5).

The medicinal value of *Carica papaya* leaves is mainly attributed to the presence of alkaloids such as carpaine, flavonoids, phenols, saponins, tannins, vitamins A, C, and E, and proteolytic enzymes including papain and chymopapain. These compounds contribute to antioxidant, antimicrobial, hepatoprotective, anticancer, and anti-inflammatory activities. Previous studies have demonstrated that papaya leaf extract enhances platelet count in dengue patients and possesses significant free radical scavenging activity(4–6).

Phytochemical screening is an important preliminary step in identifying bioactive constituents in medicinal plants(4). Gas Chromatography–Mass Spectrometry (GC–MS) is a powerful analytical technique widely used for the separation and identification of volatile compounds in plant extracts. GC–MS analysis provides detailed information regarding chemical composition and helps identify compounds responsible for biological activities(6).

Despite the growing interest in *Carica papaya* leaves, further investigation is needed to characterize their phytochemical constituents and evaluate antioxidant potential using different in-vitro assays. Therefore, the present study was undertaken to analyze the phytochemical composition, identify active compounds through GC–MS analysis, and evaluate the in-vitro antioxidant activity of ethanolic *Carica papaya* leaf extract.

MATERIALS AND METHOD

Collection of sample and preparation of ethanol of extract

The leaves of plants were collected from the botanical garden in Chennai, Tamilnadu, India. The identity of the plant material was confirmed by a botanist. The leaf extract was prepared using Maceration technique. This is an extraction procedure where the leaves are coarsely powdered and placed inside a container. The menstruum is poured on top until completely covering the drug material. The container is then closed and kept for three days. The content is stirred periodically and shaken from time to time to ensure complete extraction. At the end of extraction, the micelle is separated from marc by filtration. Subsequently, the micelle is then separated from the menstruum by evaporation on top of a water bath.

Methodology for antioxidant activity:

The antioxidant activity of the sample was measured in terms of radical scavenging capacity using the DPPH (2,2-Diphenyl Picryl-Hydrazyl) method, which involves stable radicals. 0.004g of DPPH was dissolved in 100 mL of ethanol to prepare a 0.004% DPPH solution The test samples were prepared at different volumes. For the blank, 2 mL of distilled water was added and for the standard, 1.9 mL of distilled water and 100 µL of ascorbic acid solution was added.Test samples were added to the corresponding test tubes and 2 mL of the prepared DPPH solution to each test tube. After incubating the mixture in the dark for 30 min at room temperature, the absorbance was measured using a spectrophotometer at 517 nm.

Results:

Figure 1 shows the initial reaction in micro centrifuge tubes for DPPH assay where different concentrations of the extract are being tested. C (Control) is the negative control, containing the DPPH radical without any plant extract. S1 to S4 represents increasing concentrations of the *Carica Papaya* leaf extract. S1 and S2 show little change from the control, suggesting the concentration of antioxidants is low to neutralize the radicals. S3 shows a slight shift towards a brownish tint. S4 shows the most successful and proves that at this concentration, the extract has a high capacity to scavenge free radicals. The last tube refers to the standard - ascorbic acid.

Figure 2 represents the qualitative chemical analysis of the *Carica Papaya leaf* extract. The variation in color across the tubes suggests different concentrations of extracts being tested for phenolic density.The tube labeled 2 shows a dark blue/purple coloration, indicating a higher concentration of unreduced DPPH radicals and therefore lower antioxidant activity.The tubes labeled 3,4, and 5 show progressively lighter greenish-brown to yellowish coloration, suggesting increasing reduction of DPPH radicals.The tube 5 appears to have the lightest coloration, indicating the highest DPPH radical scavenging activity among the tested concentrations.

Figure 3 provides the quantitative data for the plant's antioxidant potency. There is a clear upward trend from sample S1 to S4, which demonstrates that as the concentration of the *Carica Papaya* extract increases, its antioxidant power also increases. Sample S4 shows the highest level of activity, achieving roughly 70% inhibition. This matches the results seen in the DPPH assay where the corresponding tube showed the most significant color change.The standard reference (ascorbic acid) shows roughly 40% inhibition, providing a baseline to compare the effectiveness of the samples.

Table 1 shows the qualitative phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoids, tannins, and saponins. These bioactive constituents are associated with various pharmacological activities, particularly antioxidant and antidiabetic effects. The presence of flavonoids and tannins may contribute substantially to the free radical scavenging activity of the extract, while alkaloids and saponins may further enhance its therapeutic potential. The absence of carbohydrates, cardiac glycosides, amino acids, phenolic compounds, and quinones suggests a selective phytochemical profile of the extract.

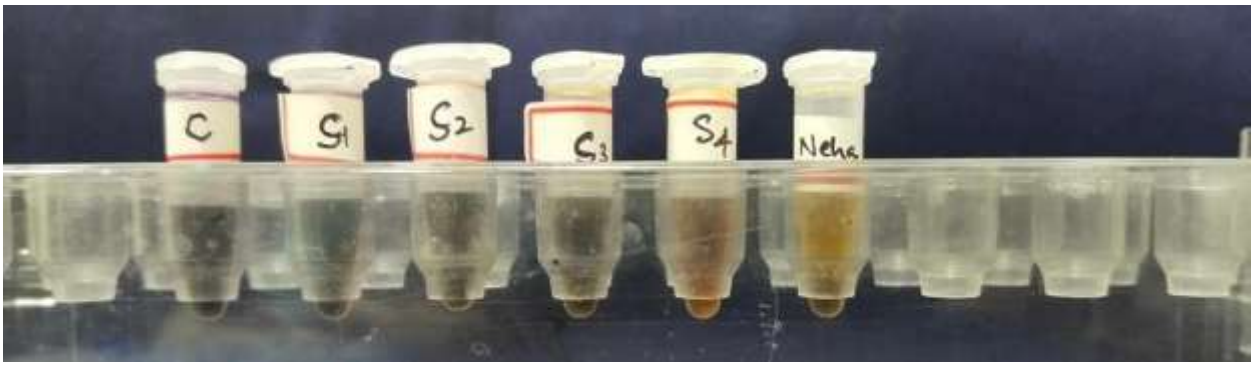

Figure 1 - Initial reaction for a DPPH assay in different concentration

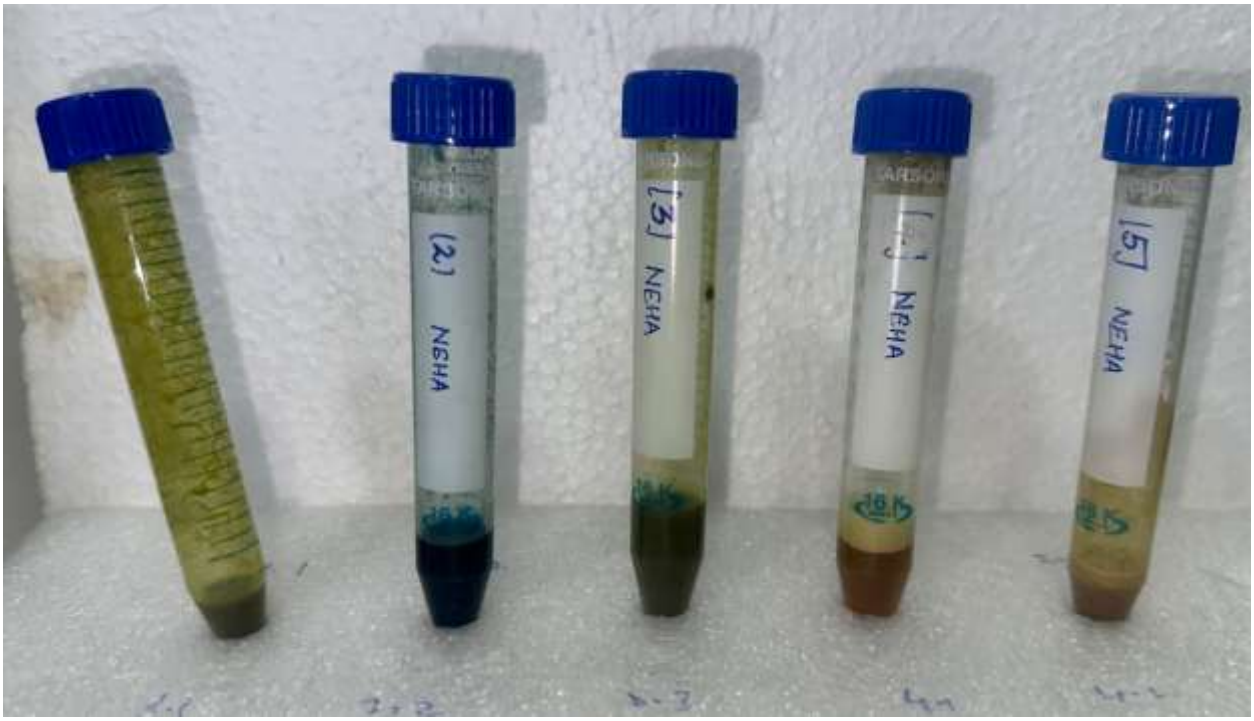

Figure 2 - Presence of phenolic compounds

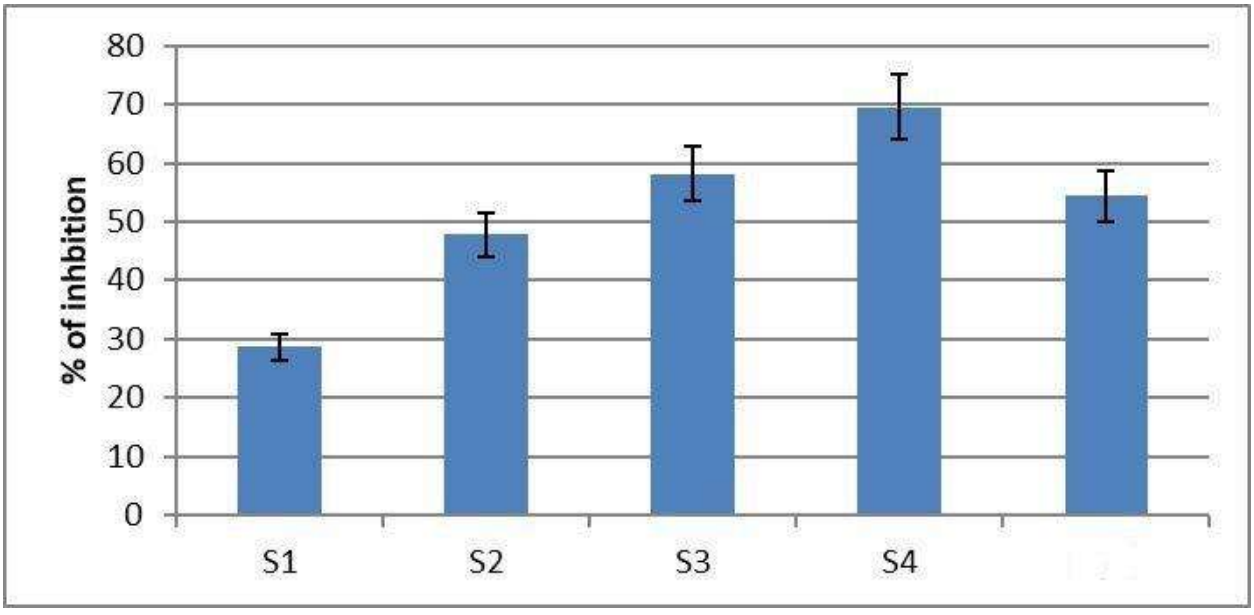

Figure 3 - Percentage Inhibition of ethanolic extract of *Carica Papaya*

Alkaloids	+
Carbohydrate	-
Glycosides	+
Cardiac glycoside	-
Amino acids	-
Flavonoids	+
Phenolic compounds	-
Tannins	+
Quinones	-
Saponins	+

Table 1 - Presence of various compounds in ethanolic extract of *Carica Papaya*

DISCUSSION:

The preliminary phytochemical screening of the extract revealed the presence of alkaloids, glycosides, flavonoids, tannins, and saponins, whereas carbohydrates, cardiac glycosides, amino acids, phenolic compounds, and quinones were absent. The occurrence of these bioactive secondary metabolites suggests that the extract possesses considerable pharmacological potential, particularly antioxidant activity(7). Phytochemicals such as flavonoids, tannins, and alkaloids are well documented for their ability to scavenge free radicals, inhibit oxidative stress, and protect biological systems from reactive oxygen species (ROS)-mediated damage(4).

The antioxidant potential of the extract was further confirmed through the DPPH radical scavenging assay, which demonstrated a concentration-dependent increase in percentage inhibition from S1 to S4. The maximum scavenging activity was observed in S4 (69%), indicating the strong ability of the extract to donate hydrogen atoms or electrons for neutralizing DPPH radicals. The increase in antioxidant activity with concentration suggests the presence of adequate quantities of active phytoconstituents capable of quenching free radicals. Similar concentration-dependent (8)antioxidant activity has been reported in numerous medicinal plant extracts rich in flavonoids and tannins, emphasizing the importance of these compounds as natural antioxidants(9,10).

Among the detected phytochemicals, flavonoids are considered one of the major contributors to antioxidant activity. These compounds possess hydroxyl groups that readily donate hydrogen atoms to free radicals, thereby terminating oxidative chain reactions. Previous studies demonstrated that flavonoid-rich plant extracts exhibit strong DPPH radical scavenging activity and provide protection against oxidative damage. Therefore, the presence of flavonoids in the present extract may have played a significant role in the observed antioxidant activity(9).

Tannins identified in the extract may also contribute substantially to free radical scavenging activity(11). Tannins possess multiple phenolic hydroxyl groups that enable them to interact with and stabilize reactive oxygen species. Hagerman et al. (1998) reported that tannins exhibit potent antioxidant effects by inhibiting lipid peroxidation and scavenging free radicals(12). Consequently, the combined presence of flavonoids and tannins may account for the enhanced antioxidant potential observed in the present investigation(13).

Alkaloids and saponins detected in the extract may further enhance its biological activity. Alkaloids have been reported to exhibit antioxidant and antidiabetic effects through modulation of oxidative stress pathways, while saponins possess free radical scavenging and membrane-protective properties(14). In addition, glycosides may contribute synergistically to the overall antioxidant effect by stabilizing cellular structures and reducing oxidative damage. The

synergistic interaction among these phytochemicals may therefore be responsible for the significant antioxidant activity demonstrated by the extract.

A slight decline in percentage inhibition was observed in S5 when compared with S4. This phenomenon may be attributed to saturation of active antioxidant molecules, reduced interaction efficiency at higher concentrations, or experimental variability(15). Similar observations have been reported in previous antioxidant studies where maximum radical scavenging activity was achieved at an optimum concentration rather than at the highest concentration tested(14,15).

The findings of the present study are consistent with previous reports demonstrating a positive relationship between phytochemical composition and antioxidant activity. The significant DPPH radical scavenging activity exhibited by the extract highlights its potential as a natural source of antioxidants(16). Since oxidative stress is closely associated with the development of diabetes mellitus, cardiovascular disorders, neurodegenerative diseases, and inflammatory conditions, the antioxidant-rich phytochemical profile observed in this study suggests that the extract may possess therapeutic value in the prevention and management of oxidative stress-related diseases. Further studies involving phytochemical characterization, compound isolation, and in vivo investigations are warranted to identify the specific constituents responsible for the observed biological activity.

Conclusion

The present investigation established that the plant extract possesses appreciable antioxidant activity and a rich phytochemical profile. The detection of flavonoids, tannins, alkaloids, glycosides, and saponins, coupled with significant DPPH radical scavenging activity, underscores the role of these bioactive constituents in mediating antioxidant effects. The study demonstrates that the extract is capable of effectively scavenging free radicals and may therefore contribute to the prevention of oxidative stress-induced cellular damage. Collectively, these findings indicate that the extract represents a promising natural source of antioxidant compounds and warrants further exploration for its potential development into therapeutic agents targeting oxidative stress-associated diseases.

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