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Phytochemical Composition and Antioxidant Potential of *Carica papaya* Leaf and Stalk Litter

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Abstract

This study evaluated the phytochemical content and in-vitro antioxidant capacity of *Carica papaya* leaf and stalk litter extracts using spectrophotometric methods. Four solvents—aqueous, chloroform, methanol, and ethanol—were employed for extraction. Antioxidant assays, including total antioxidant capacity, reducing power assay, nitric oxide scavenging, and 1,1-diphenyl-2-picrylhydrazyl radical scavenging, were conducted with ascorbic acid, gallic acid, aspirin, and catechin used as standard. Qualitative analysis revealed the presence of saponins, terpenes, and alkaloids in both leaf and stalk litter extracts. Quantitative phytochemical analysis indicated the presence of phenols, tannins, flavonoids, and alkaloids in the various extracts. The chloroform extract of the leaf litter exhibited the highest alkaloid and phenolic content, while the methanol extract of the leaf litter was richest in flavonoids. The chloroform extract of the stalk litter contained the highest tannin levels. The antioxidant analysis showed significant activity in both leaf and stalk litter extracts compared to the standards. The IC50 values indicated that the methanol extract of the stalk litter exhibited more potent antioxidant properties than the methanol extract of the leaf litter and other extracts. These results suggest that the leaf and stalk litter of *Carica papaya* are viable sources of bioactive compounds with strong antioxidant properties. This study reveals the higher antioxidant potency of the methanol extract of *Carica papaya* stalk litter over the *Carica papaya* leaf and stalk litter extracts analyzed.

Keywords: *Carica papaya*, Phytochemicals, Antioxidant capacity, Leaf and stalk litter, Extraction methods

Introduction

Carica papaya, commonly known as pawpaw, is a highly valued plant in tropical and subtropical regions, cultivated both for its fruit and for its medicinal properties. Nigeria, one of the top five producers globally, has extensive papaya cultivation (Olubode *et al.*, 2016). The plant's nutritional value is well recognized, particularly for its high vitamin C content and natural antioxidants. However, beyond its fruits, other parts of the plant, such as its leaves and stalks, contain a wealth of bioactive compounds that have significant therapeutic potential (Alara *et al.,* 2020). Various parts of *Carica papaya*, including its leaves, fruits, seeds, and roots, have been studied for their pharmacological activities. These activities include antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer effects, among others (Sharma *et al.*, 2022).

The plant is rich in phytochemicals such as alkaloids, flavonoids, tannins, saponins, and phenols, which contribute to these medicinal benefits (Dwivedi *et al.*, 2020). The leaves of *Carica papaya* are particularly notable for their medicinal use, with compounds like carpaine aiding digestion and antimicrobial activity being reported against pathogens such as *Pseudomonas aeruginosa* (Koul *et al.,* 2022). Papaya leaves are also consumed as vegetables, and their boiled extracts are traditionally used to treat conditions such as dengue fever (Teh *et al.*, 2022).

While significant research has been conducted on the pharmacological properties of *Carica papaya*'s leaves and fruits, less attention has been paid to the leaf and stalk litter of the plant. These parts of the plant, often discarded, may represent an untapped source of valuable bioactive compounds (Wanapat *et al.*, 2024). The stalk litter, in particular, could serve as a sustainable source of phytochemicals with potential industrial and medicinal applications. To fully understand the plant's utility, it is important to investigate the antioxidant potential and phytochemical composition of these less-explored parts of *Carica papaya*. Phytochemicals are naturally occurring compounds in plants that contribute to their defense against environmental stresses, pathogens, and herbivores. These compounds also provide health benefits when consumed by humans, helping to neutralize harmful free radicals and reduce the risk of chronic diseases (Kumar *et al.,* 2020). Among the key phytochemicals found in *Carica papaya* are alkaloids, flavonoids, tannins, and phenolic compounds, which are known to have strong antioxidant properties (Dwivedi *et al.,* 2020). The antioxidant potential of these compounds is of particular interest because antioxidants play a crucial role in protecting the body from oxidative damage, which is linked to diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions (Rudrapal *et al.,* 2022).

Extraction methods play a critical role in determining the types and amounts of phytochemicals that can be obtained from plant materials. Solvents like water, methanol, ethanol, and chloroform are commonly used in extraction processes to isolate various bioactive compounds (Jha and Sit, 2022). By utilizing multiple solvents, researchers can identify the most effective extraction method for isolating specific phytochemicals and maximizing antioxidant activity. This study employs four solvents—aqueous, chloroform, methanol, and ethanol—to extract bioactive compounds from *Carica papaya* leaf and stalk litter and evaluate their antioxidant potential.

Given the increasing demand for natural antioxidants in the food, pharmaceutical, and cosmetic industries, there is a growing interest in identifying plant-based sources of these compounds. The current study aims to fill the gap in literature by focusing on the leaf and stalk litter of *Carica papaya*, parts of the plant that have been under-explored in previous research. The findings from this study could provide valuable insights into the potential use of *Carica papaya* leaf and stalk litter in nutraceuticals, pharmaceuticals, and other industrial applications. By investigating the phytochemical composition and antioxidant capacity of *Carica papaya* leaf and stalk litter, this research seeks to highlight the broader utility of the plant beyond its fruit. The results could support the development of new, sustainable sources of antioxidants and further reinforce the

medicinal importance of *Carica papaya* in both traditional and modern medicine.

MATERIALS AND METHODS

Plant Collection and Reagents

The fallen yellow leaf and stalk litter of *Carica papaya* plant were harvested from a farmland in Obiaruku, Delta State, Nigeria in November 2022. The plant was identified and authenticated by Mr Micheal Ozioma Emmanuel at the Department of Botany at Delta State University, Abraka, Nigeria. A voucher specimen (DELSUH127) was provided and deposited in the departmental herbarium for future reference. The stalk and leaf litter of *Carica papaya* were collected, washed with tap water, and cut into small pieces. They were then shade dried and pulverized before being stored in airtight bags for future use. All reagents used were of analytical grade and were purchased from the Central Drug House, India; Loba Chemie, India; and Sigma Aldrich, USA.

Method

Preparation of Various Extracts of *Carica papaya* Leaf and Stalk Litter

One hundred grams (100g) of the powdered stalks were weighed and transferred to a beaker, to which 850ml of solvent was added. Four different solvents were used separately: methanol, ethanol, chloroform, and water. The extract was left to sit for 48 hours, after which it was filtered through cheesecloth. The filtrate was then filtered again using Whatman No. 1 filter paper. The resulting filtrate was concentrated using a rotary evaporator and stored in a refrigerator at 4^0C. The same extraction procedure was followed using the leaf litter.

Qualitative Phytochemical Analysis of *Carica papaya* Leaf and Stalk Litter

Qualitative phytochemical analysis of the various extracts (methanol, ethanol, chloroform and aqueous) of *Carica papaya* leaf and stalk litter were carried out using standard methods as described by Njoku and Chidi, (2009) and Borokini and Omotayo, (2012) to screen for the presence of various phytochemical constituents namely alkaloids, saponins, phlobatannins, cardiac glycosides, flavonoids, tannins, phenols, steroids, terpenes.

Quantitative Phytochemical Analysis

Quantitative phytochemical analysis of various solvent extracts of *Carica papaya* leaf and stalk litter was conducted to determine the levels of total phenols, total tannins, total flavonoids, and total alkaloids. The total phenol and tannin contents were measured following the method outlined by Singleton and Rossi, (1965), with the results expressed as milligrams of gallic acid equivalents (GAE)/g extract and milligrams of tannic acid equivalents (TAE)/g extract, respectively. The total flavonoid content was estimated using the procedure described by Jia *et al.,* (1999), with the results expressed as milligrams of catechin equivalents (CAE)/g extract. Alkaloid content was determined following the method of Shamsa *et al.,* (2008).

In-Vitro Antioxidants Activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was determined using the method described by Manzocco *et al*., (1998). Nitric oxide (NO) free radical scavenging activity of the various extracts was measured following the procedure outlined by Marcocci *et al.,* (1994). The

reducing power assay was conducted according to the method of Oyaizu (1986). The total antioxidant capacity was determined using the method described by Prieto *et al*., (1999), with gallic acid (20-100 µg/mL) serving as the reference standard.

Statistical Analysis

All analyses were performed in triplicates and values were expressed as mean and standard deviations (Mean ± SD). All results were considered significant at p-values of less than 0.05, that is, at 95% confidence level ($p<0.05$). The comparison of IC50 values was performed using one-way analysis of variance (ANOVA). Tukey post hoc tool was used as basis for comparison for level of significance using the statistics package for social sciences (SPSS) 16.0. Paired t-test was used to compare the statistical difference between leaf and stalk litter.

Results

Qualitative Phytochemical Screening of Extracts of *Carica papaya* Leaf and Stalk Litter

The phytochemicals in the various extracts of *Carica papaya* leaf and stalk litter are shown in Table 1. Various phytochemicals such as tannins, phenol, saponins, terpenes, and alkaloids were found to be present as depicted in Table 1. Aqueous extract of *Carica papaya* leaf and methanol extract of the stalk had the highest amounts of phytochemicals.

Table 1. Qualitative Phytochemical Screening of Extracts of *Carica papaya* Leaf and Stalk Litter

Phytochemical	Aqueous		Methanol		Ethanol		Chloroform	
	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk
Saponins	-	-	-	+	-	-	-	-
Phlobatannin	-	-	-	-	-	-	-	-
Cardiac glycoside	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	-	-
Tannins	+	+	-	+	-	+	-	-
Phenol	+	-	-	-	-	-	+	-
Terpenes	+	+	-	+	-	+	-	-
Steriods	-	-	-	-	-	-	-	-
Alkaloids	+	+	-	+	-	+	+	+

Key: + = positive; - = Negative

Quantitative Phytochemical Analysis of Extracts of *Carica papaya* Leaf and Stalk Litter

The results from the quantitative phytochemical analysis of *Carica papaya* extracts of the leaf and stalk litter are presented in table 2. The results showed that methanol, ethanol, aqueous, and chloroform extracts of *Carica papaya* leaf and stalk litter were rich in alkaloid content, with the highest amount found in the chloroform extract of the leaf litter (3.82 mg/gATE). The chloroform extract of the stalk litter had the highest tannin content (0.993 mg/gTAE). Methanol extract of the leaf litter had the highest number of flavonoids (1.412 mg/CAE) and phenolic content (1.009 mg/gGAE) was found to be highest in the chloroform extract of the leaf.

Flavonoids

In the leaf litter, flavonoids were present in decreasing order of content as follows:

Methanol extract (1.412 mg/gCAE) > Chloroform extract (0.814 mg/gCAE) > Ethanol extract (0.797 mg/gCAE) > Aqueous extract (0.703 mg/gCAE)

In the stalk litter, flavonoids were present in decreasing order of content as follows:

Methanol extract (0.943 mg/gCAE) > Chloroform extract (0.923 mg/gCAE) > Ethanol extract (0.465 mg/gCAE) > Aqueous extract (0.369 mg/gCAE)

Stalk extracts generally showed lower concentrations of flavonoids compared to leaf extracts with the exception of choroform extracts (0.814 mg/gCAE for leaf extract is less than 0.923 mg/gCAE for stalk extract). The flavonoid concentration followed a similar decreasing order in the various extracts of the leaf and stalk litter.

The paired t-test was used to calculate the p-value, which indicates whether the difference between leaves and stalks is statistically significant for each solvent. This shows how the phytochemical distribution varies between the leaf and stalk for a particular solvent. For instance, in the case of the ethanol extracts, the following values were observed:

Leaf: 0.797 ± 0.176^{a} mg/g GAE,

Stalk: 0.465 ± 0.033^{a} mg/g GAE,

Superscripts: "a" (leaves) and "a" (stalks).

Since the p-value > 0.05 (p value = 0.09, thus, same superscripts), there is no significant difference in flavonoid content between leaf and stalk of the ethanol extract.

Phenol

In the leaf litter, phenol were present in decreasing order of content as follows:

Chloroform (1.287 ± 0.058 mg/g GAE) > Methanol (1.009 ± 0.04 mg/g GAE) > Aqueous (0.915 ± 0.04 mg/g GAE) > Ethanol (0.826 ± 0.013 mg/g GAE).

In the stalk litter, phenol were present in decreasing order of content as follows:

Chloroform (1.039 ± 0.045 mg/g GAE) > Ethanol (0.805 ± 0.041 mg/g GAE) > Aqueous (0.764 ± 0.02 mg/g GAE) > Methanol (0.729 ± 0.04 mg/g GAE).

Chloroform shows the highest extraction efficiency for phenol. Phenol content is generally higher in the leaf than in the stalk across all solvents except for ethanol, where it is almost equal.

The paired t-test was used to calculate the p-value, which indicates whether the difference between leaf and stalk is statistically significant for each solvent. This shows how the phytochemical distribution varies between the leaf and stalk for a particular solvent. For instance, in the case of the aqueous extracts, the following values were observed:

Leaf: 0.915 ± 0.04^{a} mg/g GAE,

Stalk: 0.764 ± 0.02^{b} mg/g GAE,

Superscripts: "a" (leaves) and "b" (stalks).

Since the p-value < 0.05 (p = 0.02, thus, different superscripts), there is a significant difference in phenol content between leaf and stalk of the aqueous extract.

In contrast, the following values were observed for the ethanol extracts:

Leaf: 0.826 ± 0.013^{a} mg/g GAE

Stalk: 0.805 ± 0.041^{a} mg/g GAE

Superscripts: "a" (leaves) and "a" (stalks).

Since the p-value > 0.05 (p = 0.34, thus, same superscripts), there is no significant difference in phenol content between leaf and stalk of the ethanol extract.

Tannins

In the leaf litter, tannin were present in decreasing order of content as follows:

Aqueous (0.920 ± 0.07 mg/g TAE) > Ethanol = Chloroform (0.864 mg/g TAE)> Methanol (0.744 ± 0.423 mg/g TAE).

The aqueous extract has the highest tannin content in leaf litter, indicating it is the most efficient solvent for extracting tannins from leaves. Ethanol and chloroform have equal tannin contents in leaf litter, suggesting their extraction efficiencies are similar. Methanol has the lowest tannin content in leaf litter.

In the stalk litter, tannin were present in decreasing order of content as follows:

Chloroform (0.993 ± 0.028 mg/g TAE) > Methanol (0.512 ± 0.035 mg/g TAE) > Ethanol (0.505 ± 0.008 mg/g TAE) > Aqueous (0.402 ± 0.06 mg/g TAE)

Chloroform extract has the highest tannin content in stalk litter, making it the most efficient solvent for extracting tannins from stalks. Methanol and Ethanol have moderate tannin content in stalk litter, but their close values suggest similar efficiencies, with Methanol being slightly higher. Aqueous extract has the lowest tannin content in stalk litter, indicating it is the least efficient solvent for extracting tannins from stalks.

The paired t-test was used to calculate the p-value, which indicates whether the difference between leaf and stalk is statistically significant for each solvent. This shows how the phytochemical distribution varies between the leaf and stalk for a particular solvent. For instance, in the case of the aqueous extracts, the following values were observed:

Leaf: 0.920 ± 0.07^{a} mg/g GAE,

Stalk: 0.402 ± 0.06^{b} mg/g GAE,

Superscripts: "a" (leaves) and "b" (stalks).

Since the p-value < 0.05 (p = 0.002, thus, different superscripts), there is a significant difference in tannin content between leaf and stalk of the aqueous extract.

In contrast, the following values were observed for the methanol extracts:

Leaves: 0.744 ± 0.423[a] mg/g GAE

Stalk: 0.512 ± 0.035[a] mg/g GAE

Superscripts: "a" (leaves) and "a" (stalks).

Since the p-value > 0.05 (p = 0.43, thus, same superscripts), there is no significant difference in tannin content between leaf and stalk litter of the ethanol extract.

Alkaloid

In the leaf litter, alkaloid was present in decreasing order of content as follows:

Chloroform (3.824 ± 0.069 mg/g ATE) > Methanol (3.773 ± 0.04 mg/g ATE) > Ethanol (3.723 ± 0.059 mg/g ATE) > Aqueous (3.302 ± 0.09 mg/g ATE)

In the stalk litter, alkaloid was present in decreasing order of content as follows:

Chloroform (3.554 ± 0.138 mg/g ATE) > Ethanol (3.429 ± 0.075 mg/g ATE) > Methanol (2.931 ± 0.422 mg/g ATE) > Aqueous (1.386 ± 0.12 mg/g ATE)

Chloroform is the most effective solvent for alkaloid extraction in both leaf (3.824 ± 0.069 mg/g ATE) and stalks (3.554 ± 0.138 mg/g ATE). The paired t-test was used to calculate the p-value, which indicates whether the difference between leaf and stalk is statistically significant for each solvent. This shows how the phytochemical distribution varies between the leaf and stalk for a particular solvent. For instance, in the case of chloroform extracts, the following values were observed:

Leaf: 3.824 ± 0.069[a] mg/g GAE,

Stalk: 3.554 ± 0.138[b] mg/g GAE,

Superscripts: "a" (leaves) and "b" (stalks).

Since the p-value < 0.05 (p = 0.02, thus, different superscripts), there is a significant difference in alkaloid content between leaf and stalk of the chloroform extract.

In contrast, the following values were observed for the methanol extracts:

Leaf: 3.773 ± 0.04 [a] mg/g GAE

Stalk: 2.931 ± 0.422 [a] mg/g GAE

Superscripts: "a" (leaves) and "a" (stalks).

Since the p-value > 0.05 (p = 0.07, thus, same superscripts), there is no significant difference in alkaloid content between leaf and stalk of the methanol extracts.

Table 2. Quantitative Phytochemical Analysis of Extracts of *Carica papaya* Leaf and Stalk Litter

Phytochemicals	Aqueous		Methanol		Ethanol		Chloroform		
	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	Leaf	Stalk	
Flavonoids (mg/gCAE) D.W	0.703 ± 0.19 [a]	0.369± 0.07 [a]	1.412 ± 0.844[a]	0.943 ± 0.101[a]	0.797 ± 0.176[a]	0.465 ± 0.033[a]	0.814 ± 0.279[a]	0.923 ± 0.083[a]	

Phenols (mg/gGAE) D.W	0.915 ± 0.04 [a]	0.764 ± 0.02 [b]	1.009 ± 0.040 [a]	0.729 ± 0.040 [b]	0.826 ± 0.013 [a]	0.805 ± 0.041 [a]	1.287 ± 0.058 [a]	1.039 ± 0.045 [b]	±
Tanins (mg/gTAE) D.W	0.920 ± 0.07 [a]	0.402 ± 0.06 [b]	0.744 ± 0.423 [a]	0.512 ± 0.035 [a]	0.864 ± 0.204 [a]	0.505 ± 0.008 [a]	0.864 ± 0.253 [a]	0.993 ± 0.028 [a]	±
Alkaloids (mg/gATE) D.W	3.302 ± 0.09 [a]	1.386 ± 0.12 [b]	3.773 ± 0.04 [a]	2.931 ± 0.422 [a]	3.723 ± 0.059 [a]	3.429 ± 0.075 [b]	3.824 ± 0.069 [a]	3.554 ± 0.138 [b]	±

Key: GAE= Gallic acid equivalent, CAE=Catechin equivalent, ATE= Atropin equivalent and TAE= Tannic acid equivalent. Values are means ± standard deviations of triplicate determinations. Different superscript characters on the same row represent significant differences at $p<0.05$ in the paired t-test of leaf and stalk for each solvent.

DPPH scavenging activity of extracts of *Carica papaya* leaf and stalk

Table 3 shows the results of the *in-vitro* DPPH scavenging activities of methanol, ethanol, aqueous and chloroform extracts of *Carica papaya* leaf and stalk litter. From Table 3, the ethanol extract of the leaf litter had the highest scavenging activity against DPPH radical in a concentration dependent manner when compared to the other extracts.

Scavenging activities of the leaf extracts

The DPPH scavenging activities of various extracts of the leaf litter decreased in the following order:

Ethanol (81.78 ± 0.13%) > Methanol (66.28 ± 0.34%) > Aqueous (46.96 ± 0.60%) > Chloroform (40.26 ± 0.78%).

The ethanol extract exhibited the highest scavenging activity, methanol extract demonstrated moderate activity, while aqueous extract showed lower activity. The chloroform extract had the least activity.

Scavenging activities of the stalk extracts

The scavenging activities of various extracts of the stalk litter decreased as follows:

Ethanol (74.24 ± 0.13%) > Methanol (66.26 ± 0.29%) > Aqueous (55.76 ± 0.44%) > Chloroform (41.05 ± 0.46%).

Similar to leaves, ethanol extract showed the highest scavenging activity. Methanol extract had slightly lower activity, while aqueous extract displayed moderate antioxidant activity. The chloroform extract had the lowest activity, indicating limited antioxidant extraction efficacy in stalks.

Statistical significance of difference

The superscripts attached to the values indicate the statistical significance of

differences between scavenging activities in leaf and stalk extracts for each solvent. Different superscripts (e.g., "a" vs. "b") means the difference between leaf and stalk extracts is statistically significant ($p < 0.05$). Same superscripts (e.g., "a" vs. "a") means there is no statistically significant difference ($p > 0.05$). For instance:

- Methanol extracts at 1.5 mg/ml: Leaf (66.28 ± 0.34^a %) vs. Stalk (66.26 ± 0.29^a %). The superscripts "a" and "a" denote no significant difference ($p > 0.05$)
- Ethanol extracts at 1.5 mg/ml: Leaf (81.78 ± 0.13^a %) vs. Stalk (74.24 ± 0.13^b %). The superscripts "a" and "b" indicate a significant difference ($p < 0.05$).

Table 3. DPPH scavenging activity of various extracts of *Carica papaya* leaf and stalk litter

DPPH Scavenging Activity (%)								
Conc. (mg/ml)	**Methanol**		**Ethanol**		**Aqueous**		**Chloroform**	
	Leaf	**Stalk**	**Leaf**	**Stalk**	**Leaf**	**Stalk**	**Leaf**	**Stalk**
0.40	53.68 ± 0.65^a	43.31 ± 0.16^b	60.60 ± 0.62^a	48.39 ± 0.52^b	39.50 ± 0.26^a	40.30 ± 0.13^b	18.67 ± 0.97^a	28.25 ± 0.43^b
0.60	55.70 ± 0.58^a	47.10 ± 0.38^b	65.05 ± 0.35^a	53.96 ± 0.13^b	40.36 ± 0.26^a	42.91 ± 0.94^b	20.06 ± 0.19^a	30.60 ± 0.23^b
0.80	58.39 ± 0.23^a	53.43 ± 0.46^b	72.51 ± 0.96^a	61.38 ± 0.35^b	43.34 ± 0.26^a	45.34 ± 0.32^b	21.79 ± 0.54^a	31.76 ± 0.19^b
1.0	61.98 ± 1.09^a	60.84 ± 0.40^a	79.09 ± 0.41^a	68.65 ± 0.13^b	45.47 ± 0.21^a	49.71 ± 0.31^b	31.50 ± 0.97^a	35.42 ± 2.07^a
1.5	66.28 ± 0.34^a	66.26 ± 0.29^a	81.78 ± 0.13^a	74.24 ± 0.13^b	46.96 ± 0.60^a	55.76 ± 0.44^b	40.26 ± 0.78^a	41.05 ± 0.46^a

Values are means ± standard deviations of triplicate determinations. Different superscript characters on the same row (same concentration) represent significant differences at $p<0.05$ in the paired t-test for each solvent

NO scavenging activity of *Carica papaya* leaf and stalk litter

Table 4.1 and table 4.2 show the nitric oxide scavenging activities of methanol, ethanol, aqueous and chloroform extracts of *Carica papaya* leaf and stalk litter.

Scavenging activities of leaf extracts

The NO scavenging activities of various extracts of the leaf litter followed this order:

Chloroform (78.81 ± 0.33%) > Aqueous (74.10 ± 0.08%) > Methanol (50.72 ± 2.9%) > Ethanol (32.62 ± 0.63%).

The chloroform extract exhibited the highest scavenging activity while the aqueous extract exhibited moderate activity, followed closely by methanol extracts. The ethanol extract had the lowest activity.

Scavenging activities of stalk extracts

The NO scavenging activities of various extracts of the stalk litter followed the order: Aqueous (53.69 ± 0.37%) > Methanol (52.17 ± 0.33%) > Ethanol (45.01 ± 0.13%) > Chloroform (24.28 ± 1.82%).

Aqueous had the highest scavenging activity, methanol and ethanol showed moderate activity, while chloroform exhibited the least efficiency in scavenging activity.

Comparism of the various extracts of stalk and leaf litter

From the table, chloroform and aqueous extracts of the leaf litter had a higher percentage inhibition in a concentration dependent manner than the various extracts of the stalk. However, the ethanol and methanol extracts of the stalk litter showed a higher percentage inhibition in a concentration dependent manner than the ethanol extract of the leaf litter.

Statistical significance of difference

The superscripts attached to the scavenging activity values indicate whether the differences between leaf and stalk extracts for each solvent are statistically significant:

- Different superscripts (e.g., "a" vs. "b"): Indicates a statistically significant difference ($p<0.05$).
- Same superscripts (e.g., "a" vs. "a"): Indicates no statistically significant difference ($p > 0.05$).

Table 4.1 NO scavenging activity of various extracts of *Carica papaya* leaf and stalk litter

NO Scavenging Activity (%)								
Conc. (mg/ml)	Methanol		Ethanol		Chloroform			
	Leaf	**Stalk**	**Leaf**	**Stalk**	**Leaf**	**Stalk**		
0.2	23.19 ± 2.29[a]	23.28 ± 0.43[a]	18.84 ± 1.48[a]	28.97 ± 0.32[b]	29.02 ± 0.48[a]	4.49 ± 0.44[b]		
0.4	29.09 ± 1.42[a]	26.83 ± 0.85	22.69 ± 1.53[a]	31.33 ± 0.34[b]	32.37 ± 0.39[a]	6.95 ± 1.23[b]		
0.6	40.97 ± 0.59[a]	32.90 ± 0.74[b]	18.48 ± 13.52[a]	36.14 ± 0.28[a]	36.93 ± 1.38[a]	11.27 ± 1.32[b]		
0.8	45.46 ± 1.08[a]	35.87 ± 0.24[a]	32.62 ± 0.63[a]	45.01 ± 0.13[b]	42.34 ± 0.27[a]	15.54 ± 0.91[b]		

1.0	50.72 2.9[a]	±	52.17 0.33[a]	±	32.62 0.63[a]	±	45.01 0.13[b]	±	78.81 0.33[a]	±	24.28 1.82[b]	±

Values are means ± standard deviations of triplicate determinations. Different superscript characters on the same row (same concentration) represent significant differences at $p<0.05$ in the paired t-test for each solvent.

Table 4.2 NO scavenging activity of aqueous extract of *Carica papaya* leaf and stalk litter

NO Scavenging Activity (%)		
Conc. (mg/ml)	**Aqueous**	
	Leaf	**Stalk**
1.5	31.38 ± 0.56[a]	27.26 ± 0.40[b]
2.0	38.33 ± 0.36[a]	32.45 ± 2.23[b]
2.5	44.62 ± 0.10[a]	42.92 ± 0.25[b]
3.0	49.28 ± 0.05[a]	47.64 ± 0.48[b]
3.5	74.10 ± 0.08[a]	53.69 ± 0.37[b]

Values are means ± standard deviations of triplicate determinations. Different superscript characters on the same row (same concentration) represents significant differences at $p<0.05$ in the paired t-test for each solvent.

Reducing Power of various extracts of *Carica papaya* leaf and stalk litter

Table 5.1 and table 5.2 shows the reducing power activities of methanol, ethanol, aqueous and chloroform extracts of *Carica papaya* leaf and stalk litter.

Reducing power of leaf extracts

The reducing power of various extracts of *Carica papaya* leaf litter followed the order:

Aqueous (0.63 ± 0.00) > Chloroform (0.54 ± 0.01) > Ethanol (0.53 ± 0.00) > Methanol (0.20 ± 0.00).

The aqueous extract demonstrated the highest reducing power, chloroform extract exhibited moderate activity, followed closely by ethanol extract. Methanol

displayed the lowest activity, indicating that it was the least effective solvent for antioxidant extraction from leaves at these concentrations.

Reducing power of stalk extracts

The reducing power of various solvents of *Carica papaya* stalk litter followed the order:
Methanol (1.87 ± 0.02) > Chloroform (0.45 ± 0.00) > Ethanol (0.41 ± 0.01) > Aqueous (0.41 ± 0.01).

The methanol extract had the highest reducing power, chloroform extract showed moderate activity, while ethanol and aqueous extracts exhibited the least reducing power.

Comparism of the various extracts of the stalk and leaf litter

As depicted in the table, it can be seen that the chloroform, ethanol, and aqueous extracts of the leaf litter had a higher reducing power activity in a concentration dependent manner than the corresponding extracts of the stalk litter. In contrast, the methanol extract of the stalk had a higher reducing power in a concentration dependent manner than methanol extract of the leaf litter.

Statistical significance of difference

The superscripts attached to the scavenging activity values indicate whether the differences between leaf and stalk extracts for each solvent are statistically significant:

- Different superscripts (e.g., "a" vs. "b"): Indicates a statistically significant difference ($p<0.05$).
- Same superscripts (e.g., "a" vs. "a"): Indicates no statistically significant difference ($p > 0.05$).

Table 5.1 Reducing power of various extracts of *Carica papaya* leaf and stalk litter

Reducing Power (OD at 700nm)						
Conc. (mg/ml)	Ethanol		Aqueous		Chloroform	
	Leaf	**Stalk**	**Leaf**	**Stalk**	**Leaf**	**Stalk**
0.5	0.14 ± 0.00^{a}	0.08 ± 0.00^{b}	0.27 ± 0.00^{a}	0.08 ± 0.00^{b}	0.13 ± 0.00^{a}	0.09 ± 0.00^{b}
1.0	0.22 ± 0.01^{a}	0.12 ± 0.00^{b}	0.32 ± 0.00^{a}	0.12 ± 0.00^{b}	0.23 ± 0.01^{a}	0.18 ± 0.01^{b}
1.5	0.31 ± 0.03^{a}	0.29 ± 0.01^{a}	0.46 ± 0.00^{a}	0.29 ± 0.01^{b}	0.36 ± 0.02^{a}	0.25 ± 0.01^{b}
2.0	0.43 ± 0.01^{a}	0.32 ± 0.00^{b}	0.59 ± 0.00^{a}	0.32 ± 0.00^{b}	0.46 ± 0.00^{a}	0.30 ± 0.00^{b}
2.5	0.53 ± 0.00^{a}	0.41 ± 0.01^{b}	0.63 ± 0.00^{a}	0.41 ± 0.01^{b}	0.54 ± 0.01^{a}	0.45 ± 0.00^{b}

Values are means ± standard deviations of triplicate determinations. Different superscript characters on the same row (same concentration) represent significant differences at $p<0.05$ in the paired t-test for each solvent.

Table 5.2. Reducing power of methanol extract of *Carica papaya* leaf and stalk litter

Reducing Power (OD at 700nm)		
Conc. (mg/ml)	**Methanol**	
	Leaves	**Stalk**
0.02	0.08 ± 0.00^{a}	1.41 ± 0.01^{b}
0.04	0.11 ± 0.00^{a}	1.53 ± 0.01^{b}
0.06	0.14 ± 0.00^{a}	1.63 ± 0.01^{b}
0.08	0.16 ± 0.00^{a}	1.75 ± 0.01^{b}
0.10	0.20 ± 0.00^{a}	1.87 ± 0.02^{b}

Values are means ± standard deviations of triplicate determinations. Different superscript characters on the same row (same concentration) represents significant differences at $p<0.05$ in the paired t-test for methanol extracts.

Total antioxidant capacity of various extracts of *Carica papaya* leaf and stalk litter

Table 6.1 and table 6.2 show the *in-vitro* total antioxidant capacities of methanol, ethanol, aqueous and chloroform extract of *Carica papaya* leaf and stalk litter.

Total antioxidant capacity of leaf extracts

The total antioxidant capacity of various extracts of *Carica papaya* leaf litter followed the order:

Chloroform (0.65 ± 0.02) > Aqueous(0.60 ± 0.00) > Ethanol (0.57 ± 0.03) > Methanol (0.56 ± 0.01).

The chloroform extract demonstrated the highest total antioxidant capacity, aqueous extract exhibited moderate activity, followed closely by ethanol extract. Methanol displayed the lowest activity.

Total antioxidant capacity of stalk extracts

The total antioxidant capacity of various solvents of *Carica papaya* stalk litter followed the order:
Chloroform (0.89 ± 0.00) > Methanol (0.68 ± 0.01) > Ethanol (0.55 ± 0.01) > Aqueous (0.46 ± 0.02).

The chloroform extract had the highest total antioxidant capacity, methanol extract showed moderate activity, while ethanol and aqueous extracts exhibited the least total antioxidant capacity .

Comparism of the various extracts of the stalk and leaf litter

From table 6, the ethanol and aqueous extracts of the leaf litter had a higher antioxidant capacity in a concentration dependent manner than the corresponding extract of the stalk litter. In contrast, the chloroform and methanol extract of the stalk litter had a higher antioxidant capacity in a concentration dependent manner than the corresponding extract of the leaf litter.

Statistical significance of difference

The superscripts attached to the scavenging activity values indicate whether the differences between leaf and stalk extracts for each solvent are statistically significant:

- Different superscripts (e.g., "a" vs. "b"): Indicates a statistically significant difference ($p<0.05$).
- Same superscripts (e.g., "a" vs. "a"): Indicates no statistically significant difference ($p > 0.05$).

Table 6.1. Total antioxidant capacity of various extracts of *Carica papaya* leaf and stalk litter

Total Antioxidant Capacity (OD at 695nm)												
Conc. (mg/ml)	**Methanol**			**Ethanol**			**Chloroform**					
	Leaf		**Stalk**		**Leaf**		**Stalk**		**Leaf**		**Stalk**	
0.2	0.24 0.00[a]	±	0.28 0.00[b]	±	0.19 0.01[a]	±	0.22 0.01[a]	±	0.29 0.00[a]	±	0.45 0.02[a]	±
0.4	0.32 0.01[a]	±	0.32 0.02[b]	±	0.25 0.01[a]	±	0.27 0.03[a]	±	0.33 0.01[a]	±	0.54 0.00[b]	±
0.6	0.42 0.01[a]	±	0.44 0.01[a]	±	0.35 0.04[a]	±	0.35 0.03[a]	±	0.43 0.02[a]	±	0.67 0.03[b]	±
0.8	0.50 0.00[a]	±	0.52 0.00[a]	±	0.48 0.01[a]	±	0.43 0.05[a]	±	0.51 0.01[a]	±	0.73 0.02[b]	±
1.0	0.56 0.01[a]	±	0.68 0.01[b]	±	0.57 0.03[a]	±	0.55 0.01[a]	±	0.65 0.02[a]	±	0.89 0.00[b]	±

Values are means ± standard deviations of triplicate determinations. Values are means ± standard deviations of triplicate determinations. Different superscript characters on the same row (same concentration) represents significant differences at $p<0.05$ in the paired t-test for each solvent.

Table 6.2. Total antioxidant capacity of aqueous extracts of *Carica papaya* leaf and stalk litter

Total Antioxidant Capacity (OD at 695nm)

Conc. (mg/ml)	Aqueous	
	Leaves	Stalk
0.02	0.25±0.00[a]	0.11±0.01[a]
0.04	0.32 ± 0.02[a]	0.18 ± 0.01[b]
0.06	0.42 ± 0.01[a]	0.28 ± 0.01[b]
0.08	0.58 ± 0.01[a]	0.36 ± 0.00[b]
0.10	0.60 ± 0.00[a]	0.46 ± 0.02[b]

Values are means ± standard deviations of triplicate determinations. Different superscript characters on the same row (same concentration) represents significant differences at $p<0.05$ in the paired t-test for aqueous extracts.

Half Maximum Inhibitory Concentration (IC50) of *Carica papaya* Leaf and Stalk Litter

According to the data in Table 7, the methanol extract of the stalk litter has the lowest IC50 value (-0.14), indicating that a smaller concentration is required to achieve 50% of the desired antioxidant effect, compared to the methanol extract of the leaf litter, which has a higher IC50 value (0.32). The methanol extract also has a lower IC50 value when compared to the other extracts of *Carica papaya* leaf and stalk litter analyzed.

Table 7 Half Maximum Inhibitory Concentration (IC50) of *Carica papaya* Leaf and Stalk Litter

Assays	Aqueous			Chloroform			Methanol			Ethanol		
	Leaves	Stalk	Standard	leaves	Stalk	Standard	leaves	Stalk	Standard	leaves	Stalk	Standard
Reducing Power	1.03 ± 0.00[a]	1.89 ± 0.05[b]	0.22 ± 0.02[c]	1.31 ± 0.01[a]	1.79 ± 0.01[b]	0.22 ± 0.02[c]	0.32 ± 0.01[a]	-0.14 ± 0.01[b]	0.22 ± 0.02[c]	1.35 ± 0.00[a]	1.89 ± 0.04[b]	0.22 ± 0.02[c]
Total Antioxidant	0.07 ± 0.00[a]	0.11± 0.00[b]	0.08 ± 0.00[c]	1.04 ± 0.02[a]	0.51 ± 0.01[b]	0.08 ± 0.00[b]	1.12 ± 0.05[a]	1.01 ± 0.00[b]	0.08 ± 0.00[c]	1.24 ± 0.04[a]	1.89 ± 0.04[a]	0.08 ± 0.00[b]

Nitric Oxide	2.63 ± 0.02[a]	3.18 ± 0.03[b]	4.68 ± 0.09[c]	1.00 ± 0.01[a]	2.81 ± 0.30[b]	4.68 ± 0.09[c]	1.38 ± 0.12[a]	1.44 ± 0.00[a]	4.68 ± 0.09[b]	2.09 ± 0.10[a]	0.29 ± 0.01[b]	4.68 ± 0.09[c]
DPPH	0.51 ± 0.04[a]	0.24 ± 0.00[a]	14.28 ± 1.21[b]	0.53 ± 0.02[a]	0.65 ± 0.05[a]	14.28 ± 1.21[b]	1.0 ± 0.09[a]	2.15 ± 0.03[a]	14.28 ± 1.21[b]	0.57 ± 0.09[a]	1.65 ± 0.01[a]	14.28 ± 1.21[b]

Values are means ± standard deviations of triplicate determinations. Bars bearing different letters at the same concentration differ significantly($p<0.05$). Standard compounds are as follows: DPPH and reducing power = ascorbic acid, total antioxidant capacity= gallic acid, nitric oxide= catechin

Discussion

Phytochemicals are bioactive compounds with health-promoting properties that play a crucial role in a plant-based diet rich in fruits, grains, vegetables, nuts, and legumes (Claus Leitzmann,2016). The qualitative phytochemical screening of methanol, ethanol, chloroform, and aqueous extracts of *Carica papaya* leaf litter revealed the presence of tannins, phenols, terpenes, and alkaloids. Similarly, the qualitative analysis of extracts from the stalk litter demonstrated the presence of saponins, tannins, phenols, terpenes, and alkaloids. These findings are consistent with the research conducted by Ayoola and Adeyeye, (2010), which identified alkaloids in green, yellow, and brown leaves of *Carica papaya*, and corroborated by Sharma *et al*., (2020), who reported the presence of tannins and alkaloids in the leaves.

Quantitative phytochemical screening of the various extracts showed notable levels of flavonoids, phenols, tannins, and alkaloids. The methanolic extract of the leaf litter exhibited the highest flavonoid content, aligning with the findings of Fatma *et al.,* (2019), who reported elevated flavonoid levels in methanolic extracts of mature *Carica papaya* leaves. The chloroform extract of the leaf litter was found to be rich in both phenol and alkaloid content, while the stalk litter's chloroform extract had the highest tannin concentration. This supports the results of Joseph *et al*., (2014), who indicated that chloroform extracts of the leaf litter were abundant in phenol, alkaloid, and tannin compounds.

Flavonoids, terpenes, phenols, tannins, and alkaloids are classified as plant polyphenols and are recognized for their roles as dietary antioxidants and anti-inflammatory agents. They have been shown to combat a variety of diseases, including dengue fever, atherosclerosis, cancer, and gastrointestinal disorders. Alkaloids possess antimicrobial, antioxidant, and anti-inflammatory properties and have historically been utilized in the treatment of conditions such as cancer, diabetes, hypertension, asthma, allergies, and neurodegenerative diseases (Aryal *et al.,* 2022).

A diet rich in flavonoids has been associated with a decreased risk of cardiovascular diseases (Panche *et al.,* 2016; Ciumărnean *et al.,* 2020). Research by Ganugapati *et al*., (2011) indicated that epicatechin, a type of flavonoid, serves as an insulin receptor

activator and mitigates the detrimental effects of diabetes. Flavonoids also function as therapeutic agents for metabolic dysregulation, modulating lipid metabolism and influencing other metabolic parameters associated with metabolic syndrome (Mulvihill *et al.,* 2016).

Tannins are a class of polyphenolic compounds known for their antimicrobisal properties, effectively inhibiting the growth of various fungi, yeasts, bacteria, and viruses. This characteristic positions tannins as a natural defense mechanism against microbial infections by interfering with extracellular microbial enzymes (Souna *et al.,* 2024). The antimicrobial efficacy of tannic acid has prompted its application in enhancing food quality during processing, underscoring its potential in food preservation (Pinto *et al.,* 2023).

Beyond their antimicrobial effects, tannins exert a range of physiological benefits. They have been shown to accelerate blood clotting, reduce blood pressure, and modulate immune responses (Sruthi and Indira, 2016; Jing *et al.,* 2022). These diverse actions make tannins not only beneficial for human health but also vital for maintaining overall well-being. Their antioxidant capabilities further contribute to their health-promoting properties, as they can interfere with oxidation reactions, chelate metal ions, and donate hydrogen atoms to oxidants (Souna *et al.,* 2024).

A diet rich in polyphenols, such as tannins, offers significant nutritional advantages and is linked to the prevention of chronic diseases by inhibiting enzymes associated with these conditions (Minatel *et al.,* 2017). The incorporation of these compounds into plant-based diets has been widely advocated. For instance, phenolic compounds have been shown to inhibit the activity of angiotensin-converting enzyme (ACE), which is pivotal in managing hypertension (Paiva *et al.,* 2023). Additionally, phenol-rich plants have been utilized in treating type 2 diabetes by inhibiting carbohydrate-hydrolyzing enzymes like α-amylase and α-glucosidase (Oboh *et al.,* 2014; Gong *et al.,* 2020).

Furthermore, phenolic compounds play a crucial role in developing therapeutics for neurodegenerative disorders, such as Alzheimer's disease, by inhibiting cholinesterase and enhancing cognitive function (Hanafy *et al.,* 2017). The anti-inflammatory properties of phenolic compounds are also noteworthy, as they have been employed in treating skin diseases, rheumatoid arthritis, and inflammatory bowel disease (Rahman *et al.,* 2022).

The in-vitro antioxidant analysis of various extracts from *Carica papaya* leaf litter demonstrated significant antioxidant activity in a concentration-dependent manner against DPPH radicals, nitric oxide, and total antioxidant capacity. The ethanol extract showed the highest scavenging activity (81.78%) against DPPH, followed by the stalk extract (74.24%) and other solvent extracts. These findings align with the study conducted by Yap *et al*., (2021), which reported that older *Carica papaya* leaves exhibited higher DPPH scavenging activities compared to stalks. Sharma *et al.,* (2022) further support this observation, indicating that the elevated flavonoid and phenolic content in the ethanol extract of *Carica papaya* leaves contributes to its potent DPPH radical scavenging activity.

The chloroform extract of the *Carica papaya* leaf litter demonstrated notable nitric oxide

scavenging activity, which can be attributed to the substantial alkaloid and phenolic content present in the chloroform extract. Various studies have consistently linked the antioxidant activities of plant leaves to their phenolic and alkaloid constituents (Imaga *et al.,* 2013; Majeed *et al.,* 2021).

Interestingly, the chloroform extract of the stalk litter exhibited superior total antioxidant capacity compared to other extracts. This observation may be influenced by the extraction solvent and the elevated tannin content in the chloroform extract. Imaga *et al*., (2013) reported that the chloroform fraction of *Carica papaya* extracts showed heightened total antioxidant activity relative to other extracts. The presence of tannins, known for their antioxidant properties—acting through interference with oxidation reactions, metal ion chelation, and the donation of hydrogen atoms to oxidants—likely contributes to the observed high total antioxidant capacity in this extract (Souna *et al.,* 2024).

In addition, the methanolic extract of the stalk litter exhibited strong reducing power compared to other extracts, suggesting strong antioxidant potential. This finding correlates with the high flavonoid content observed in both leaf and stalk methanolic extracts, which was significantly higher than in other solvent extracts. Sharma *et al*., (2022) noted that polar solvents such as methanol are particularly effective in extracting flavonoids, which are known to enhance the ferric reducing power of plant extracts.

The IC50 (half-maximal inhibitory concentration) refers to the concentration of a substance needed to elicit 50% of its maximum effect. Consequently, the potency of a bioactive compound is inversely correlated with its IC50 value, such that compounds with lower IC50 values demonstrate greater potency (Manye *et al.,* 2023). The IC50 values of the extracts indicate that *Carica papaya* leaf and stalk litter possess noteworthy antioxidant properties, comparable to standard antioxidants like gallic acid. The methanol extract of stalk litter has the lowest IC50 value (-0.14), indicating that a smaller concentration is required to achieve 50% of the desired antioxidant effect, compared to the methanol extract of the leaf litter and other extracts analyzed. This highlights the potential of *Carica papaya* stalk litter as natural sources of antioxidants for dietary and medicinal applications.

Conclusion

This study demonstrated that the leaves and stalk litter of *Carica papaya* are rich in bioactive compounds, including flavonoids, alkaloids, tannins, and phenolics, which significantly contribute to their antioxidant activities. The chloroform extract of the leaf litter exhibited the highest alkaloid and phenolic content, while the stalk litter showed the highest tannin levels. The low IC50 value of the methanol extract of the stalk litter indicates that it has the highest antioxidant effect when compared to other analyzed extracts. The utilization of *Carica papaya* leaf and stalk litter presents an opportunity for enhancing dietary health while promoting the sustainable use of agricultural by-products. Its application in nutraceutical and medicinal products contributes to waste reduction by transforming what would otherwise be discarded biomass into valuable resources, thereby supporting environmental sustainability.

Conflict of interest

The authors declare no conflict of interest.

Authors' declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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