

PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF *CARICA PAPAYA* AND *URTICA DIOICA* LEAVES CULTIVATED IN CÔTE D'IVOIRE: IMPLICATIONS FOR BIOCONTROL AND PLANT HEALTH

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ABSTRACT

Crop protection against pests and diseases remains a critical challenge for sustainable agricultural production, particularly in tropical regions. This study investigated the phytochemical composition and antioxidant properties of leaf extracts from *Carica papaya* and *Urtica dioica* cultivated in Côte d'Ivoire, aiming to assess their potential as natural biopesticides. Phytochemical screening revealed the presence of polyphenols, flavonoids, tannins, alkaloids, sterols, and polyterpenes in both species. Quantitative analyses showed significantly high contents of total polyphenols (158.8 to 176.9 mg GAE/g dry matter) and flavonoids (152.7 to 153.8 mg QE/g dry matter). Antioxidant activities evaluated by DPPH and ABTS assays highlighted strong free radical scavenging capacity, especially in *Urtica dioica* extracts. The bioactive secondary metabolites identified are known for their antimicrobial, antifungal, and insecticidal effects, supporting their application in integrated pest management. These findings underscore the potential of *Carica papaya* and *Urtica dioica* leaf extracts as eco-friendly, low-cost biopesticides to reduce reliance on synthetic chemicals and promote sustainable crop protection in Côte d'Ivoire.

Keywords: Phytochemical compounds, antioxidants, *Carica papaya*, *Urtica dioica*, Natural biopesticides.

INTRODUCTION

Crop protection against diseases and pests remains one of the major challenges in global agricultural production. The intensive use of synthetic pesticides has undoubtedly contributed to reducing yield losses; however, it is increasingly associated with severe adverse effects on human health, ecosystems, and biodiversity (Isman, 2017; Damalas and Koutroubas, 2018). Within the current paradigm of sustainability and agroecology, the search for

alternative, accessible, and environmentally friendly solutions has become a strategic priority to reduce reliance on chemical inputs. In this context, plant extracts rich in secondary metabolites including flavonoids, tannins, alkaloids, saponins, phenolics, and terpenoids represent promising candidates for the development of low-cost, biodegradable biopesticides (Castillo-Sánchez *et al.*, 2015). Medicinal and aromatic plants constitute a valuable reservoir of bioactive molecules that can be harnessed as biopesticides. Among them, stinging nettle (*Urtica dioica*)

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L.) and papaya (*Carica papaya* L.) have attracted growing interest due to the abundance of their leaves in diverse secondary metabolites such as flavonoids, tannins, alkaloids, saponins, and phenolic compounds (Singh *et al.*, 2020). These compounds are well documented for their antibacterial, antifungal, insecticidal, and repellent properties, underscoring their potential in plant disease management (Okwu & Nnamdi, 2011; Rafieian-Kopaei *et al.*, 2014). Previous studies have shown that nettle leaf extracts exert significant antifungal and antibacterial activity against a wide range of phytopathogens, mainly attributed to the presence of caffeic acid, quercetin, and other polyphenols (Chandrashekara *et al.*, 2018). Similarly, papaya leaves have long been used in traditional medicine and smallholder agriculture for their insecticidal and fungistatic effects, linked to the presence of papain, alkaloids, and glycosides (Akinmoladun *et al.*, 2019). These properties suggest that both plants could provide effective natural alternatives to chemical pesticides, particularly in tropical agricultural systems where access to conventional crop protection products is limited. Exploring the antimicrobial and antiparasitic potential of nettle and papaya leaf extracts thus opens promising avenues for the development of plant-based biopesticides. The present study aims to characterize these extracts (secondary metabolite profiles, extraction methods) and to evaluate their effectiveness against a panel of phytopathogens and insect pests, with the ultimate goal of proposing practical formulations suitable for sustainable agriculture.

MATERIAL AND METHODS

Plant material

The leaves of *Carica papaya* and *Urtica dioica* were collected in Bouake (7° 41' 37.9" north, 5° 1' 49.1" central town of Côte d'Ivoire). The identification was confirmed by the National Flora Center in Abidjan (5° 20' 11" Nord, 4° 01' 36" Ouest), in accordance with existing Herbaria.

Sample preparation and extraction

The leaves of *Carica papaya* and *Urtica dioica* were removed and gently washed to remove any debris, air-dried under shade at room temperature for 5 to 10 days in the laboratory. The dried plant samples were ground to a fine powder using an electric grinder, sieved and packed in a polyethylene plastic bag wrapped with aluminum foil. One hundred grams of powder from each plant material samples were extracted using 500 ml of general-purpose grade petroleum ether (40°C) in an extraction flask with periodic stirring for 3 days at room temperature (20–25°C). The mixture was filtered over Whatman N° 1 paper using Büchner funnel. The residue was dried in the fume hood until the smell of petroleum-ether was removed. The dried residue was soaked in 70% ethanol, stirred for 30 min and left for 24 h with periodic shaking. Then the extracts were filtered using Whatman N°1 filter paper and the filtrate evaporated to dryness under vacuum using a rotary

evaporator at 60°C. The crude extract was stored in a refrigerator below 4°C for subsequent analysis.

Phytochemical screening

Phytochemical screening of *Carica papaya* and *Urtica dioica* leaves of secondary metabolites such as polyphenols, flavonoids, sterols and terpenes, alkaloids, tannins, saponins were screened according to the method described by Bidié *et al* (2011), Békro *et al* (2007) and Bagre *et al* (2007). The presence of a bioactive compound was indicated by a color change and a precipitate.

Test for polyphenols

A drop of 2% alcoholic ferric chloride solution was added to 2 mL of the extracts (aqueous and methanolic solution). The appearance of blackish-blue or dark green coloring indicates a positive reaction.

Test for flavonoids

A volume of 2 mL of the extracts was dried on a sand bath. The dry residue obtained was cooled and taken up in 5 mL of hydrochloric alcohol (mixture of 10 mL of ethanol, 10 mL of distilled water and 10 mL of concentrated hydrochloric acid). Then two to three magnesium shavings were added to it. The appearance of a pink-orange or purple color after adding 3 drops of isoamyl alcohol indicates the presence of flavonoids.

Catechetical tannins

A volume of 15 mL of STIASNY reagent (10 mL of 40% formaldehyde added with 5 mL of concentrated HCl) was added to 1 g of dry extract. The mixture was kept in a water bath at 80 °C for 30 min and cooled under flowing water. The appearance of large precipitates in form of flakes indicates the presence of catechetical tannins.

Gallic tannins

The solution containing the flakes was filtered and the collected filtrate was then saturated with sodium acetate. To the mixture, 3 drops of 2 ferric chloride were added to it. The appearance of an intense blue-black color indicates the presence of gallic tannins.

Test for leucoanthocyanins

A volume of 2 mL of the extracts was dried on a sand bath. The dry residue obtained was cooled and taken up in 1 mL of hydrochloric alcohol and 1 mL of isoamyl alcohol. The mixtures were kept in a water bath at 80 °C for 15 min and cooled under flowing water. The appearance of a red-cherry or purplish color indicates the presence of leucoanthocyanins.

Test for sterols and polyterpenes

Liebermann's reagent was used for this demonstration. A mass of 0.1 g of dry extract of the leaves was dissolved at

hot in 1 mL of acetic anhydride and collected in a test tube. Then, 0.5 mL of concentrated sulfuric acid was added. The appearance of a purple or violet ring at the interphase, turning blue and then green, indicates the presence of polyterpenes and sterols.

Test for saponins

A mass of 0.1 g of dry extract was dissolved in 10 mL of distilled water. The solutions obtained were stirred vigorously for 45 seconds. After stirring, the solutions were allowed to stand for 15 minutes. The observation of persistent foam, greater than 1 cm in height, indicates the presence of saponins.

Test for quinones

A volume of 2 mL of the extracts was added to 5 mL of HCl, mixed and diluted to 1/5. The mixtures were kept in a water bath at 80 °C for 30 min and cooled under flowing water. The hydrolyzate solutions were extracted with 20 mL of chloroform. A volume of 1 mL of Borntraeger's reagent (ammonia diluted twice) was added to the chloroform phase. The appearance of an intense red or purple color indicates the presence of quinones.

Test for alkaloids

A mass of 1 g of dry extract was dissolved in 6 mL of 60° absolute ethanol. 2 drops of DRAGENDORFF reagent (aqueous solution of potassium iodobismuth) were added to the alcoholic solution thus obtained. The appearance of a precipitate or an orange color indicates the presence of alkaloids.

Test for Coumarins

2 ml of the crude extract (taken up with ethanol) are poured into 2 test tubes. To one tube 0.5 ml of NaOH is added (10%, m/v), then the 2 tubes are heated in a water bath until boiling. After cooling, 4 ml of distilled water are added to each tube. If the liquid in the tube to which the alkaline solution is added is transparent yellow compared with the liquid in the tube containing no alkali, the reaction is positive. By acidifying the transparent solution with a few drops of concentrated HCl, it loses its yellow color, becomes cloudy or forms a precipitation.

Polyphenols Contents

Total Polyphenols

The determination of polyphenol content was carried out following the colorimetric method using Folin-Ciocalteu's reagent as described by Wood *et al.* (2002). In a test tube, 2.5 ml of 10% diluted Folin-Ciocalteu reagent was added to 30 µl of extract (2.5 mg/ml). The mixture was let to react for 2 min in the dark. Then, 2 ml of 7.5% Na₂CO₃ were added. The tube was incubated in water bath set at 50°C for 15 min. The tube was rapidly cooled under tap water. The absorbance was measured spectrophotometrically at 760 nm against distilled water as blank. The contents were

expressed as milligram of gallic acid equivalent per gram of CAB (mg GAE/g CAB).

Total flavonoids

The flavonoid content determination was carried out by the colorimetric method using AlCl₃ reagent as described by Marinova *et al.* (2005). In 25 ml volumetric flasks containing 2.5 ml of extract (2.5 mg/ml), 0.75 ml of 5% NaNO₂ and 0.75 ml of 10% AlCl₃ were successively added. The mixture was let to react in the dark at laboratory temperature (22 ± 2°C) for 6 min. Then, 5 ml of NaOH 1N were added. The volume was completed up to the mark with distilled water. The solution was well mixed and the absorbance was measured spectrophotometrically at 510 nm using distilled water as blank. The flavonoid contents were expressed as milligram of quercetin per gram of CAB (mg QE/g CAB).

Determination of condensed tannins (CT) and total tannins (TT) contents

Catechins tannins

The quantification of catechins tannins is performed using the method described by Broadhurst & Jones (1978) as well as Heilmer *et al.* (2006). To 400 µl of each crude extract (300 mg/ml), 3 ml of vanillin solution (4% in methanol) and 1.5 ml of concentrated HCl are added. The mixture is incubated for 15 minutes, and absorbance is measured at 500 nm. The concentrations of condensed tannins are determined from calibration curves established with catechin (0-500 µg/ml) and are expressed in micrograms of catechin equivalent per milligram (µg CE/mg).

Total tannins

The total tannin contents were measured following the spectrometric method using Folin-Ciocalteu's reagent as described by Ci and Indira (2016). 100 µl of extract were added to a test tube containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu's reagent. Then, 1 mL of 35% Na₂CO₃ was added. The volume was completed to 10 ml by adding 900 µl of distilled water. The tube was mixed and let to react for 30 min at laboratory temperature (22 ± 2°C). The absorbance was measured spectrophotometrically at 700 nm using distilled water as blank. Tannin contents were expressed as milligram of tannic acid equivalent per gram of CAB (mg TAE/g CAB).

Antioxidant activity

DPPH Free Radical Scavenging

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was solubilized in methanol to obtain a solution with a concentration of 0.1 mg/ml. Different concentration ranges (6.25, 12.5, 25, 50, 100 and 200 µg/mL) of methanol plant extract were prepared in the same solvent. 1 mL of methanol plant extract and 1.5 mL of DPPH methanolic solution were introduced into test tubes. After shaking, the tubes were placed in a dark place for 30 min. The absorbance of the mixture was then read at 517 nm with a

UV-visible spectrophotometer (Jasco V-530) against a blank consisting of 1 mL methanol and 1.5 mL DPPH. The positive reference control is quercetin. The percentage reduction (% I) of DPPH was calculated according to the following equation (Sánchez-Moreno, 2000):

$$I (\%) = \left(1 - \frac{A_S}{A_W}\right) \times 100$$

As: absorbance of the extract; Aw: absorbance of the white.

This parameter was used to determine the median effective reduction concentration (EC₅₀) of DPPH, determined graphically using Graph Pad Prism.

ABTS Assay

The 2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical assay was performed using the method described by Teow (2007), with modifications. The ABTS^{•+} radical cation was produced by mixing an 8 mM ABTS salt solution with a 3 mM potassium persulfate solution. This solution was then incubated at room temperature (30 ± 2 °C) for 16 hours in the dark. The analytical solution was prepared before each dosing series by diluting the ABTS stock solution with methanol until an absorbance of 0.7 ± 0.02 at a wavelength of 734 nm was obtained. A volume of 3.9 mL of the ABTS^{•+} mixture, diluted in methanol, was added to a small volume (0.1 mL) of the sample (standard or extract). After vigorous stirring, the mixture was incubated in the dark at room temperature for 6 minutes. The absorbance of the mixture was measured using a UV-visible spectrophotometer at a wavelength of 734 nm. The results were expressed as micromoles of Trolox equivalent per gram of extract (μmol/g TE), obtained by comparing the percentage degradation of ABTS^{•+} by Trolox with that of

the sample. The percentage degradation of ABTS^{•+} (A) was calculated as follows:

$$A (\%) = (A_{\text{blank}} - A_{\text{extract}}) / (A_{\text{blank}} \times 100)$$

With, A_{734blanc} = absorbance of the blank; A_{734extract} = absorbance of the extract after incubation.

RESULTS AND DISCUSSION

Based on the identification of phytonutrients using appropriate reagents, the detection tests were able to identify several phytochemical families by the appearance of specific colors visible to the naked eye. The results of the detection tests are summarised below (Table 1). Five families of metabolites were detected in *Carica papaya* and *Urtica dioica* ethanolic extracts: polyphenols, flavonoids, catechin tannins, alkaloids and sterols and polyterpenes. The presence of all these phytochemicals in *Carica papaya* and *Urtica dioica* extracts would justify the pharmacological properties attributed to these in non-conventional medicine. Total polyphenols of *C. papaya* and *U. dioica* leaves are shown in Table 2. The highest value is obtained by *U. dioica* leaves with a value of 176.9 ± 3.3 mg GAE/g DM while the lowest value is obtained by *C. papaya* (158.8 ± 1.1 mg GAE/g DM). As shown in Table 2, total flavonoids varied from 152.7 ± 2.7 to 153.8 ± 3.3 mg QE/g DM. The highest amount of flavonoids was also observed in extracts of *U. dioica* while the lowest amount was detected in *C. papaya*. Catechic tanins content in the two plants ranged from 4.74 ± 0.07 to 9.47 ± 0.05 mg/g catechin equivalent dry matter. *U. dioica* leaves exhibited the highest amount of CT. The total tannin content ranged from 72.69 ± 0.24 to 85.15 ± 0.28 mg GAE /g with *U. dioica* exhibited the highest value.

Table 1. Phytochemical composition of *Carica papaya* and *Urtica dioica* extracts by detection tests.

Plant materiel	<i>Carica papaya</i>	<i>Urtica dioica</i>
Polyphenols	+	+
Flavonoids	+	+
Catechic tanins	+	+
Gallic tanins	+	+
Alkaloids	+	+
Anthocyanins	-	-
Saponins	-	-
Sterols and polyterpenes	+	+
Quinones	-	-
Coumarins	-	-

(+) = presence; (-) = absence

Table 2. Total phenolic compounds, total flavonoids, catechic and total tannins of *C. papaya* and *U. dioica* extracts.

	<i>Carica papaya</i>	<i>Urtica dioica</i>
Total polyphenols (mg/g GAE)	158.8 ± 1.1	176.9 ± 3.3
Total flavonoids (mg/g QE)	152.7 ± 2.7	153.8 ± 3.3
Catechic tannins (mg/g CE)	4.74 ± 0.07	9.47 ± 0.05

Total tannins (mg/g GAE)	72.69 ± 0.24	85.15 ± 0.28
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n=3 values are presented as mean±SD.

Table 3. Antioxidant activity of *Carica papaya* and *Urtica dioica* extracts.

	<i>Carica papaya</i>	<i>Urtica dioica</i>
DPPH IC ₅₀ (mg/ml)	0.54 ± 0.01	0.38 ± 0.01
ABTS (μmol. L ⁻¹ TE)	4.49 ± 0.02	5.1 ± 0.3

n=3 values are presented as mean ± SD.

The DPPH radical scavenging of the two plants determined as IC₅₀ (mg/ml) ranged from 0.38 ± 0.01 to 0.54 ± 0.01 compared to 3.26 for the standard quercetin used (Table 3). The extract with the best antioxidant capacity is that of the leaves of *Urtica dioica* (5.1 ± 0.3 μmol. L⁻¹ TE) followed by that of *C. papaya* (4.49 ± 0.02 μmol. L⁻¹ TE). Crop protection via management of phytopathogens and pests is essential for global food security. However, extensive synthetic pesticide use has caused increasing adverse effects on human health, ecosystems, biodiversity, and soil and water quality (Damalas and Koutroubas, 2018). Consequently, sustainable alternatives such as plant-derived biopesticides have garnered significant interest in agricultural systems. Among plants with promising bioactive properties, *Urtica dioica* L. (nettle) and *Carica papaya* L. (papaya) stand out due to their leaves' richness in secondary metabolites, including polyphenols, flavonoids, tannins, alkaloids, sterols, and terpenoids, many of which exhibit antimicrobial, antifungal, and insecticidal activities (Singh *et al.*, 2020). Phytochemical screening using specific reagents identified five major metabolite families in both species: polyphenols, flavonoids, tannins (catechic and gallic), alkaloids, sterols, and polyterpenes, while saponins were absent. These results concur with recent studies (Alhodieb *et al.*, 2025; El Kahkahi *et al.*, 2025), emphasizing the therapeutic and pesticidal potential of these extracts. The active compounds influence plant-damaging organisms through multiple mechanisms, including repellent, antifeedant, growth regulation, attractant, and toxic effects (Purba *et al.*, 2024). Specifically, the papain enzyme from papaya leaf extract effectively controls sap-sucking pests (Purba *et al.*, 2024). Similarly, *Urtica dioica* exhibits diverse biological activities, notably antimicrobial effects (Alaboo *et al.*, 2023). Quantitative analyses revealed high total phenolic contents ranging from 158.8 ± 1.1 mg GAE/g dry matter in *C. papaya* to 176.9 ± 3.3 mg GAE/g in *Urtica dioica*. Previous studies reported comparable or higher values (Premalatha & Lakshmi, 2018; Suli & Papadaki, 2024), reflecting species variance, environmental factors, plant parts used, and extraction solvents (Dwivedi *et al.*, 2020; Dakhli *et al.*, 2025). Phenolic compounds primarily function as antioxidants, antiseptics, and anti-inflammatory agents. Flavonoid contents were 152.7 ± 2.7 mg/g QE and 153.8 ± 3.3 mg/g QE in papaya and nettle, respectively exceeding values reported in other investigations (Dulta *et al.*, 2020; Elsherif *et al.*, 2023). Flavonoids contribute to characteristic sensory attributes of plants and function as natural insecticides, displaying antifeedant or toxic effects

that are often environmentally safer than synthetic chemicals. Tannin concentrations ranged from 4.74 to 9.47 mg/g CE (catechic) and 72.69 to 85.15 mg/g GAE (total), surpassing amounts reported in similar studies (Otutu and Achuba, 2025; Wafa *et al.*, 2022). Geographic distribution and soil nutrient variability are likely to explain these differences. Tannins possess anti-inflammatory, wound healing, antibacterial, and anticancer-preventative properties. Antioxidant assays revealed significant free radical scavenging activities. DPPH values ranged from 0.38 to 0.54 mg/mL, and ABTS scavenging ranged from 4.49 to 5.1 μmol L⁻¹ TE, comparable to prior data for papaya leaves (Premalatha & Lakshmi, 2018) and consistent with findings by Dakhli *et al.* (2025) demonstrating *U. dioica*'s antioxidant efficacy across multiple assays (DPPH, ABTS, FRAP). Given their antioxidant and antimicrobial profiles, *Urtica dioica* and *Carica papaya* constitute promising plant resources for natural biopesticide development. Their extracts exhibit broad spectrum antimicrobial effects effective against insect pests and fungal or bacterial phytopathogens, mainly mediated by secondary metabolites. Experimental evidence reveals that papaya leaves and powders demonstrate potent insecticidal or repellent activities against pests such as *Bemisia tabaci* on chili, *Spodoptera litura*, *Aedes aegypti* mosquitoes, and pea seed beetles, often achieving mortality rates exceeding 70-80% at practical concentrations. Similarly, nettle (*Urtica dioica*) exhibits insecticidal effects on aquatic vector insects (e.g., *Culex pipiens*) and stored grain pests (*Plodia interpunctella*), with enzymatic inhibition (cholinesterase) suggesting biochemical modes of action beyond toxicity. Collectively, these data support that the secondary metabolites, including phenols, flavonoids, alkaloids, and tannins, possess multifaceted antimicrobial and insecticidal properties, reinforcing their role in sustainable pest management strategies.

CONCLUSION

The present study highlights the significant phytochemical richness and antioxidant potential of *Carica papaya* and *Urtica dioica* leaves cultivated in Côte d'Ivoire. The high levels of phenolic compounds, flavonoids, and tannins, coupled with their strong free radical scavenging activities, underscore their potential as sustainable biopesticides. These secondary metabolites are known to exhibit broad-spectrum antimicrobial, antifungal, and insecticidal properties, which could be leveraged to develop eco-

friendly pest management solutions. Consequently, these findings support the further investigation and formulation of plant-based biopesticides from these species, contributing to sustainable agriculture by reducing reliance on synthetic chemicals and mitigating their adverse environmental impacts.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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