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PHYTOCHEMICAL EVALUATION AND ANTIBACTERIAL ACTIVITY OF *ERIGERON FLORIBUNDUS* LEAVES: INFLUENCE OF EXTRACTION METHODS AND OPTIMIZATION PERSPECTIVES

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ABSTRACT
This study evaluates the *Erigeron floribundus* plant by examining extracts from its leaves. Maceration yielded 2.16 ± 0.01% for the aqueous extract and 0.41 ± 0.01% for the ethanolic extract, indicating that distilled water is more effective than ethanol for compound extraction. Phytochemical tests revealed the presence of flavonoids, coumarins, tannins, free quinones, and triterpenes/steroids in the aqueous extract, while the ethanolic extract contained these compounds along with polyterpenes. The presence of flavonoids, coumarins, tannins, and free quinones in both extracts suggests potential antioxidant and anti-inflammatory properties. The ethanolic extract, richer in polyphenols, tannins, and flavonoids, may exhibit better antioxidant activity, with measured concentrations of total polyphenols (0.441 mgGAE/gDM), condensed tannins (0.052 mgCTE/gDM), and total flavonoids (0.117 mgQE/gDM) surpassing those of the aqueous extract. However, no antibacterial activity was observed, necessitating further research to optimize extraction conditions and test higher concentrations. Alternative extraction methods could also be explored to enhance the recovery of bioactive compounds. Although *Erigeron floribundus* leaves are rich in bioactive compounds, the absence of antibacterial activity highlights the need for continued research to fully exploit their therapeutic potential.

KEYWORDS: *Erigeron floribundus*, phytochemical, antibacterial activity, secondary metabolite, leaf extract.

INTRODUCTION
Medicinal plants, regarded as living libraries of natural remedies, have a long and universal tradition dating back to the dawn of humanity (Fouché *et al.*, 2000). Across ages and cultures, these plants have been used for their healing properties to alleviate various ailments and treat a multitude of diseases (Perotto, 2013). Today, this botanical heritage generates increasing scientific interest, not only for its diverse therapeutic potential but also for the opportunities it offers in the development of new therapies (Duport, 2001). Technological advancements now allow for precise identification and characterization of active plant compounds, paving the way for promising pharmacological discoveries and confirming the efficacy of many extracts in treating conditions such as hypertension, diabetes, infections, and even certain cancers (Fézan *et al.*, 2008). For instance, quinine, extracted from the bark of cinchona, revolutionized malaria treatment worldwide (Chast, 2020). Moreover, the holistic approach to medicinal plants often promotes environmental sustainability, contrasting with synthetic drugs whose environmental impact can be detrimental (Annie, 2008). In this context, traditional plant-based medicine represents a promising avenue for addressing public health needs, particularly in Africa, where plants play a crucial role in traditional medicine. *Erigeron floribundus*, a plant often used in traditional remedies for treating various ailments such as toothaches, headaches,

and female infertility (Biholong, 1986; Telefo *et al.*, 2011), will be the focus of our study. The general objective of this study is to evaluate the therapeutic potential of the *Erigeron floribundus* plant by analyzing the bioactive compounds present in its leaves.

I. MATERIALS AND METHODS

I.1. Plant Material

The plant material used in this study consisted of *Erigeron floribundus* leaves. The collection was carried out on the campus of Jean Lorougnon Guédé University in Daloa, Côte d'Ivoire. The leaves were dried away from sunlight for five days in a room at ambient temperature. Once dried, they were ground into a fine powder using an agate mortar, then sieved. This powder was then used to prepare various extracts for testing.

I.2. Bacterial Strains

Five bacterial strains from the Pasteur Institute of Côte d'Ivoire were used:

- *Staphylococcus aureus*: *S. aureus* ATCC 25922 and *S. aureus* 1568 UB/23 CNRa.
- *Pseudomonas aeruginosa*: *P. aeruginosa* ATCC 27853 and *P. aeruginosa* 1587 UB/23 CNRa.
- *Escherichia coli*: *E. coli* ATCC 25922.

II. Methods

II.1. Extractions

Aqueous extracts were obtained following the method used by Zirihi *et al.* (2003) and Tahiri et al. (2022). Two portions of ten grams (10 g) of *Erigeron floribundus* powder were placed in two separate 250 mL Erlenmeyer flasks. The first Erlenmeyer received 120 mL of distilled water, and the second 120 mL of ethanol. Both mixtures were subjected to continuous agitation for 2 hours. After maceration, the mixtures were filtered through cotton using a funnel, and the resulting solutions were collected in two separate beakers. These solutions were then dried to obtain crude extracts (dry residues). These extracts were used for phytochemical screening and antibacterial activity evaluation.

II.2. Phytochemical Study

For phytochemical screening, we employed methodologies as described in the works of Ladyguina *et al.* (1983), Dohou *et al.* (2003), Lebreton *et al.* (1967), Koffi *et al.* (2008), Kadja (2009), and Tahiri *et al.* (2022).

II.2.1. Detection of Tannins

The extracts obtained from the two solutions were placed in separate test tubes, each containing 5 mL of the corresponding extract. A few drops of an aqueous solution of iron(III) chloride (1%) were added to each tube. The presence of gallic or catechin tannins was indicated by the appearance of a greenish or blue-black coloration.

II.2.2. Detection of Flavonoids

Test tubes containing 5 mL of each extract were treated with a few drops of concentrated hydrochloric acid and three magnesium chips. The presence of flavonoids in the extracts was indicated by a color change to red or yellow.

II.2.3. Detection of Coumarins

Ten drops of 10% potassium hydroxide (KOH) were added to 5 mL of each extract in test tubes. The resulting solution was neutralized with a 10% hydrochloric acid solution. The formation of turbidity or precipitate in the tubes indicated the presence of coumarins in the extracts.

II.2.4. Detection of Free Quinones

A few drops of 1% sodium hydroxide were added to tubes containing 5 mL of various extracts. The development of a yellowish-green tint indicated the presence of free quinones.

II.2.5. Detection of Sterols and Triterpenes

A reagent composed of an equal volume mixture of acetic anhydride and sulfuric acid was prepared. A few drops of this reagent were added to 5 mL of each extract. After 15 minutes of incubation, the presence of a color change (green or violet) indicated a positive test.

II.2.6. Detection of Polyterpenes

An appropriate volume of the crude ethanolic extract was dissolved in 1 mL of hot acetic anhydride in a test tube. Next, 0.5 mL of concentrated sulfuric acid was slowly added along the walls of the test tube. The formation of a violet color that evolved into blue and then green indicated a positive reaction.

II.2.7. Detection of Terpenoid

A reagent composed of 2 mL of chloroform and 3 mL of sulfuric acid was prepared. A few drops of this reagent were added to 5 mL of each extract. The presence of terpenoids was confirmed by the formation of two phases and the appearance of a brown tint.

II.2.8. Detection of Saponins

After adding 5 mL of distilled water to 5 mL of each extract, the test tubes were capped and vigorously shaken for 15 seconds. The presence of saponins was indicated by the formation of a persistent foam with a height greater than 1 cm.

II.2.9. Detection of Reducing Compounds

Fehling's reagent, composed of solution A (copper sulfate in distilled water) and solution B (tartaric acid, sodium hydroxide, and distilled water), was used. A few drops of Fehling's reagent were added to 5 mL of each extract. A brick-red precipitate indicated a positive test.

II.2.3. Quantitative Analysis by Assay

II.2.3.1. Determination of Total Polyphenols

The Folin-Ciocalteu colorimetric method was used to determine the amount of total phenols in the extracts. It

is based on the oxidation of polyphenolic compounds by the Folin-Ciocalteu reagent in a basic medium. The resulting blue reduction products absorb at 760 nm, and the intensity of this absorption is proportional to the amount of polyphenols present in the sample. For each crude extract tested (0.01 g), a stock solution was prepared by diluting it in 10 mL of distilled water, followed by a 1/10 dilution. To 1 mL of the diluted solution, 0.5 mL of Folin-Ciocalteu reagent (0.5 N) and 1.5 mL of sodium carbonate (Na_2CO_3) (17%, m/v) were added. The mixture was incubated in the dark for 30 minutes. The absorbance was then measured at 760 nm using distilled water as a reference blank. Calibration curves were established using gallic acid solutions at different concentrations and treated similarly to the samples. This procedure was repeated three times for each sample, and the concentration of polyphenols was expressed as micrograms per gram of extract equivalent to gallic acid (mg GAE/g) according to the methods described by Singleton *et al*. (1999) and Heilerova *et al.* (2003). The total phenol content was determined using the following formula:

Quantity (mg/g) = (V_f × Q × D) / M

Where:

- V_f : final volume of the extract
- Q : quantity of phenols in gallic acid equivalent (mg/mL)
- M : mass of the extract (g)
- D : dilution factor

The concentration of total phenols was determined from the calibration curve equation: y=6.7469x−0.0353, with R^2=0.9942R^2 = 0.9942R2=0.9942, where x = Concentration and y = Absorbance.

II.2.3.2. Determination of Total Flavonoids

For each crude extract (0.01 g), a stock solution was prepared by diluting it in 10 mL of distilled water, followed by a 1/10 dilution. To 2 mL of the diluted solution, 2 mL of 2% aluminum chloride ($AlCl_3$) in methanol was added. The mixture was incubated in the dark for 15 minutes. The absorbance was measured at 415 nm using distilled water as a reference blank. Calibration curves were established using quercetin solutions at different concentrations and treated similarly to the samples. This procedure was repeated three times for each sample, and the concentration of flavonoids was expressed as micrograms per gram of extract equivalent to quercetin (mg QE/g) (Arvouet-Grand *et al.*, 1994). The total flavonoid content was determined using the following formula:

Quantity (mg/g) = (Q×D) / C

Where:

- Q: quantity of flavonoids in quercetin equivalent (mg/mL)
- D: dilution factor
- C: initial concentration of the stock solution

The concentration of total flavonoids was determined from the calibration curve equation: y = 22.779x−0.0159 with R^2 = 0.9994, where x = Concentration and y = Absorbance.

II.2.3.3. Determination of Total Condensed Tannins

The determination of condensed tannins was performed using the method described by Broadhurst & Jones (1978), modified by Heilmer *et al.* (2006). For each sample at a concentration of 1 mg/mL (0.2 mL), 1.5 mL of 4% vanillin solution in methanol and 0.75 mL of concentrated hydrochloric acid were added. The mixture was incubated for 15 minutes, and the absorbance was measured at 500 nm. The concentrations of condensed tannins were calculated from calibration curves established with catechin at concentrations ranging from 0.15 to 0.009375 mg/mL. The results were expressed as micrograms of catechin equivalent per milligram of extract (mg CE/g). The total tannin content was determined using the calibration curve equation: y=1.6546x+0.0086 with R^2=0.9983, where x = Concentration and y = Absorbance.

II.2.4. Biological Study: Evaluation of the Antibacterial Activity of Erigeron floribundus Leaves

An experimental method was implemented to evaluate the antibacterial activity of *Erigeron floribundus* leaf extracts. First, an isolated bacterial colony was taken after 18 to 24 hours of incubation and homogenized in 2 mL of sterile physiological water to achieve an optical density of 0.5 McFarland, equivalent to approximately 10^6 CFU/mL. This inoculum was used to inoculate MH agars by streaking them with a swab. Wells prepared on the inoculated agars received a solution of *Erigeron floribundus* extract at a concentration of 134 mg/mL. Positive controls included antibiotics such as amoxicillin + clavulanic acid, ticarcillin + clavulanic acid, and cefoxitin. Microplates were set up with 100 µL of bacterial inoculum per well, to which 100 µL of plant extract to be tested was added at decreasing concentrations. The growth control wells received only sterile distilled water. After 24 hours of incubation at 37°C, the Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of extract where no turbidity was observed to the naked eye, and this procedure was repeated three times. To evaluate the Minimum Bactericidal Concentration (MBC), successive dilutions of the initial inoculum were plated on MH agars and incubated. The microplate wells without turbidity were transferred onto new MH agars starting from the first experimental well without turbidity (MIC). The MBC was defined as the lowest concentration of extract allowing a reduction in bacterial survival to less than 0.01%. This experimental approach provides a precise evaluation of the inhibitory and bactericidal effects of *Erigeron floribundus* leaf extracts against the tested bacteria, offering reliable data on their antibacterial potential (Guessennd *et al.*, 2005).

II.2.5. Statistical Analysis

The analysis of the measurements obtained during the various manipulations was conducted using EXCEL software. It was used to plot the various diagrams of the extract.

III. RESULTS AND DISCUSSION

III.1. Results

III.1.1. Extraction of *Erigeron floribundus* Leaves

Maceration was chosen as the extraction method in this study to recover a diverse range of organic compounds from the leaves of *Erigeron floribundus*. This technique, widely used by healers and practitioners of traditional medicine, effectively extracts the plant's active ingredients to prepare medicinal formulations. The yields obtained after the different extractions are recorded in Table I. Specifically, the yields of the extracts by maceration were 2.16 ± 0.01% for the aqueous extract and 0.41 ± 0.01% for the ethanolic extract from the leaves of *Erigeron floribundus*.2.16 ± 0.01% for the aqueous extract and 0.41 ± 0.01% for the ethanolic extract from the leaves of *Erigeron floribundus*.3

Table I: Extraction Yields.

Extraction Solvents	Distilled Water	Ethanol
Extraction Yields (%)	2.16 ± 0,01	0.41 ± 0,01

III.1.2. Phytochemical Study

III.1.2.1. Phytochemical Screening of *Erigeron floribundus* Leaves

The results of the phytochemical screening for flavonoids, coumarins, tannins, reducing compounds, saponins, free quinones, sterols, and polyterpenes in the hydroethanolic extracts of *Erigeron floribundus* leaves are presented in Table II. These results are interpreted as follows: (+) presence and (-) absence.

Table II: Phytochemical Screening: Aqueous and Ethanolic Extracts.

Secondary Metabolite Families	Aqueous Extract	Ethanolic Extract
Flavonoids	+	+
Coumarins	+	+
Tannins	+	+
Free Quinones	+	+
Polyterpenes	-	+
Triterpenes and Steroids	+	-
Reducing Compounds	-	-
Saponins	-	-

II.1.2.2. Quantification by Assay

III.1.2.2.1. Total Polyphenols Assay

The results of the total phenolic content assay, presented in table III, demonstrate varying amounts of total phenols in the crude extracts of *Erigeron floribundus* leaves. Specifically, the ethanolic extract exhibits a higher concentration of total phenolic compounds, measured at 0.441 mg GAE/g MS, compared to the aqueous extract, which shows a concentration of 0.168 mg GAE/g MS.

Table III: Total polyphenols in ethanolic and aqueous extracts of *Erigeron floribundus.*

Extracts	Total polyphenol content (mg EAG/g MS)
Ethanolic	0.441 ± 0.003
Aqueous	0.168 ± 0.005

III.1.2.2.3. Total Condensed Tannin Content

The results of the quantitative analysis of total condensed tannins in *Erigeron floribundus* leaves are shown in table IV. Higher concentrations of total condensed tannins are observed in the ethanolic extract (0.052 mgECT/g MS) compared to the aqueous extract (0.023 mgECT/g MS).

Table IV: Total condensed tannins in ethanolic and aqueous extracts of *Erigeron floribundus.*

Extracts	Total condensed tannins ontent (mg ECT/g MS)
Ethanolic	0,052 ± 0,003
Aqueous	0,023 ± 0,004

III.1.2.2.4. Total Flavonoid Content

Table V illustrates the total flavonoid content in the ethanolic and aqueous extracts of *Erigeron floribundus* leaves. The total flavonoid content in the ethanolic extract, measured at 0.117 mgEQ/g MS, is significantly higher than that observed in the aqueous extract, which shows a value of 0.047 mgEQ/g MS.

Table V: Total flavonoids in ethanolic and aqueous extracts of *Erigeron floribundus.*

Extracts	Total flavonoid content (mg EQ/g MS)
Ethanolic	0.117 ± 0.005
Aqueous	0.047 ± 0.003

III.1.3. Antibacterial Activity

Table VI shows that the plant extracts of *Erigeron floribundus* do not inhibit the growth of the bacteria *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Table VI: Diameter of inhibition zones of the extract against bacterial strains.

	Diameter of Inhibition Zones at 134 mg/mL (mm)			
Bacterial strains		Extraits	FOX	EDS
Staphylococcus aureus	ATCC 25923	06 ± 0.30	35 ± 1.30	06± 0.30
	1568UB/23 CNRa	06± 0.30	28 ± 1.41	06± 0.30
AMC				
Escherichia coli	1562UB/23 CNRa	06 ±0.30	18 ± 0.35	06± 0.30
TCC				
Pseudomonas aeruginosa	ATCC 27853	06 ± 0.30	17± 0.35	06± 0.30
	1587UB/23 CNRa	06 ± 0.30	00± 0.00	06± 0.30

FOX: Reference antibiotic (positive control)
EDS: Ethanol disinfectant solution (negative control)

III.2. DISCUSSION

This study is part of the valorization of plant species from the Ivorian flora. It involved conducting a triphytochimic analysis, quantifying certain secondary metabolites, and evaluating the antibacterial activities of various extracts from the leaves of *Erigeron floribundus*. The objective was to provide a reliable database on the chemical and biological parameters of *Erigeron floribundus* leaves.

The yields obtained after maceration indicate that the aqueous extract presents a yield of 2.16 ± 0.01%, while the ethanolic extract shows a yield of 0.41 ± 0.01%. These results demonstrate that maceration with distilled water is more effective than ethanol in extracting soluble compounds from the leaves.

Phytochemical analysis of the hydro-ethanolic extracts revealed the presence of several classes of secondary metabolites, including flavonoids, coumarins, tannins, free quinones, polyterpenes, triterpenes, steroids, reducing compounds, and saponins. The presence of flavonoids in both types of extracts suggests that this plant may possess antioxidant and anti-inflammatory properties (Koné, 2018). Additionally, the detection of coumarins and tannins indicates potential for antimicrobial and anti-inflammatory applications (Bamba *et al.*, 2023). The free quinones, present in both extracts, also show potential antimicrobial activity (Chitra *et al.*, 2003). On the other hand, the presence of polyterpenes only in the ethanolic extract suggests that these compounds are more soluble in ethanol, while the detection of triterpenes and steroids in the aqueous extract indicates that these compounds can be effectively extracted with water. The absence of reducing compounds and saponins in both extracts might indicate a low concentration or inefficient extraction by the method used.

Furthermore, the results show that the ethanolic extract contains a higher concentration of total phenolic compounds (0.441 mgGAE/g MS) compared to the aqueous extract (0.168 mgGAE/g MS). Since phenolic compounds are known for their antioxidant properties (Bruneton, 1999), this concentration difference could explain a potentially superior antioxidant efficacy of the ethanolic extract. Additionally, total condensed tannins are also more concentrated in the ethanolic extract (0.052 mgCTE/g MS) than in the aqueous extract (0.023 mgCTE/g MS), indicating increased therapeutic efficacy in astringent and anti-inflammatory areas (Kabran *et al.*, 2011). The total flavonoid content is significantly higher in the ethanolic extract (0.117 mgQE/g MS) compared to the aqueous extract (0.047 mgQE/g MS), suggesting that the ethanolic extract could offer more robust antioxidant protection.

However, despite these high concentrations, the extracts did not inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. This lack of inhibition could be due to an insufficient concentration of active compounds or the natural resistance of the tested bacteria (Ponce *et al.*, 2003; Guessennd *et al.*, 2005). Therefore, it would be useful to explore alternative extraction methods or increase the concentration of the extracts to better understand their antibacterial potential.

CONCLUSION

The study highlights the richness of bioactive compounds in the leaves of *Erigeron floribundus*, with ethanol proving to be more effective in extracting phenolic compounds, tannins, and flavonoids. However, the observed lack of antibacterial activity suggests the need for further research to optimize extraction conditions or to test more concentrated fractions in order

to better assess the potential therapeutic properties of this plant.

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