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Phytochemical characterization and antioxidant potential of the leaves of *Calyptrochilum christyanum* (Rchb.f.) Summerh. collected in Man, Western Côte d'Ivoire

Ebalah Delphine MONYN-KOUAME*

Faculty of Agronomic, Forestry and Environmental Engineering, University of Man, BP 20 Man, Côte d'Ivoire
e-mail: ebalahmo@gmail.com

Kouamé Séraphin KOUASSI

Faculty of Fundamental and Applied Sciences, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire
e-mail: monsieurkouassi01@gmail.com

Lanciné TRAORE

Faculty of Science and Technology, University of Man, BP 20 Man, Côte d'Ivoire
e-mail: lancine.traore@univ-man.edu.ci

Mamidou Witabouna KONE

Swiss Center for Scientific Research in Côte d'Ivoire, 01 BP 1303 Abidjan 01, Côte d'Ivoire
e-mail: mamidou.kone@csrs.ci

Abstract

Calyptrochilum christyanum (Rchb.f.) Summerh. is an orchid species reported to be used in traditional medicine in Côte d'Ivoire, but its phytochemical composition and biological properties remain poorly documented. This study aimed to characterize the phytochemical profile and evaluate the antioxidant activity of aqueous and hydroethanolic leaf extracts of *C. christyanum*. Thin-layer chromatography (TLC) was used for phytochemical screening, while total phenolic compounds, flavonoids, and condensed tannins were quantified using colorimetric methods. Antioxidant activity was evaluated by TLC-DPPH bioautography and spectrophotometric DPPH assays. TLC revealed the presence of tannins, flavonoids, coumarins, and anthraquinone/anthrone derivatives. The aqueous extract exhibited the highest levels of total phenolic content (157.75 ± 3.89 mg GAE/g extract), flavonoids (87.53 ± 6.11 mg QE/g extract), and condensed tannins (17.35 ± 1.26 mg CE/g extract). However, the hydroethanolic extract showed stronger antioxidant activity (IC_{50} = 0.197 mg/mL) than the aqueous extract (IC_{50} = 0.739 mg/mL). These findings highlight *C. christyanum* leaves as a potential source of antioxidant phytochemicals.

Introduction

Medicinal plants play an important role in primary healthcare, particularly in developing countries where access to conventional medicines remains limited. In sub-Saharan Africa, traditional medicine relies extensively on plant resources because of their accessibility, cultural acceptance, and therapeutic potential. These plants represent valuable sources of bioactive secondary metabolites, including phenolic compounds, flavonoids, tannins, alkaloids, and terpenoids, which are associated with various biological activities, notably antioxidant, anti-inflammatory, and antimicrobial effects [1–3]. Among the biological properties attributed to medicinal

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*Corresponding author

plants, antioxidant activity has received considerable attention due to the involvement of oxidative stress in the development of several chronic diseases. Oxidative stress results from an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, leading to cellular damage and contributing to the pathogenesis of disorders such as diabetes, cardiovascular diseases, neurodegenerative diseases, and cancer. Consequently, the search for natural antioxidants, particularly plant-derived phenolic compounds such as flavonoids and tannins, has increased due to their ability to scavenge free radicals and mitigate oxidative damage [3,4]. The growing interest in plant-based antioxidants has encouraged the investigation of underexplored medicinal species, including members of the Orchidaceae family. *Calyptrochilum christyanum* (Rchb.f.) Summerh. (Orchidaceae) is a monopodial epiphytic orchid widely distributed throughout tropical Africa, including Côte d'Ivoire, where it occurs in various ecological environments [5]. In western Côte d'Ivoire, particularly in the Man region, this species is associated with traditional medicinal practices. However, despite its ethnomedicinal relevance, scientific information regarding its phytochemical composition and biological properties remains limited. Previous investigations on *C. christyanum* have mainly focused on its pharmacognostic characteristics and biological potential, with preliminary phytochemical studies reporting the presence of several classes of bioactive compounds, including flavonoids, phenolic compounds, tannins, terpenoids, and glycosides [6,7]. Nevertheless, data concerning the phenolic composition and antioxidant properties of aqueous and hydroethanolic leaf extracts of this species remain scarce [6–8]. Since the biological activities of plant extracts are strongly influenced by their chemical composition, the choice of extraction solvent represents an important factor in the recovery of bioactive compounds. Water and hydroethanolic mixtures are among the most commonly used solvents for medicinal plant extraction because of their ability to extract a wide range of polar phytoconstituents, particularly phenolic compounds, and their relevance in pharmacological investigations [2,9]. Therefore, the present study aimed to investigate the phytochemical profile, phenolic composition, and antioxidant potential of aqueous and hydroethanolic leaf extracts of *Calyptrochilum christyanum*. The findings provide new insights into the bioactive constituents of this poorly investigated species and contribute to the scientific validation of its traditional medicinal use.

Material and Methods

Plant Material

Leaves of *Calyptrochilum christyanum* (Rchb.f.) Summerh. (Figure 1) were collected in March 2026 from a natural habitat located approximately 5 km from Man, in western Côte d'Ivoire. The collected leaves were thoroughly washed with tap water to remove adhering impurities and subsequently air-dried in an air-conditioned room maintained at 18 °C for two weeks. The dried plant material was then finely powdered using an electric grinder and stored in airtight glass containers at room temperature until further analysis. The plant material was botanically authenticated at the National Floristic Center of Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire. A voucher specimen was deposited in the herbarium collection of the institution under voucher number UCJ013611 for future reference.

Figure 1. Leafy stem of *Calyptrochilum christyanum* (Rchb.f.) Summerh collected in Man, western Côte d'Ivoire.

Extraction procedures

Aqueous extraction

Aqueous extraction was performed with slight modifications according to a previously reported method [10]. Briefly, 25 g of powdered *Calyptrochilum christyanum* leaves were extracted with 250 mL of distilled water. The mixture was heated to boiling and maintained under continuous mechanical stirring for 30 min. The extract was then filtered under vacuum through Whatman No. 1 filter paper. The filtrate was concentrated and dried in an oven at 50 °C for 24 h. The dried extract was weighed, and the extraction yield was calculated according to the following equation:

$$\textbf{Extraction yield (\%)} = (\text{Mass of dried extract} / \text{Mass of plant powder}) \times 100$$

Hydroethanolic extraction

Hydroethanolic extraction was performed according to a previously described method [11], with slight modifications. Briefly, 25 g of powdered leaves were macerated in 250 mL of 70% ethanol (v/v) under continuous mechanical stirring for 24 h. The resulting macerate was filtered under vacuum using Whatman No. 1 filter paper. The filtrate was evaporated to dryness in an oven at 40 °C for 24 h. The dried extract was weighed, and the extraction yield was calculated using the same formula described above. The obtained crude extracts were stored in airtight containers at 4 °C until further phytochemical and antioxidant analyses.

Phytochemical screening by thin-layer chromatography (TLC)

Thin-layer chromatography (TLC) was performed to identify the main groups of secondary metabolites present in the aqueous and hydroethanolic extracts of *Calyptrochilum christyanum* leaves. Extract solutions

were prepared at a concentration of 10 mg/mL by dissolving 10 mg of crude extract in 1 mL of methanol. Twenty microliters of each extract solution were applied onto silica gel 60 F254 plates (aluminium support, 0.2 mm thickness). The plates were developed in a saturated chromatographic chamber containing a solvent system composed of chloroform/ethyl acetate/acetic acid/water/ammonia (2.5:7:0.2:1:2 drops, v/v/v/v/v).

After development, the plates were dried and examined under ultraviolet light at 254 and 365 nm, as well as under visible light. Different specific spray reagents were used for the detection of phytochemical groups according to a previously described procedure [12].

Determination of total phenolic content

The total phenolic content was determined using the Folin–Ciocalteu method according to a previously described procedure [13], with slight modifications. Briefly, 1 mL of each extract solution was mixed with 0.5 mL of Folin–Ciocalteu reagent (0.5 N) and 1.5 mL of sodium carbonate solution (17%, w/v). The reaction mixture was incubated at 37 °C for 30 min, and the absorbance was measured at 760 nm using a spectrophotometer against a reagent blank. Quantification was performed using a calibration curve established with gallic acid as the reference standard at concentrations ranging from 0 to 1000 µg/mL under the same experimental conditions. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

Determination of total flavonoid content

The total flavonoid content was determined using the aluminum chloride method according to a previously described procedure [14], with slight modifications. Briefly, 500 µL of each extract solution was mixed with 500 µL of 2% aluminum chloride solution prepared in methanol. After incubation for 10 min at room temperature, the absorbance was measured at 415 nm using a spectrophotometer. Quantification was performed using a calibration curve established with quercetin as the reference standard at concentrations ranging from 0 to 100 µg/mL. The total flavonoid content was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract).

Determination of condensed tannin content

The condensed tannin content was determined according to the vanillin–hydrochloric acid method [15], with slight modifications. Briefly, 400 µL of each extract solution or catechin standard solution was mixed with 3 mL of 4% vanillin solution prepared in methanol and 1.5 mL of concentrated hydrochloric acid. The mixture was incubated for 15 min at room temperature, and the absorbance was measured at 500 nm.

The condensed tannin content was calculated using a calibration curve established with catechin as the reference standard (0–300 µg/mL). Results were expressed as milligrams of catechin equivalents per gram of extract (mg CE/g extract)

TLC-DPPH bioautography assay

The antioxidant activity was evaluated by TLC-DPPH bioautography according to a previously described method [16], with slight modifications. Briefly, 10 µL of each extract solution were applied onto silica gel 60 F254 TLC plates (aluminium support). The plates were developed using the solvent system chloroform/ethyl

acetate/acetic acid/water/ammonia (2.5:7:0.2:1:2 drops, v/v/v/v/v). After development, the chromatograms were dried and sprayed with a freshly prepared ethanolic DPPH solution. Antioxidant compounds appeared as yellowish or pale yellow spots against a purple background after 30 min of incubation, indicating the reduction of the DPPH radical. The retention factor (Rf) values of active compounds were calculated according to the following equation:

$$\mathbf{Rf} = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent}}$$

Spectrophotometric DPPH radical scavenging assay

The DPPH radical scavenging activity was determined according to a previously described method [17], with slight modifications. A DPPH solution was prepared by dissolving DPPH in absolute ethanol to obtain a final concentration of 0.03 mg/mL. Different concentrations of each extract (0.015625–0.5 mg/mL) were prepared in absolute ethanol. Subsequently, 1 mL of each extract solution was mixed with 2 mL of DPPH solution. The mixtures were incubated in the dark for 30 min at room temperature, and the absorbance was measured at 517 nm against a reagent blank. A control consisting of 2 mL of DPPH solution and 1 mL of absolute ethanol was used to determine A_0. Ascorbic acid (vitamin C) was used as the positive control under the same experimental conditions. The percentage of DPPH radical inhibition was calculated using the following equation:

$$\textbf{DPPH inhibition (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 represents the absorbance of the DPPH control and A_1 represents the absorbance of the sample. The IC_{50} value, defined as the concentration of extract required to inhibit 50% of DPPH radicals, was determined from the concentration–response curves. All measurements were performed in triplicate.

Data processing

Data were entered and processed using Microsoft Excel 2007. The results are expressed as mean ± standard deviation (SD) of three independent measurements (n = 3). Differences between aqueous and hydroethanolic extracts were evaluated using a two-tailed Student's t-test for independent samples, with statistical significance set at $p < 0.05$. Calibration curves for the determination of total phenolic, flavonoid, and condensed tannin contents were generated by linear regression using Excel. IC_{50} values were determined from the concentration–response curves of DPPH radical inhibition. The concentration-dependent antioxidant response was analyzed by nonlinear four-parameter logistic regression after logarithmic transformation of the concentrations. The half-maximal inhibitory concentration (IC_{50}), defined as the concentration required to inhibit 50% of DPPH radicals, was calculated from the fitted dose–response curve. Lower IC_{50} values indicate stronger antioxidant activity. The IC_{50} values were calculated using the same procedure for both plant extracts and the ascorbic acid reference standard.

Results

Extraction yields

The extraction yields obtained from *Calyptrochilum christyanum* leaves varied according to the extraction solvent. The aqueous extraction showed the highest yield (11.20 ± 0.32%), whereas the hydroethanolic

extraction resulted in a lower yield (3.60 ± 0.17%) (Table 1). These results indicate that water extracted a greater quantity of soluble compounds from the plant material compared with the hydroethanolic solvent under the experimental conditions used.

Table 1. Extraction yields of aqueous and hydroethanolic extracts from *Calyptrochilum christyanum* leaves

Plant Species	Type of Extract	Powder Weight (g)	Extract Weight (g)	Yield (%)
Calyptrochilum	Aqueous	25	2.8± 0.08	11.20 ± 0.32
christyanum	Hydro-ethanolic	25	0.9± 0.04	3.60 ± 0.17

Values are expressed as mean ± SD (n = 3).

Phytochemical screening by TLC

Thin-layer chromatography (TLC) revealed the presence of several classes of secondary metabolites in both aqueous and hydroethanolic extracts of *Calyptrochilum christyanum* leaves (Table 2). Ferric chloride reagent revealed the presence of tannins through the appearance of black spots in both extracts. Yellow-orange spots observed after spraying with potassium hydroxide confirmed the presence of anthraquinones and anthrones. Treatment with aluminum chloride produced yellow fluorescence under UV light at 365 nm, indicating the presence of flavonoids in the hydroethanolic extract, whereas no fluorescent spot was detected for the aqueous extract under the same conditions. Likewise, observation under UV light after spraying with 25% ammonia solution revealed yellow fluorescent spots characteristic of coumarins.

Overall, the phytochemical profile demonstrated that both extracts contain a diversity of phenolic secondary metabolites known for their antioxidant properties.

Table 2. Phytochemical profile of aqueous and hydroethanolic leaf extracts of *Calyptrochilum christyanum* determined by thin-layer chromatography

Reagents / **Extracts**	Ferric chloride 10 %		Potassium hydroxide 5 %		Aluminum chloride 5 %				Ammonia 25 %	
	Visible		Visible		Visible		365nm		365 nm	
	Rf	Color	Rf	Color	Rf	Color	Rf	Color	Rf	Color
Aqueous extract	0.22	Black	0.94	Yellow	0.95	yellow	Nd		0.08	yellow
Hydroethanolic extract	0.09	Black	0.94	Yellow	0.95	yellow	0.44	yellow	0.13 0.63 0.94	yellow green yellow

Nd = Not detected.

Total phenolic, flavonoid and condensed tannin contents

Quantitative analysis showed that both extracts contained appreciable amounts of phenolic compounds (Table 3).

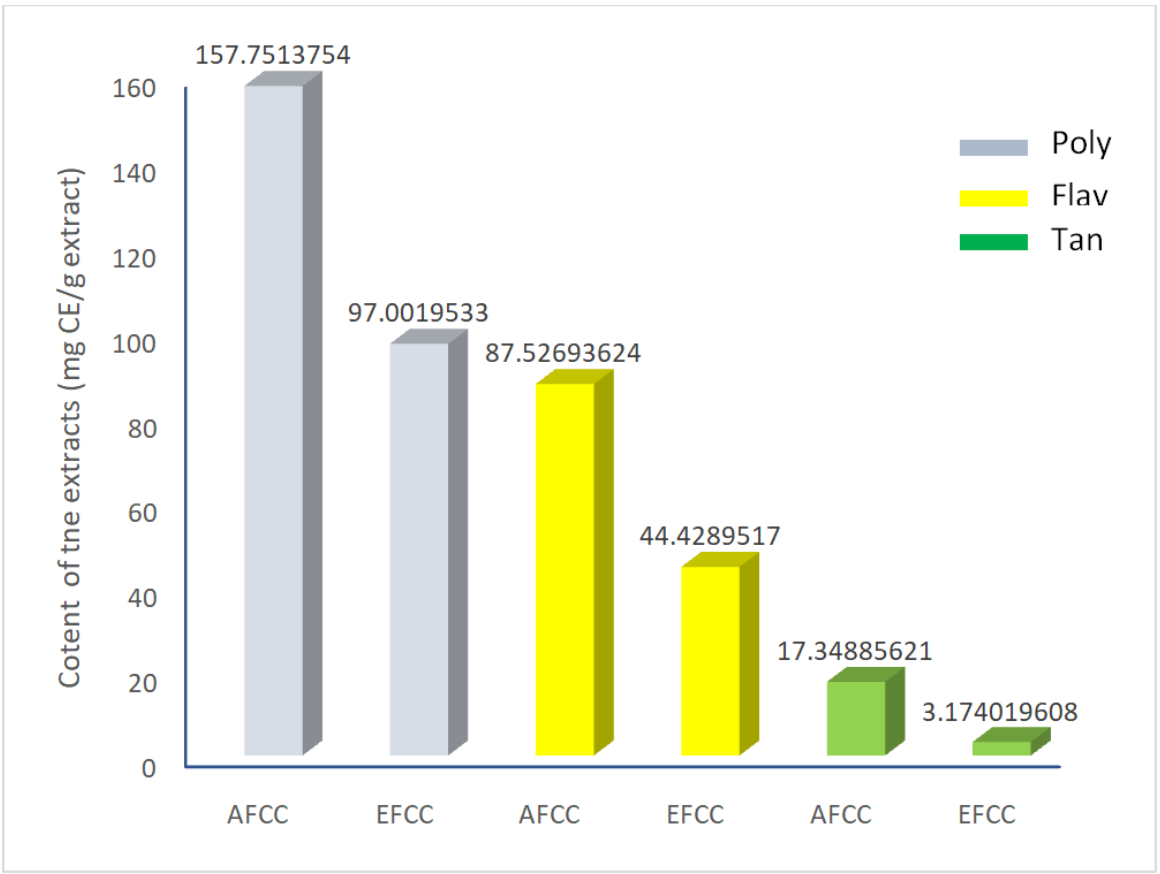
Table 3. Total phenolic, flavonoid, and condensed tannin contents of aqueous and hydroethanolic extracts of *Calyptrochilum christyanum* leaves

Parameters	Aqueous (n=3)	Hydroethanolic (n=3)	T	df	p
Total polyphenols (mg GAE/g extract)	157.75 ± 3.89	97.00 ± 3.78	19.41	4	**0.0000415**
Total flavonoids (mg QE/g extract)	87.53 ± 6.11	44.43 ± 0.63	12.15	4	**0.000263**
Condensed tannins (mg CE/g extract)	17.35 ± 1.26	3.17 ± 1.18	14.23	4	**0.000142**

Values are expressed as mean ± standard deviation (SD) (n = 3). t = Student's t-test statistic; df = degrees of freedom; p = p-value; GAE = gallic acid equivalents; QE = quercetin equivalents.

Differences were considered statistically significant at $p < 0.05$.

The aqueous extract showed the highest total phenol content, reaching 157.75 ± 3.89 mg GAE/g extract, along with the highest concentrations of flavonoids and condensed tannins (Table 3). These results indicate that water was more efficient than 70% ethanol in extracting phenolic compounds from C. christyanum leaves under the experimental conditions employed. The histogram (Figure 2) makes it easier to appreciate this clear difference.



AFCC = Aqueous extract of *Calyptrochilum christyanum* leaves; EFCC = Hydro-ethanolic extract of *Calyptrochilum christyanum*

Figure 2. Comparison of total phenolic, flavonoid, and condensed tannin contents between aqueous and hydroethanolic extracts of *Calyptrochilum christyanum* leaves.

Antioxidant activity

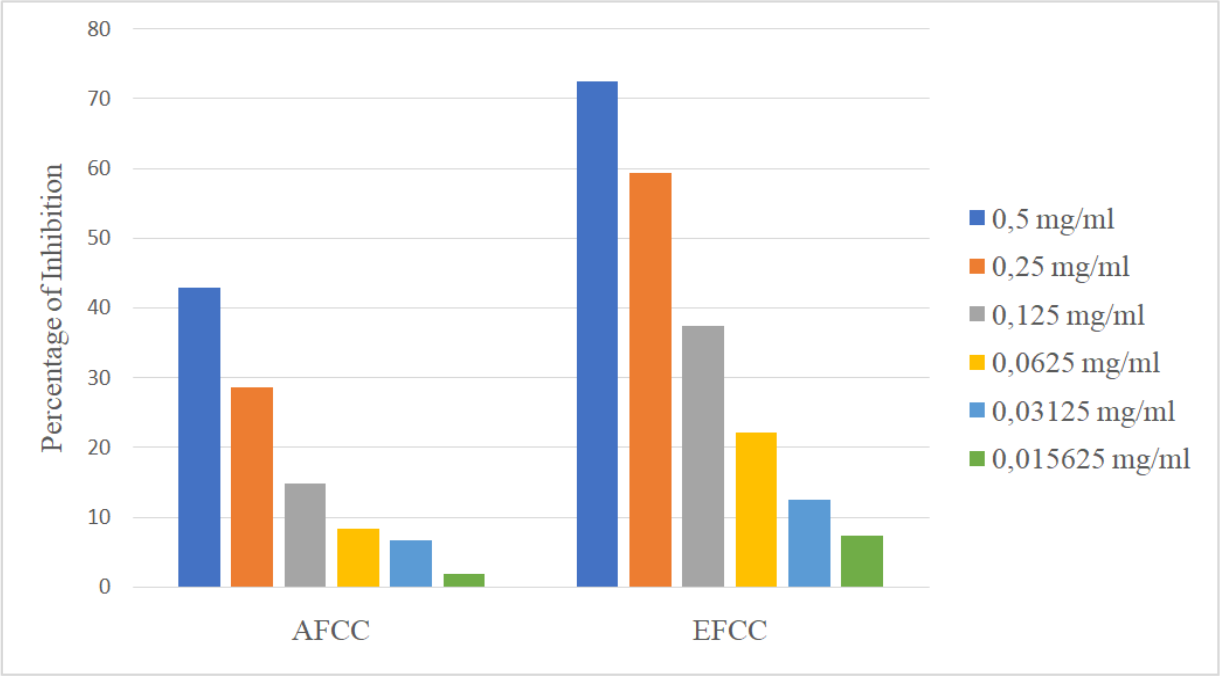
Antioxidant activity evaluated by TLC-DPPH bioautography

The TLC-DPPH bioautography assay revealed yellow spots against a purple background for both aqueous and hydroethanolic extracts, indicating the presence of antioxidant compounds capable of scavenging DPPH free radicals. The distribution of these active spots suggests that several phytochemicals contribute to the antioxidant activity of the plant.

DPPH radical scavenging activity

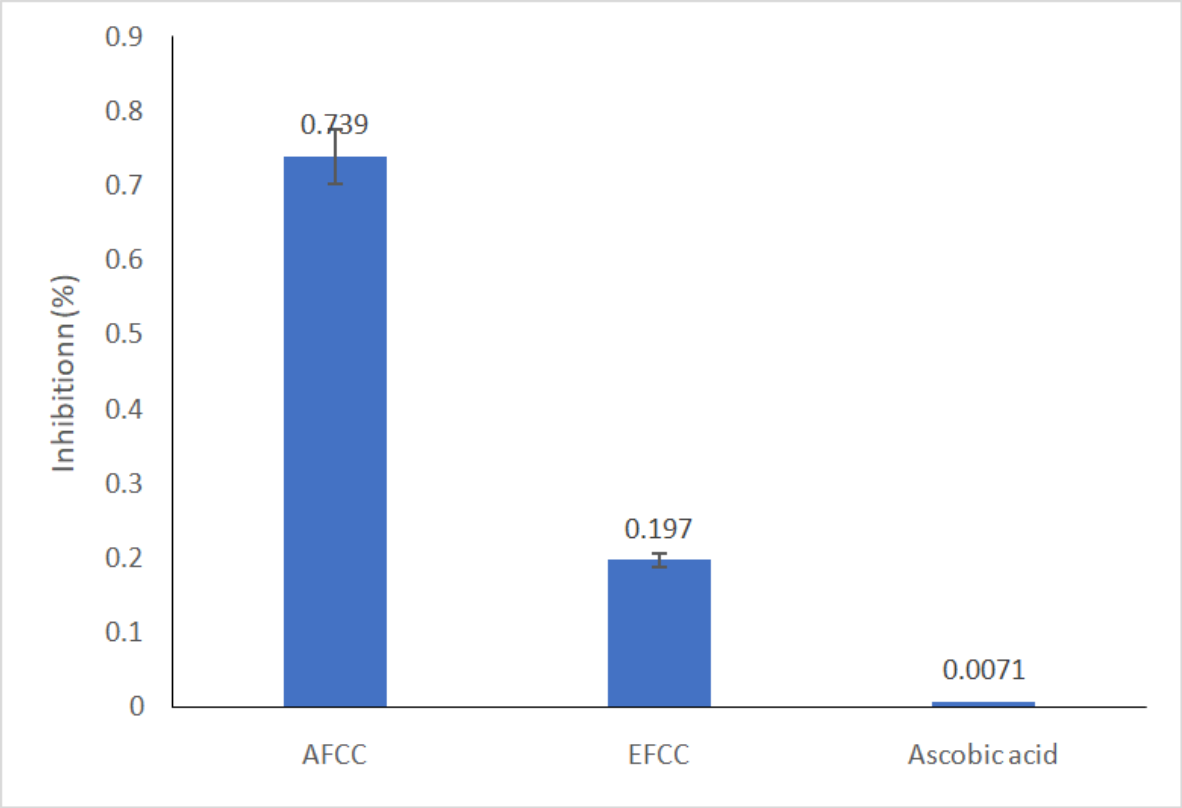
Both extracts exhibited concentration-dependent DPPH radical scavenging activity (Figure 3). The percentage of inhibition increased progressively with increasing extract concentration.

The hydroethanolic extract exhibited the strongest antioxidant activity with an IC_{50} value of 0.197 mg/mL, whereas the aqueous extract showed a lower activity with an IC_{50} of 0.739 mg/mL. Nevertheless, both extracts were less active than the reference antioxidant, ascorbic acid, which presented an IC_{50} value of 0.007 mg/mL (Figure 4).



AFCC = Aqueous extract of *Calyptrochilum christyanum* leaves; EFCC = Hydro-ethanolic extract of *Calyptrochilum christyanum*

Figure 3. DPPH radical scavenging activity of aqueous and hydroethanolic extracts of *Calyptrochilum christyanum* leaves at different concentrations.



AFCC = Aqueous extract of *Calyptrochilum christyanum* leaves; EFCC = Hydro-ethanolic extract of *Calyptrochilum christyanum*

Figure 4. IC_{50} values of *Calyptrochilum christyanum* extracts compared with ascorbic acid.

Discussion

The present study provides new insights into the phytochemical composition and antioxidant potential of *Calyptrochilum christyanum* leaves collected in western Côte d'Ivoire. Thin-layer chromatography revealed several classes of secondary metabolites, including phenolic compounds, flavonoids, tannins, coumarins and anthraquinones. These phytochemical groups are widely associated with antioxidant activity through their ability to donate electrons or hydrogen atoms and to neutralize reactive oxygen species [3,18]. The extraction process strongly influenced the recovery of phytochemicals. The aqueous extract showed a higher extraction yield (11.20%) than the hydroethanolic extract (3.60%), suggesting that the aqueous extraction conditions recovered a greater amount of extractable material from the leaves. This observation is consistent with the influence of solvent polarity on the extraction of plant secondary metabolites, particularly phenolic acids, flavonoid glycosides and tannins [2,9]. Quantitative analysis further showed that the aqueous extract contained higher levels of total phenolic compounds (157.75 ± 3.89 mg GAE/g extract), flavonoids (87.53 ± 6.11 mg QE/g extract) and condensed tannins (17.35 ± 1.26 mg CE/g extract). These findings confirm the richness of C. christyanum leaves in phenolic constituents and are consistent with previous reports describing flavonoids, tannins and other secondary metabolites in this species [6,7]. Interestingly, the higher phenolic content of the aqueous extract did not correspond to the strongest antioxidant activity. The hydroethanolic extract exhibited a lower IC_{50} (0.197 mg/mL) than the aqueous extract (0.739 mg/mL), indicating stronger DPPH radical-scavenging activity. Both extracts were less active than ascorbic acid (IC_{50} = 0.007 mg/mL). This difference suggests that antioxidant activity is not determined solely by the total amount of phenolic compounds, but may

also depend on the chemical nature, relative abundance and interactions of individual antioxidant constituents [3,18]. The antioxidant activity observed in the present study is also noteworthy when compared with previously published results. A methanolic extract of C. christyanum reported by Sidiq et al. [19] showed an IC_{50} of 0.0505 ± 0.0006 mg/mL, indicating stronger activity than the hydroethanolic extract obtained in the present study (0.197 mg/mL). Similarly, the methanolic tuber extract of the orchid Satyrium nepalense exhibited an IC_{50} of 0.0308 mg/mL [20]. In contrast, the antioxidant activity of the hydroethanolic extract of C. christyanum was comparable to that reported for the hydroethanolic extract of Sonchus oleraceus (0.19 ± 0.08 mg/mL) and was within the same order of magnitude as that reported for the methanolic leaf extract of Picralima nitida (0.24 ± 0.03 mg/mL) [21]. These comparisons indicate that the hydroethanolic extract of C. christyanum possesses a relevant radical-scavenging capacity. Although its activity was lower than that reported for some methanolic orchid extracts, it was comparable to that of other medicinal plants, supporting the antioxidant potential of this poorly investigated species. However, differences in plant organs, geographical origin, extraction solvents and experimental conditions should be considered when comparing IC_{50} values among studies. The stronger antioxidant activity of the hydroethanolic extract, despite its lower total phenolic content, may therefore reflect the selective extraction of compounds with higher radical-scavenging efficiency or possible synergistic interactions among moderately polar constituents [2,3]. The TLC-DPPH bioautography results, which revealed antioxidant spots in both extracts, further support the presence of several compounds potentially contributing to the observed activity.

From a biological perspective, these findings provide preliminary evidence supporting the interest of C. christyanum as a source of natural antioxidant compounds. The results also contribute to the scientific valorization of an under-investigated orchid species used in traditional medicine in Côte d'Ivoire. Further studies should combine chemical and biological approaches, including HPLC-DAD, LC-MS/MS and NMR analyses to identify and characterize the compounds responsible for the antioxidant activity, together with in vitro and in vivo biological assays and toxicity evaluations to clarify their biological relevance and safety.

Conclusion

This study provides valuable information on the phytochemical composition and antioxidant potential of *Calyptrochilum christyanum* (Rchb.f.) Summerh. leaves collected in western Côte d'Ivoire. Thin-layer chromatography revealed the presence of several classes of secondary metabolites, including flavonoids, tannins, coumarins and anthrones/anthraquinones derivatives, indicating the presence of diverse phytochemical constituents in this species. The quantitative analysis demonstrated significant levels of phenolic compounds, with the aqueous extract exhibiting the highest contents of total phenolics, flavonoids and condensed tannins. Both aqueous and hydroethanolic extracts showed antioxidant activity in the TLC-DPPH and spectrophotometric DPPH assays. Although the aqueous extract contained higher amounts of phenolic compounds, the hydroethanolic extract displayed stronger radical-scavenging activity, suggesting that antioxidant potential depends not only on the total phenolic content but also on the nature and bioactivity of individual compounds present in the extracts. These findings contribute to the scientific valorization of C. christyanum, an under-investigated orchid species from Côte d'Ivoire, and provide preliminary evidence supporting its potential as a source of natural antioxidant compounds. Further studies involving the isolation, identification and structural characterization of the active molecules, as well as in vivo biological evaluation and toxicity assessment, are necessary to confirm its biological potential.

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