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Phytochemical and Biological Investigation of *Nephrolepis biserrata*, a Fern Variety From Côte D'Ivoire

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ABSTRACT

This research work was carried out on the species *Nephrolepis biserrata* (Sw.) Schott (Nephrolepidaceae), a fern from Côte d'Ivoire with the aim of establishing its phytochemical, antioxidant and biological profile. The results obtained from the phytochemical screening show that said species contains alkaloids, coumarins, flavonoids, polyphenols, sterols, terpenes, and tannins. The antioxidant activity was evaluated spectrophotometrically against the DPPH radical in comparison with that of vitamin C. The determination of the antibacterial parameters indicates that *N. biserrata* doesn't exhibit any bactericidal action against the bacterial strains tested. Regarding the vermifugal activity against earthworms, decocts of *N. biserrata* showed an anthelmintic effect, which however remains less pronounced than that of albendazole, the anthelmintic antiparasitic taken as a reference.

Keywords: *Nephrolepis biserrata*, phytochemical screening, antioxidant, antibacterial, vermicide.

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INTRODUCTION

Nephrolepis biserrata (Sw.) Schott commonly known as the oil palm fern, can reach 2 m in height and has tufted fronds¹. It can also be terrestrial. Ferns are one of the plants without flowers and without seeds, and reproduce by hidden organs. *N. biserrata* is used in the South-East of Côte d'Ivoire as a traditional medicinal product in various forms of medicinal preparations in the fight against various health problems (whitlow, wounds, boils, fontanel, malaria, umbilical hernia in babies), for the extraction of thorns, and to fight against infantile nonchalance¹. This plant finds a decorative application. The fronds of *N. biserrata* are used in local friction to combat febrile muscle aches². Among the Krobou (department of Agboville / Côte d'Ivoire), the fresh leaves, kneaded with grains of *Aframomum meleguetta* K. Schum (Zingiberaceae) give a paste, which diluted in water, constitutes a solution recommended as a beverage against hiccups³.

The present study aims to identify the phytochemical families on which depend the pharmacological properties attributed to *N. biserrata*, a pteridophyte (vascular plant) of the fern class growing in Côte d'Ivoire.

MATERIALS AND METHOD

Material

Plant material

The aerial part (leaves and stems) of terrestrial and epiphytic *Nephrolepis biserrata* was the study plant material. It was harvested on August 10, 2020 in a palm grove in Ebimpé (5 ° 29'34 " North, 4 ° 04'30 " West), in the town of Anyama (5 ° 29 ' 40 " North, 4 ° 03 ' 06 " West) in Côte d'Ivoire. The material was authenticated at Centre National de Floristique (CNF) of Abidjan, in accordance with the herbarium number 4CJ018137. The harvested organs were cleaned of impurities with tap water, then dried in an air-conditioned room (18 ° C) for 2 weeks. After grinding the organs using an electric grinder, the powders obtained were stored in hermetically sealed jars for the preparation of the crude extracts.

Biological material

The biological material consists of *Lumbricus terrestris* Linn. (Earthworms) collected on the campus of NANGUI ABROGOUA University (5 ° 23 ' 21 " North, 4 ° 01 ' 09 " West) in Abidjan and from bacterial strains, namely *Escherichia coli* (466 TR/20 CNRa, 470 UB/20 CNRa), *Salmonella* sp (109 UB/20 CNRa), *Acinetobacter baumannii* (531 UB/20 CNRa), *Klebsiella pneumoniae* (471 UB/20 CNRa), *Enterobacter cloacae* (543 T/20 CNRa), *Staphylococcus aureus*

(ATCC 25923, 211 UB/20 CNRa, 483 UB/20 CNRa) et *Pseudomonas aeruginosa* (ATCC 27853, 469 UB/20 CNRa, 551 UB/20 CNRa) from the bio-bank of the Pasteur Institute of Côte d'Ivoire.

Methods

Phytochemical investigations

Extraction by decoction

To 5 g of vegetable powder introduced into a ground-necked flask connected to a condenser, are added 100 mL of distilled water. The solution was boiled for 30 min in a heat cap. After cooling, filtration of the supernatant was carried out. This operation was repeated three times with the same marc. The filtrates collected were concentrated in vacuo, and the concentrates (crude extracts) coded S (for the terrestrial species), E (for the epiphytic species) and M (for the equimassic mixture of the 2 species) were kept in a oven at 50 ° C for 48 h, then stored in a refrigerator (4 ° C).

Selective extractions

To 1 g of crude extract (E, S and M) dissolved in 25 mL of distilled water was successively exhausted with solvents of varying polarity (n-hexane, chloroform, ethyl acetate, n-butanol). The different organic fractions obtained were used for the phytochemical screening by thin layer chromatography (TLC).

Phytochemical screening

Phytocompounds were detected using color reaction and TLC tests^{4,5,6}.

Quantification of some phytophenols

The amount of total phenols in the extracts was determined by the Folin-Ciocalteu colorimetric method^{7,8}. For total flavonoids, the assay was performed according to Hariri et al.⁹ and Kadja (2014)¹⁰.

The dosage of condensed tannins (catechetical tannins) was done according to Broadhurst and Jones (1978)¹¹ and Heilmer et al.¹²

Evaluation of antioxidant activity by spectrophotometry

The quantitative evaluation of the antioxidant potential of selective extracts was made in comparison with vitamin C¹³. The oxidant used was the DPPH radical (2,2-diphenyl 1-picrylhydrazyl).

Biological investigations

Antibacterial tests

Efficiency test

The commercial antibiotics Ceftriaxone (CRO), Imipenem (IPM) or Cefoxitin (FOX) were placed on the agar, and served as positive controls^{14,15}.

Determination of antibacterial parameters MBC, MIC, MBC/MIC

The minimum bactericidal (MBC) and inhibitory (MIC) concentrations were determined using the liquid dilution method^{16,17}. The MBC / MIC ratio made it possible to specify the modality of action of the extracts against bacterial strains¹⁸.

Vermicidal tes

3 batches of 6 worms were made:

- batch 1 (negative control) treated with distilled water,
- batch 2 was treated respectively with 5 different concentrations of extracts of *N. biserrata* (100, 60, 50, 40 et 20 mg/mL).
- batch 3 (positive control) treated at the same concentrations as the crude extracts with albendazole (propylthio-5 *I*H-benzimidazolyl-2 methyl carbamate), an anthelmintic antiparasitic sold in pharmacies under the name Zentel.

Earthworm lethality was observed for 24 h. The 100% lethal time (time at which the administered dose results in the death of 100% of the worms) was noted^{19,20}. For each concentration, the experiment is repeated 3 times.

RESULTS AND DISCUSSION

Yield of crude extracts

The results (Table I) indicate the yields of phytochemicals extracted from the various study organs.

Table I: Yield of crude extracts

Vegetable extract	M (g)	M (g)	Yield (%)
Terrestrial (S)	5	0.95	19.0
Mixed (M)	5	1.01	20.2
Epiphyte (E)	5	0.92	18.4

Phytochemical composition

Phytoconstituents detected by color reactions

Based on the detection of phytoconstituents through the action of appropriate reagents, detection tests have revealed some phytochemical families by the appearance of characteristic colorings, visible to the naked eye^{4,21}. The results are summarized in Table II.

Table II: Phytochemicals detected in the crude extracts

Researched phytochemicals	Reagents	E	S
Alkaloids	Dragendorff	-	-
	Picric acid	-	-
Coumarins	KOH	+	+
Flavonoids	Mg in HCl	+	+
	(Shinoda Test)		
	Zn in HCl	+	+
	(Pew Test)		
Cardiotonic glycosides	Vanillin	+	+
	NH ₄ OH	+	+
Polyphenols	Liebermann-Bürchard	-	-
Protein	FeCl ₃ (2%)	+	+
Quinones	Biuret reaction	+	+
Saponins	Borntraëger	-	-
Sterols and Terpenes	Test de mousse	-	-
Tannins	Liebermann-Bürchard	+	+
	Stiasny	+	+

(+) : positive test; (-) : negative test

The metabolites detected (Coumarins, flavonoids, polyphenols, tannins) are endowed with various biological properties which could justify the use of the plant in unconventional medicine in the treatment of various pathologies. An absence of saponosides in the extracts is attested by the height of foam obtained^{10,22}. Indeed, no tube presented a height of persistent foam greater than or equal to 1 cm, to allow the calculation of the foam index. Also the absence of alkaloids, cardiotonic glycosides and quinones was observed in the various samples. These results corroborate those obtained by Adou et al.¹ who note the presence of polyphenols, flavonoids and catechetal tannins as well as the absence of quinones in the aqueous extract of *N. biserrata*. Flavonoids have been demonstrated with three characterization tests. The ammonia test revealed the presence of flavones, flavanones, flavonols and flavononols²³. Catechins derived from floroglucins and resorcins were also detected with 1% hydrochloric vanillin solution in all extracts. In addition, the Shinoda test or reaction known as cyanidin²³, is specific for the characterization of flavanones and dihydroflavonols. The positive result obtained after the appearance of orange-pink color shows the coexistence of flavanones and dihydroflavonols, among the flavonoids detected by the modified cyanidin test (Pew test) in the extracts²⁴. Furthermore, Table II suggests that the two types of *N. biserrata* have similar phytochemical compositions.

Phytochemicals identified by TLC

Tables (III, IV, V, VI) show the different chromatographic profiles obtained respectively at the end of the TLC carried out on the selective extracts with hexane, chloroform, ethyl acetate and n-

butanol. Anthocyanins, coumarins, flavonoids, sterols, tannins and terpenes have been identified. As for alkaloids, they appear to be present only in the ethyl acetate extract of terrestrial *N. biserrata* (Table V).

Table III: Phytochemicals identified in the hexane extracts

Extract	Possible phytochemical: [Rf], color, revealing
S ₁	Sterols: [0.20] V ^c ; [0.70] V ^c ; [0.75] Bf ^c Terpenes: [0.43] Vi ^c ; [0.81] Vi ^c Triterpenes- lupane: [0.68] Or ^e ;
E ₁	Sterols: [0.20] Ve; [0.70] Ve; [0.75] Ve; [0.87] Bf ^c Terpenes: [0.43] Vi ^c Triterpenes-lupane: [0.68] Or ^e ; [0.76] Or ^e

c: Sulfuric vanillin (visible); e: Liebermann-Burchard (under UV at 365 nm); Gold: orange, Bl: blue, Bf: fluorescent blue, Vi: purple, V: green; S₁: Terrestrial *N. biserrata*, E₁: epiphytic *N. biserrata*.

Table IV: Phytochemicals identified in the chloroform extracts.

Extract	Possible phytochemical: [Rf], color, revealing
S ₂	Anthocyanins: [0.37] Vi ^g ; [0.5] Bl ^{g1} ; [0.67] Bl ^{g1} ; [0.93] Bl ^{g1} Coumarins: [0.12] Bl ^d ; [0.3] Bl ^d ; [0.37] Bl ^a ; [0.48] Bl ^a ; [0.54] Bl ^d ; [0.68] Bl ^a Flavonoids: [0.075] J ^g ; [0.32] J ^g ; [0.6] V ^{g1} ; [0.35] V ^b ; [0.37] Bl ^b ; [0.67] V ^b
E ₂	Anthocyanins: [0.37] Vi ^g ; [0.5] Bl ^{g1} ; [0.93] Bl ^{g1} Coumarins: [0.12] Bl ^d ; [0.3] Bl ^d ; [0.37] Bl ^a ; [0.48] Bl ^a Flavonoids: [0.075] J ^g ; [0.32] J ^g ; [0.6] V ^{g1} ; [0.35] V ^b ; [0.37] Bl ^b ; [0.67] V ^b

a: Without UV developer / 365nm; b: AlCl₃ at UV / 365nm d: KOH at UV / 365nm; g: visible NH₃; g1: NH₃ at UV / 365nm; Gold: orange, Bl: blue, J: yellow, Bf: fluorescent blue, Vi: purple, V: green; S₂: Terrestrial *N. biserrata*;

E₂: Epiphytic *N. biserrata*.

Table V: Phytochemicals identified in ethyl acetate extracts.

Extract	Possible phytochemical: [Rf], color, revealing
S ₃	Anthocyanins: [0.9] Bf ^{g1} Coumarins: [0.16] Bru ^g ; [0.2] Bl ^a ; [0.31] Bl ^d ; [0.86] Bl ^a Flavonoids: [0.08] J ^g ; [0.26] Bf ^b ; [0.37] J ^g ; [0.51] J ^g ; [0.57] V ^{g1} ; [0.93] Bf ^b Tannins: [0.13] G ^f ; [0.5] G ^f Alkaloids [0.03] Or ^h
E ₃	Anthocyanins: [0.71] Bf ^{g1} Coumarins: [0.16] Bru ^g ; [0.2] Bl ^a ; [0.31] Bl ^d ; [0.86] Bl ^a Flavonoids: [0.08] J ^g ; [0.26] Bf ^b ; [0.37] J ^g ; [0.51] J ^g ; [0.57] V ^{g1} Tannins: [0.13] G ^f ; [0.5] G ^f

a: Without UV developer / 365nm; b: AlCl₃ at UV / 365nm; d: KOH at UV / 365nm; f: FeCl₃ visible; g: visible NH₃; g1: NH₃ at UV / 365nm; h: visible Dragendorff reagent; Gold: orange; Bl:

blue, J: yellow, Bf: fluorescent blue, Bru: brown, Vi: purple, V: green, G: gray; S3: Terrestrial *N. biserrata*; E3: Epiphytic *N. biserrata*.

Table VI: Phytocompounds identified in the n-Butanol extracts

Extract	Possible phytocompound: [Rf], color, revealing
S ₄	Coumarins: [0.2] B1 ^d ; [0.46] B1 ^d ; [0.5] B1 ^a ; [0.62] B1 ^a . Flavonoids: [0.18] J ^g ; [0.25] J ^g ; [0.63] J ^g ; [0.7] J ^g Tannins: [0.22] G ^f
E ₄	Coumarins: [0.2] B1 ^d ; [0.46] B1 ^d ; [0.5] B1 ^a ; . Flavonoids: [0.18] J ^g ; [0.25] J ^g ; [0.55] J ^g ; [0.63] J ^g ; [0.7] J ^g ; [0.83] J ^g Tannins: [0.22] G ^f

Without UV developer / 365nm; b: AlCl₃ at UV / 365nm; d: KOH at UV / 365nm; f: FeCl₃ visible; g: visible NH₃; g1: NH₃ at UV / 365nm; B1: blue, J: yellow; G: gray; S4: Terrestrial *N. biserrata*; E4: Epiphytic *N. biserrata*

If the chromatographic profiles seem similar, we note some differences. This is the case with the hexane extract (**Table III**) where the phytocompounds with Rf 0.87 and 0.76 were observed only in E1. The same is true for the n-butanol extract where the TLC shows spots at Rf 0.55; 0.7 and 0.83 observable exclusively in E4 (**Table VI**).

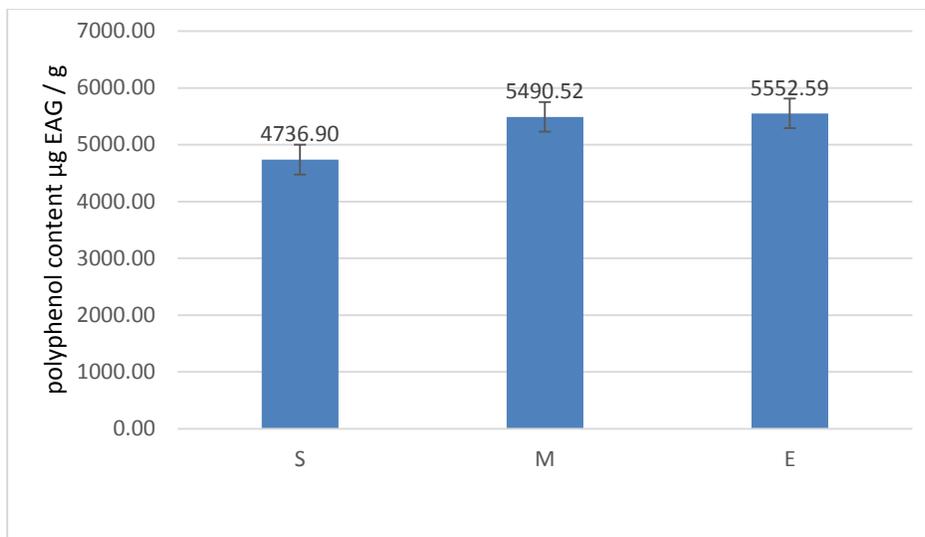
Appropriate reagents described in the literature have contributed to the determination of these secondary metabolites. Indeed, the Liebermann-Burchard reagent reveals sterols visible in brown and green, and in yellow and yellow-green under UV light at 365 nm. s for terpenes, it highlights them in the visible form in the form of blue and purple molecular spots (triterpene genins), and yellow-orange molecular spots (triterpenes-lupane) under UV exposure at 365 nm^{6,21}. The sulfuric vanillin solution detects in the visible and under UV light at 365 nm, terpenes in the form of molecular fingerprints in purple, pink, orange and sterols in blue²⁵. With the solution of aluminum chloride (AlCl₃), the flavonoids under yellow coloring are visible to the naked eye or under UV at 365 nm^{4,26}. The ammoniacal solution not only detects the flavonoids, perceptible to the naked eye and under UV at 365 nm in yellow and green²⁷, but also the anthocyanins in blue or purple colorings²¹. On contact with KOH (5% in methanol, m / v), coumarins appear as a yellow coloration in the visible range, and under UV exposure at 365 nm, the yellow intensifies or becomes blue or green²⁵. The colored tannins are highlighted in gray or brown in the visible by FeCl₃ (at 2%)^{27,28}.

Phytocomponent content

Total polyphenol content

The different amounts of polyphenols determined in the S, M and E extracts (**Figure 1**) vary between 4736.896 and 5552.586 µg EAG / g. The minimum quantity of polyphenols was obtained

in the decoct of terrestrial *N. biserrata* (4736.896 $\mu\text{g EAG} / \text{g}$). On the other hand, the extract of epiphytic *N. biserrata* has the highest polyphenol content (5552.586 $\mu\text{g EAG} / \text{g}$). The results of the work carried out by Shah *et al.*²⁹ are different. Indeed, they indicate a total polyphenol content equal to 127280 $\mu\text{g EAG} / \text{g}$, obtained from the methanol extract of *N. biserrata*. This difference may be due to the solvent and the extraction technique.

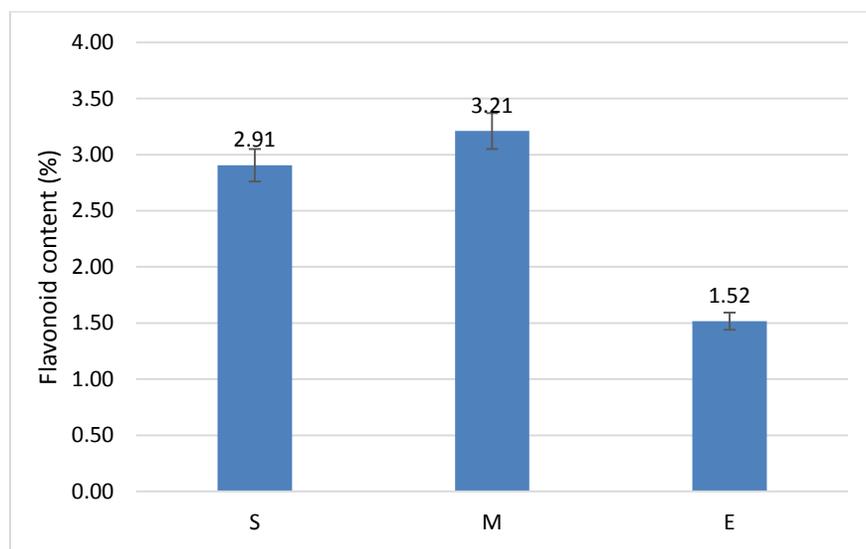


S: terrestrial *N. biserrata*; E: epiphytic *N. biserrata*; M: Equimassic mixture

Figure 1: Histograms of total polyphenol contents

Total flavonoid content

The estimated amounts of total flavonoids in the S, M and E extracts are shown in Figure 2.



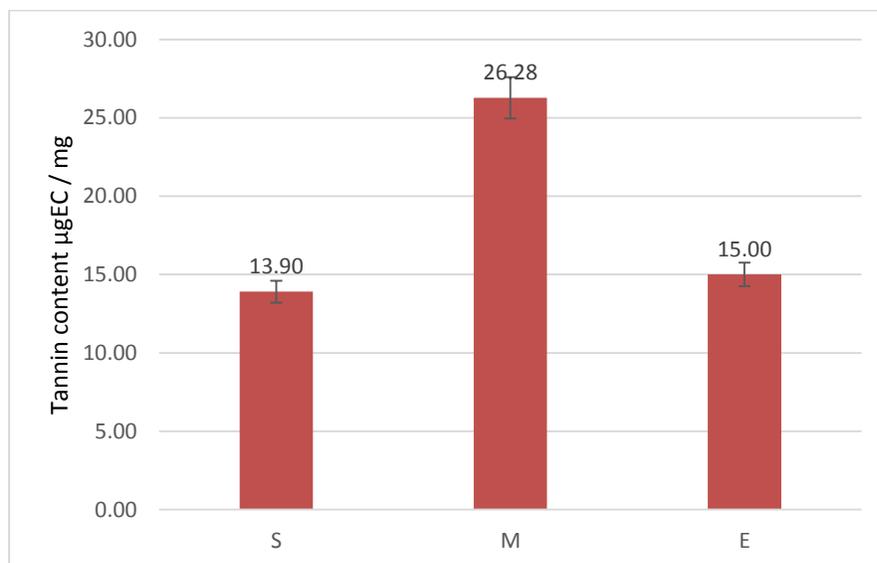
S: terrestrial *N. biserrata*; E: epiphytic *N. biserrata*; M: Equimassic mixture

Figure 2: Histograms of total flavonoid content

The minimum and maximum values were obtained respectively in E (15.166%) and M (32.111%). The S extract has a total flavonoid content of around 29%. From these results, it emerges that the M extract would be better indicated as a source of flavonoids.

Condensed tannin content

The content of condensed tannins (**Figure 3**) in $\mu\text{g EC} / \text{mg}$ of extract is obtained from the catechin calibration line ($y = 0.004x + 0.006$; $R^2 = 0.998$). Values range from 13 to 27 $\mu\text{g EC} / \text{mg}$ extract. Extract M provides the highest content of condensed tannins (26.280 $\mu\text{g EC} / \text{mg}$) compared to 13.904 $\mu\text{g EC} / \text{mg}$ for extract S and 15.00 $\mu\text{g EC} / \text{mg}$ for extract E.

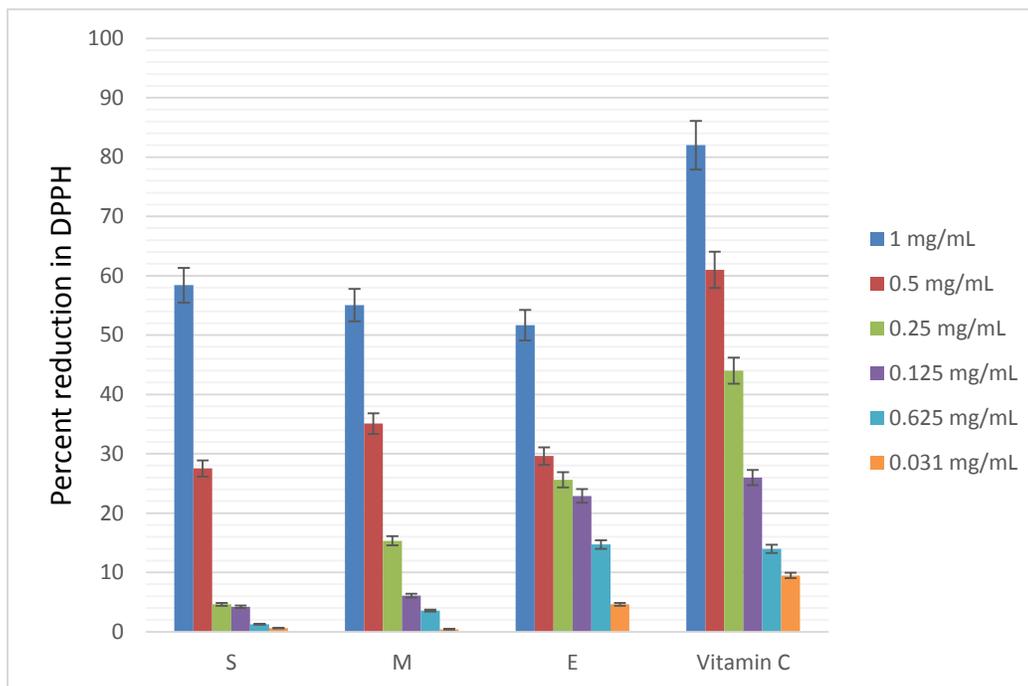


S: terrestrial *N. biserrata*; E: epiphytic *N. biserrata*; M: Equimassic mixture

Figure 3: Histograms of condensed tannin content

Antioxidant profile of crude extracts

The antioxidant activity towards the DPPH radical of the S, M and E extracts was estimated by spectrophotometry in comparison with ascorbic acid (vitamin C), taken as a reference (**Figure 4**).



S: terrestrial *N. biserrata*; E: epiphytic *N. biserrata*; M: Equimassic mixture

Figure 4: Antioxidant capacity of S, M and E extracts and vitamin C

Extracts showed lower DPPH reduction percentages (RP) than vitamin C. The RP greater than 50% of the extracts was obtained at 1 mg / mL. However, according to Shah *et al.*²⁹, the antioxidant potential of *N. biserrata* is high enough to confer significant protective effects against hepatotoxicity induced by carbon tetrachloride or tetrachloromethane (CCl₄) in rats. The various regressions obtained from the antioxidant potentials made it possible to determine the concentrations of reduction at 50% (CR₅₀) of DPPH by the extracts (Table VII). The CR₅₀ is the parameter that shows the reducing efficiency of a plant extract with respect to DPPH. The lower this parameter, the more the extract manifests an effective antioxidant³⁰.

Table VII: CR₅₀ of the different samples

Extract	S	M	E	Vitamin C
CR ₅₀ (mg/mL)	1.042	1.078	1.293	0.476

S: terrestrial *N. biserrata*; E: epiphytic *N. biserrata*; M: Equimassic mixture

Regarding the study extracts, Table VII shows that terrestrial *N. biserrata* (S) exhibits the most effective antioxidant activity with an CR₅₀ = 1.042%.

Antibacterial activity

Efficacy of crude extracts E, M and S

Sensitivity of crude extracts and antibiotic against fermentative enterobacteria

For fermentative Enterobacteriaceae, only extract E was effective against bacterial strains of *K. pneumoniae* and *A. baumannii* with diameters of the zone of inhibition between 9.18 ± 0 and 10.41 ± 0 mm (Table VIII). The negative control was sterile distilled water.

Regarding antibiotics, all bacterial strains have shown resistance, with the exception of the genus Enterobacter which naturally exhibits resistance to CRO.

Table VIII: Sensitivity of crude extracts and Ceftriaxone against enterobacteria

Bacterial strain	Diameter of inhibition zone at ATB 200 mg / mL (mm)				
	S	E	M	CRO	EDS
<i>Salmonella</i> sp 109UB/20 CNRa	0	6.93±0	0	15.60	
<i>E. coli</i> 466TR/20 CNRa	0	0	0	0	
<i>E. coli</i> 470 UB/20 CNRa	0	0	0	0	
<i>E. cloacae</i> 543 T/20 CNRa	0	0	0	10	
<i>K. pneumoniae</i> 471 UB/20 CNRa	0	9.18±0	0	0	0
<i>A. baumannii</i> 531UB/20 CNRa	6.56±0	10.41±0	9±0	0	

S: terrestrial *N. biserrata*; E: epiphytic *N. biserrata*; M: equimassic mixture; ATB: Antibiotic;

CRO: Ceftriaxone; EDS: Sterile distilled water

Sensitivity of extracts and antibiotic to staphylococci

Bacterial strains are resistant to crude extracts, with the exception of extract E which inhibited *S. aureus* strain 211 UB / 20 CNRa (Table IX).

The FOX positive control, used in the treatment of pathologies linked to staphylococci, gives diameters of the inhibition zone ranging from 20 to 26 mm. Bacterial strains have shown resistance, with the exception of the reference ATCC 25923¹⁵.

Table IX: Sensitivity of crude extracts and Cefoxitin to staphylococci

Bacterial strain	Diameter of inhibition zone at 200 mg / mL (mm)			ATB	
	S	E	M	FOX	EDS
<i>S. aureus</i> 211UB/20 CNRa	0	12.68±0	0	26	
<i>S. aureus</i> 483UB/20 CNRa	0	0	0	26	0
<i>S. aureus</i> ATCC 25923	0	0	0	20	

S: terrestrial *N. biserrata*; E: epiphytic *N. biserrata*; M: equimassic mixture;

ATB: Antibiotic; FOX: Cefoxitin; EDS: Sterile distilled water

Sensitivity of extracts and antibiotic to non-fermentative enterobacteria

Crude S, E and M extracts had no effect against bacterial strains of *Pseudomonas aeruginosa*. The strains were resistant to the various crude extracts (6 and 7 mm diameters of the inhibition zone).

On the other hand, a sensitivity to imipenem (IPM) of the strains was observed with diameters of

zone of inhibition ranging from 26 to 33 mm. According to the standard¹⁵, these strains show resistance to the antibiotic.

The MIC and MBC were determined for the extracts which exhibited inhibitory activity.

Characteristics of extracts effective by diffusion in liquid medium

After 24 h of incubation at 37 ° C, a progressive decrease in bacterial growth in the wells of the microplates according to the different concentrations of the extracts tested, compared to the growth control Tc was observed. The MIC of the extracts could be determined unlike the MBC due to the growth of the colonies on the striped streaks. Subsequently, the MBC / MIC ratio which makes it possible to specify the modality of action of the extracts¹⁸ could not be calculated.

The values determined with regard to the bacterial strains are grouped together in Table X.

Table X: Antibacterial parameters of effective extracts

Bacterial strain	Extract	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
<i>K. pneumoniae</i> 471 UB/20 CNRa	E	50	> 50	-
<i>A.baumannii</i> 531UB/20 CNRa	E	50	> 50	-
	M	50	> 50	-
<i>S.aureus</i> 211 UB/20 CNRa	E	25	> 25	-

S: terrestrial *N. biserrata*; E: epiphytic *N. biserrata*; M: equimassic mixture

Extract E has been shown to be more effective against bacterial strains. Thus, the inaction of M would probably be linked to the low concentration of these phytochemicals in said decoct or to their antagonistic action. Also, the results of the antibacterial analyzes of the samples could be explained by unitization of the plant in the treatment of pathologies linked to the various germs tested. The polyphenols contained in the extracts endowed with beneficial biological activities for humans, did not have any bactericidal action against bacterial strains. We also notice that the absent saponins are endowed with hemolytic, antimicrobial, anti-inflammatory activities, and are known for their cytotoxic properties²². The antibacterial activity could therefore be explained by a synergistic action between the activity of phenolic phytochemicals and that of saponins in medicinal plants.

Vermicidal activity

This activity of the extracts was evaluated against earthworms by comparison with that of albendazole which is an anthelmintic antiparasitic drug of the benzimidazole therapeutic class. The results obtained are presented in figure 5. The vermicide effect is proven when the lethality time is short (0 to 360 min). The shorter the 100% lethality time, the more the extract has a high vermicide activity³¹. Distilled water kills all earthworms after 1440 min.

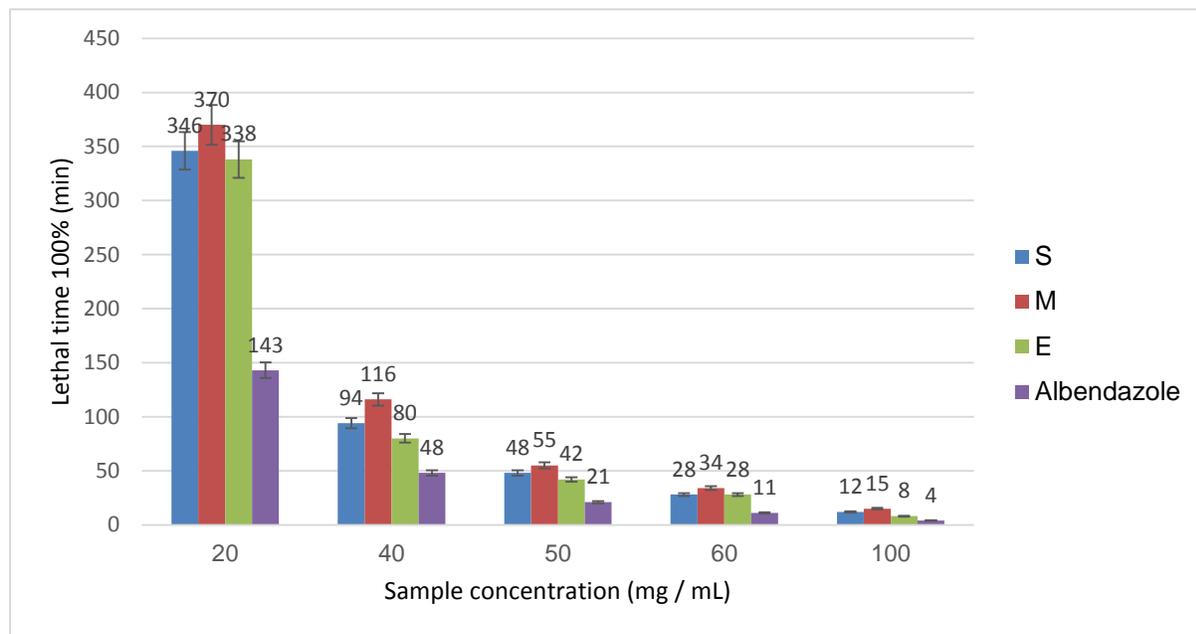


Figure 5: Vermicidal activity of crude extracts S, M, E

The results obtained show that the extracts have vermicidal activity. However, in all concentration ranges (20, 40, 50, 60 and 100 mg / mL), this activity remains lower than that of albendazole. At the same concentration, the vermicidal activity of E was found to be greater than that of extract S. Extract M showed the lowest vermicidal activity. In fact, at 20 mg / mL, the 100% lethality of earthworms is observed at 338 min for epiphytic *N. biserrata*, at 346 min for the terrestrial species and at 370 min for the equimassic mixture of epiphytic and terrestrial *N. biserrata*. As the concentration of the sample increases, the 100% lethality time is shorter (E40 at 80 min, E50 at 42 min and E100 at 8 min). This is because the 100% lethality time is concentration-dependent, the higher the concentration of the extract, the shorter the 100% lethal time³¹. These results are in accordance with those obtained by Muhammad *et al.*³² According to these authors, the time taken for parasitic leech mortality as a function of concentration was less at 100 mg / mL (4.88 min) than at 50 mg / mL (11.91 min) and 25 mg / mL (25.11 min) of methanol extract of *N. biserrata* from Malaysia. They argue that no mortality was noted in the normal control group throughout the 720 min observation. However, the antiparasitic potential of the various aqueous extracts of *N. biserrata* growing in Côte d'Ivoire appears to be lower than that of the methanol extract of the species growing in Malaysia.

This observation may be due to several factors: the extraction solvent used, the biological material (*Lumbricus terrestris* Linn. (Earthworm)) against *Zeylanicobdella arugamensis* (a marine parasitic leech), and global ecological changes.

In sum, *N. biserrata* can act as a potential biological control agent. The presence of condensed tannins and flavonoids in the extracts of the two plants suggests that the activities shown are linked to their richness in these phenolic compounds^{33,34}.

CONCLUSION

The present work is a contribution to the phytochemical and biological valorization of *Nephrolepis biserrata* (Sw.) Schott, a variety of fern coming from the Ivorian flora. The phytochemical screening by color reactions and by TLC of the crude extracts S, E, M made it possible to show the presence of alkaloids, anthocyanins, coumarins, flavonoids, polyphenols, proteins, sterols, tannins and terpenes. The quantification of the contents of total polyphenols, total flavonoids and condensed tannins was carried out. For total polyphenols, the values vary from 4736.896 µg EAG / g to 5552.586 µg EAG / g. Those in condensed tannins are between 13.904 µg EC / mg and 26.280 µg EC / mg. As for total flavonoids, a better content was observed in the M extract (26.28%). The evaluation of the antioxidant capacity of the extracts carried out against DPPH showed that terrestrial *N. biserrata* has the highest antioxidant activity with a CR₅₀ = 1.04 mg / mL although it remains lower than that of vitamin C. Regarding the evaluation of antibacterial activity, the epiphytic *N. biserrata* plant has only bacteriostatic action against bacterial strains *K. pneumoniae* (471 UB/20 CNRa), *A. baumannii* (531UB/20 CNRa) and *S. aureus* (211 UB/20 CNRa). On the other hand, a fairly pronounced vermifugal activity of all the extracts was observed. The fern *N. biserrata* from Côte d'Ivoire cannot effectively fight bacterial infections. However, epiphytic species could traditionally be used as an excellent natural dewormer.

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