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Original Research Article

Antioxidant Profiling, Phytochemical Investigation and Pharmacognostic Evaluation of N*ephrolepis biserrata* (SW.) Schott (Nephrolepidaceae)

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ABSRTACT

Natural product chemistry has contributed immensely to the discovery of bioactive compounds and the development of pharmaceutical agents. In this study, the ethanol extract of *Nephrolepis biserrata* L. was evaluated. Qualitative and quantitative phytochemical screening was done. The proximate composition was also determined. The antioxidant property was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, Total Antioxidant Capacity (TAC), and Ferric Reducing Antioxidant Power (FRAP). The phytochemical analysis of the ethanol fraction was performed by gas chromatography-mass spectrometry (GC-MS). Macroscopy and microscopy of the plant were done using standard methods. The preliminary qualitative phytochemical screening confirmed the presence of active chemical constituents such as alkaloids, tannins, flavonoids, anthraquinones, cardiac glycosides, terpenoids, and triterpenoids. The quantification showed that the ethanol extract of *N. biserrata* is rich in phenolic, tannin, and flavonoid contents. The proximate analysis showed that the ethanol extract of *N. biserrata* has high crude fat content and a significant amount of crude protein and fiber. The antioxidant assays suggested that the extract contains antioxidant compounds. Microscopy of the plant showed a diacytic type of stomata, irregular epidermal cells, and a conspicuous absence of trichomes. The leave type is simple and alternately arranged with sori present on the serrated margin. Venation is pinnate, apex is acuminate while the base is oblong. The results demonstrated that the ethanol extract of *N. biserrata* represents a good source of antioxidants that could be used in the pharmaceutical industry. The pharmacognostic parameters could also serve to identify and authenticate the plant among other ferns.

Keywords: *Nephrolepis biserrata*, Phytoconstituents, Antioxidant, Microscopy, Macroscopy, Gas Chromatography-Mass Spectrometry.

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Introduction

Natural product chemistry is a branch of chemistry that focuses on the study of compounds derived from natural sources, such as plants, animals, fungi, and microorganisms. These compounds, often referred to as natural products, have played a significant role in human civilization, providing a wide range of therapeutic agents, agricultural products, and industrial raw materials. Natural products are incredibly diverse and can be classified into several major classes based on their chemical structures and biosynthetic origins.[1] Natural product chemistry has contributed immensely to the discovery of bioactive compounds and the development of pharmaceutical agents. Many natural products serve as lead compounds for drug discovery, providing valuable scaffolds for the development of new therapeutic agents.[2] Furthermore, natural products have found applications beyond medicine. They are used as flavors, fragrances, dyes, and agricultural products. Medicinal plants are an important component of flora and are found all over the world.

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The pharmacological examination of plant components is a well-established strategy for identifying lead compounds, which can lead to the development of innovative and safe therapeutic medicines. Medicinal plants are made up of organic compounds known as phytochemicals, which cause specific physiological effects in the human body.[3] These bioactive substances include but are not limited to, tannins, alkaloids, terpenoids, steroids, and flavonoids.[4] Pharmaceutical product development demands a thorough examination of medicinal plants to increase our understanding of their biological activities and the phytoconstituents responsible for them.[5] Furthermore, there is a greater demand for extensive research in this field which is highlighted by the fact that only a small number of medicinal plant species have undergone full scientific scrutiny.[6]

An antioxidant is a chemical that, in low concentrations, can prevent or postpone the oxidation of a substrate. A significant imbalance between the formation of reactive oxygen species (ROS) or reactive nitrogen species (RNS) and antioxidant defense is referred to as oxidative stress. All sorts of biomolecules, including DNA, proteins, and lipids, are susceptible to damage from oxidative stress.[7] Phytochemical compounds with antioxidant properties can minimize or inhibit the damage caused by ROS. The antioxidant properties of different natural products depend on the phenolic content, vitamins C and E, carotenoids, flavonoids, and other phytocomponents.[8] Because of this positive role, a lot of experimental study is focused on exploiting the potential of phytochemical constituents from various plants and establishing their association with health benefits. Free radicals are unstable molecules that can cause damage to cells and contribute to the development of chronic diseases such as cancer, heart disease, and Alzheimer's disease. Antioxidants work by neutralizing free radicals and reducing their harmful effects, which can help protect against these diseases and promote overall health.[9]

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Nephrolepis biserrata (Sw.) Schott commonly known as the oil palm fern, can reach 2 m in height and has tufted fronds.[10] It can also be terrestrial. Ferns are one of the plants without flowers and seeds and reproduce by hidden organs. *Nephrolepis biserrata* L. (Nephrolepidaceae) is a perennial fern and it is commonly known as ''Paku larat'' or ''Paku pedang'' (Malay) (Figure 1). This perennial fern with green compound leaves (fronds) grows in shady and moist areas like river banks and wetlands.[11] The plant is widely distributed in many countries including, Malaysia, Indonesia, Thailand, China, and the United States of America.[12] The tip of young shoots of *N. biserrata* is used as a vegetable by the local people in Sabah, Malaysia.[13] Traditionally, *N. biserrata* is used to cure a variety of diseases. The plant is used to cure stomach pain, bleeding, and wounds, as well as to prevent miscarriage, fetal growth, and other microbiological infections (boils, abscesses, soreness, and blisters).[14] *Nephrolepis biserrata* is also used by the local people in Sarawak, Malaysia, for skin disorders. The plant is a good source of antioxidant compounds with hepatoprotective potential.[15]

A variety of medicinal plants in Nigeria are being utilized to develop treatments for various illnesses. Numerous of these plants have been the subject of scientific investigations and there is now significant data to support their use in indigenous healthcare. Given this, this study is designed to provide qualitative and quantitative phytochemical information about the leaves of *N. biserrata* and also to provide information about its antioxidant properties. This research aims to investigate the antioxidant properties of the leaves of *Nephrolepis biserrata* and conduct phytochemical and pharmacognostic evaluations as a means of scientifically authenticating the unique qualities of the plant to avoid adulteration and substitution with similar ferns.

Materials and Methods

Plant Material

The leaves were collected in March 2023 from Dalemo, Ota, Ogun State (6°41'54.5"N 3°15'33.0"E), Nigeria, and the specimen was identified at the Herbarium of the Department of Botany, University of Lagos with voucher number LUH 9082. Fresh leaves were cleared using the peeling method and chloral hydrate solution was used for macroscopic and microscopic evaluation. The remaining leaves were air-dried at room temperature (25 ± 3°C) for two (2) weeks after which they were milled into powder.

The powdered plant material was then macerated exhaustively in 95 %v/v ethanol for 72 h in a tightly sealed aluminum pot. The pot was shaken at regular intervals and the extract was decanted into an amber-colored glass bottle and filtered. The filtrate obtained was then concentrated under reduced pressure and further air-dried to remove the remaining solvent. The dry extract was stored in sample bottles and used for further analysis.

Macroscopical Evaluation

The macroscopic characters of the fresh leaves were visually evaluated and compared with the literature. Plant venation, margin type, shape, base, apex, mid-rib, and lamina were determined which could aid in future identification.

Microscopical Evaluation

Qualitative microscopy was performed using a compound light microscope (Olympus CX, Japan). This was performed using standard procedures recommended by the WHO guidelines on quality control methods for herbal materials. Transverse sections of *N. biserrata* leaves were cut and placed into test tubes containing chloral hydrate. These sections were then mounted with glycerol under magnifications of ×10 and ×40 for identification of some features and cell contents.

Qualitative phytochemical Analysis

The qualitative phytochemical screening of *N. biserrata*'s extract was carried.[16,17] The plant extract and fractions were analyzed for alkaloids, phenols, tannins, flavonoids, saponins, anthraquinones, steroids, cardiac glycosides, terpenoids, and triterpenoids.

Quantitative Phytochemical Analysis

Quantitative Test for Alkaloids

Alkaloid content was determined by a method described in the literature. About 1g of the extract, 45 mL ethanol, and 5 mL acetic acid were added and covered for 4 hours while stirring intermittently. This solution was then filtered and concentrated to one-quarter volume. It was then basified with 30 mL ammonia solution and partitioned twice with 40 mL of chloroform. The filtrate was then dried and weighed.[18]

Quantitative Test for Flavonoids

Total flavonoid content was determined by the Aluminium chloride method using rutin as a standard.[19] About 0.5mL of the sample and 1.5 mL of methanol were added to a volumetric flask (10 mL volume). After 5 minutes, 0.1 mL of sodium acetate solution and 0.1 mL of 10 % Aluminium chloride. Then distilled water was added to make up to 10 mL. this solution was incubated at room temperature for thirty minutes and absorbance reading was taken at 415 nM using infitek Double Beam UV-Vis Spectrophotometer SP-IUV7, USA.

Quantitative Test for Phenols

Total phenolic content was determined spectrophotometrically by the Folin-Cocalteu method.[20] In this procedure, 0.1 mL of different concentrations of the standard (gallic acid) and 1 mg/mL of the test sample were mixed with 0.5 mL of Folin-Cocalteu reagent. After 5-8 minutes, 0.4 mL 7.5 % of sodium carbonate solution was added. The final volume of the tubes was made up to 10 mL with distilled water and allowed to stand for 90 minutes at room temperature. The absorbance of the sample was measured against the blank at 750 nm using a spectrophotometer.

Quantitative Test for Tannins

Tannin content was determined spectrophotometrically by the Folin-Denis method.[21] In this procedure, 0.2 mL of different concentrations of the standard (tannic acid) and 1 mg/mL of the test sample were mixed with 0.2 mL of Folin-Denis reagent. After 5 minutes, 0.4 mL 7.5% of sodium carbonate solution was added. The solution was incubated for 90 minutes at room temperature. The absorbance of the sample was measured against the blank at 760 nm using a spectrophotometer.

Quantitative Test for Cardiac Glycosides

In the determination of the amount of cardiac glycoside present, Buljet's reagent was employed.[22] Buljet's reagent is prepared by adding 95 mL of 1 % picric acid to 5 mL of 10 % NaOH. In this procedure, 1 mL of different concentrations of the standard (digoxin) and 1mg/mL of the test sample were mixed with 5 mL of Buljet's reagent. The solutions were then incubated for one hour at room temperature. The absorbance of the sample was measured against the blank at 495 nm using a spectrophotometer.

Figure 1: Photograph of Nephrolepis bisserata in its natural habitat (+06.69848, +003.25917).

Quantitative Test for Steroids
The test sample of volume 1 mL, was transferred into a 10 mL volumetric flask. Sulphuric acid (4N, 2 mL) and iron (III) chloride (0.5 %w/v, 2 mL) were added, followed by potassium hexacyanoferrate (III) solution (0.5 % w/v, 0.5 mL). The mixture was heated in a water bath maintained at 70 ^{O}C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Antioxidant studies
DPPH radical scavenging assay
The effect of the extract of *N. biserrata* leaves on the DPPH radical was estimated according to the literature method with modifications. A stock solution of the extract was prepared by dissolving 125 mg of *N. biserrata* extract in 125 ml of methanol. Varying concentrations of the extract; 10 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, and 250 µg/ml were prepared by carrying out serial dilutions. A solution of the radical was also prepared by dissolving 3.94 mg of DPPH in 100 ml of methanol.[23]
To each test tube containing 3 ml of the varying concentrations of extract, 2 ml of methanol, DPPH solution was added. The mixture was shaken vigorously and kept at room temperature in the dark for 30 min. A blank solution of the radical without extract was also prepared.
The absorbance of the reaction mixture was measured at 517 nm using a spectrophotometer. All determinations were done in triplicate. Percentage inhibition was then calculated. Ascorbic acid was used as the standard.
The DPPH radical scavenging ability was calculated using the equation 1:

$$\%\ Scavenging\ activity = \frac{Absorbance\ blank - Absorbance\ Sample}{Absorbance\ blank}\ x\ 100 \quad (1)$$

For each sample, the concentration with a 50 % scavenging effect (EC_{50}) on the DPPH radical
was determined by interpolation of linear regression analysis.

Total antioxidant capacity (Phosphomolybdenum assay)
The total antioxidant capacity of the extracts was evaluated by the Phosphomolybdenum method according to the procedure described by the literature with some modifications.[24]
The molybdate reagent was prepared by adding 1 ml each of 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate to 20 ml of distilled water. The solution was then made up to 50 ml with more distilled water.
A stock solution of extract was prepared by dissolving 125 mg of *N. biserrata* in 125 ml of methanol (1000 µg/ml) and average absorbance was taken after the triplicate determination.
Serial dilutions using distilled water were carried out to make a 3 ml solution of varying concentrations (10 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml and 250 µg/ml) of the standard.
To each concentration, 3 ml of molybdate reagent was added after which the test tubes were incubated at 95 °C for 90 min. The mixture was allowed to cool rapidly and absorbance was read at 493 nm against a blank solution containing the reagent solution and solvent used. Determinations were done in triplicate and ascorbic acid was used as reference standard. Total antioxidant capacity was deduced by plotting a standard calibration curve of the standard.

Ferric reducing antioxidant power (FRAP) assay
This was carried out by a method described in literature.[25] FRAP reagent was prepared by mixing 10 ml of 300 mM acetate buffer (pH 3.6), 1 ml TPTZ in 40 mM HCl, and 1 ml of 20 mM $FeCl_3.6H_2O$. The mixture was warmed at 37°C for about 15 min before use.
A stock solution of extract was prepared by dissolving 125mg of *N. biserrata* in 125 ml of methanol (1000 µg/ml) and average absorbance was taken after triplicate determination.
Serial dilutions using distilled water were carried out to make a 3 ml solution of varying concentrations (10 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml and 250 µg/ml) of the standard.
About 4 ml of the FRAP reagent was mixed with 5 ml of the extract and mixed thoroughly. A blue color solution was observed and the absorbance was read at 593 nm against a reagent blank containing FRAP reagent and distilled water. All determinations were performed in triplicates
and ascorbic acid was used as reference standard. The calibration curve was prepared by plotting absorbance versus concentration.

Proximate Analysis
This analysis was carried out according to standard methods.[26]

Estimation of Moisture Content
About 2 grams of a sample of *N. biserrata* leaves was taken in triplicates and dried at a temperature of 100-102 degrees Celsius. The weight of the dried leaves was noted. The moisture content (%) was estimated using Equation 2 below.

$$\frac{\text{weight of the fresh plant leaves} - \text{weight of the dry plant leaves}}{\text{weight of the fresh plant leaves}}\ \text{X}\ 100 \quad (2)$$

Total ash content
About 1 gm of air-dried plant material was incinerated using a silica crucible in an incinerator for 6 h at 600-650 °C. The ash formed was white and free from carbon. It was cooled and weighed on the ashless filter paper. This was performed in triplicates and the total ash content was obtained as an average of the three values. Total ash content is calculated with reference to the weight of the air-dried plant material, expressed in % w/w in equation 3.[27]

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Initial weight of sample}}\ \text{x}\ 100 \quad (3)$$

Estimation of Crude Fat Content
The dried powdered sample of *N. biserrata* (1 gram) was taken in a thimble and placed in a Soxhlet extractor. Approximately 150ml of petroleum ether was added up to one and a half siphons in a dried round bottom flask connected to the Soxhlet assembly. After extraction, the sample was weighed and the crude fat content (%) was calculated as follows with equation 4:

$$\text{Crude fat content} = \frac{\text{Weight of fat}}{\text{Weight of sample}}\ \text{x}\ 100 \quad (4)$$

Estimation of Crude protein content
The conventional microkjeldahl's method was used for the estimation of the crude protein content (using K_2S0_4: $CuSO_4$ (9:1), concentrated H_2SO_4 40% NaOH, N/100 H_2SO_4, Ammonia as reagents, methyl red as indicator and NaOH as titrant .[28]

Amount of nitrogen (%) in the sample
= Volume of NaOH used for blank - Volume of NaOH used for sample (5)

Crude Protein content (%) = Nitrogen content in sample x 6.25 (6)

Estimation of Crude Fiber Content
The plant leaves (1g) were weighed and transferred to a clean 250 mL beaker and added 50 mL of 2.5 %(w/w) sulphuric acid. For heating, a hot plate was used to place the beaker, and the contents
were allowed to reflux for 30 minutes, and shaking was done every 5 min. After 30 minutes beaker was removed from the hot plate and filtration was performed using an ashless filter. Washing of the residue was done with hot water till it became free from acid, then the residue was transferred to the same beaker and after the addition of 50 mL of 2.5 % NaOH solution, the contents were again refluxed for 30 min. It was followed by filtration through an ashless filter, it was washed with hot water and then transferred to a crucible and placed in an oven, allowed to dry to constant weight at 80-110°C, and recorded its weight. The residue was ashed at 550-660°C for 2-3 h, then cooled and weighed again. The loss of weight due to ignition is the weight of crude fiber.

$$\text{Crude fiber content}(\%) = \frac{\text{Weight of crude fiber}}{\text{Original weight of sample}} \text{ x } 100 \quad (7)$$

Total Carbohydrates Content
The difference is used for the calculation of Total carbohydrate content as follows:
Total carbohydrates content (%) =
100 - [Moisture (%) + Crude Fat (%) + Ash (%) - Crude Protein (%) + Crude fiber (%)] (8)

Gas Chromatography-Mass Spectrometry Analysis
GC-MS analysis of the extract was performed using an Agilent 5977B GC/MSD, USA, system coupled with Agilent 8860 auto-sampler, a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-5MS (5% diphenyl/95%dimethyl polysiloxane) fused a capillary column (30 × 0.25µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1ml/min, and an injection volume of 1µl was employed (a split ratio of 10:1).
The injector temperature was maintained at 300 °C, the ion-source temperature was 250 °C, and the oven temperature was programmed from 100 °C (isothermal for 0.5 min), with an increase of 20 °C/min to 280°C (2.5 min), Mass spectra were taken at 70 eV; a scanning interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 3 min.

Results and Discussion

Pharmacognostic evaluation
Pharmacological parameters of plant samples are important as they ensure plant identification and establish standardized parameters that will help prevent adulteration and thus improve the efficacy and safety of medicinal plants. The evaluation of *N. biserrata* focuses on macroscopic, microscopic, and physicochemical analyses of the crude drug, which are important in determining the identity, purity, and quality of plant samples.
N. biserrata has green simple type of leaves, with sori present on the margin. The arrangement is alternate, margin serrated, and pinnate venation. The base is oblong while the apex is acuminate (Table 1)
The microscopy of the leaves which could serve means of identification revealed that *N. biserrata* has a dicytic stomata type, with irregularly shaped epidermal cells but no trichome. The presence of an anticlinal wall that is thick and wavy was also observed. (Figure 2 and Table 2).

Qualitative Phytochemical Analysis
Plant phytochemical components are physiologically active molecules that perform a variety of functions such as antioxidant, anti-inflammatory, antibacterial, antifungal, and anticancer activity. The percentage yield of the extract was calculated to be 6.33% yield. The preliminary phytochemical analysis of the ethanol extract of *N. biserrata* leaves (Table 3) revealed the presence of alkaloids, tannins, phenols, flavonoids, saponins, cardiac glycosides, terpenoids, triterpenoids and anthraquinones.
Anti-cholinergic, vasodilating, anti-hypertensive, anti-bacterial, and anti-viral properties have been found for alkaloids and tannins.[29] Alkaloids are natural products with basic properties that include heterocyclic nitrogen atoms. Some alkaloids are beneficial and important in medicine, accounting for the majority of valuable medications currently in use by humans. Many indole alkaloids have antihypertensive effects, antiarrhythmic effects, antimalarial activity, and anticancer effects.[30] Steroids have been reported to possess anti-inflammatory properties.[31] Saponins have been known to exhibit coagulating and cholesterol-binding activities.[32] Triterpenoids also possess anti-inflammatory, analgesic, and antipyretic properties. Flavonoids were also found present in the extract. Flavonoids are the major nutraceutical ingredients that are in plants. They are highly effective scavengers of most oxidizing molecules. The best-described property of almost every group of flavonoids is their capacity to act as anti-oxidants. According to this study, the ethanol extract of *N. biserrata* leaves contains steroids, saponins, and triterpenoids which may be responsible for its use for wound cleaning and jaundice.[33]

Quantitative Phytochemical Analysis
The quantitative analysis (Table 4) result revealed alkaloid content as 5.3 ± 0.11 mg/g. Total Phenolic Content was obtained as 58.024 ± 0.13mg EAG/g. The content of tannins was obtained as 102.28 ± 0.16 mg EC/g. The extract has a flavonoid content of 60.56 ± 0.025mg/g. The cardiac glycoside content is obtained to be 17.379 ± 0.00mg/g and the steroid content of 14.553 ± 0.61mg/g. The ethanol extract of *N. biserrata* leaves is seen to have high phenolic, tannin, and flavonoid contents which would be responsible for some of its medicinal uses.

Table 1: Leaf morphology (Macroscopic characteristics of the leaves of N. biserrata)

Morphology	Description
Type	Simple
Sori	Present on the margin
Color	Green
Arrangement	Alternate
Venation	Pinnate
Base	Oblong
Apex	Acuminate
Margin	Serrated

Table 2: Leaf Anatomy (Adaxial Surface)

Features	Description
Epidermal cells	Irregular in shape
Stomata	Diacytic
Trichome	Not seen
Anticlinal wall	Thick and wavy

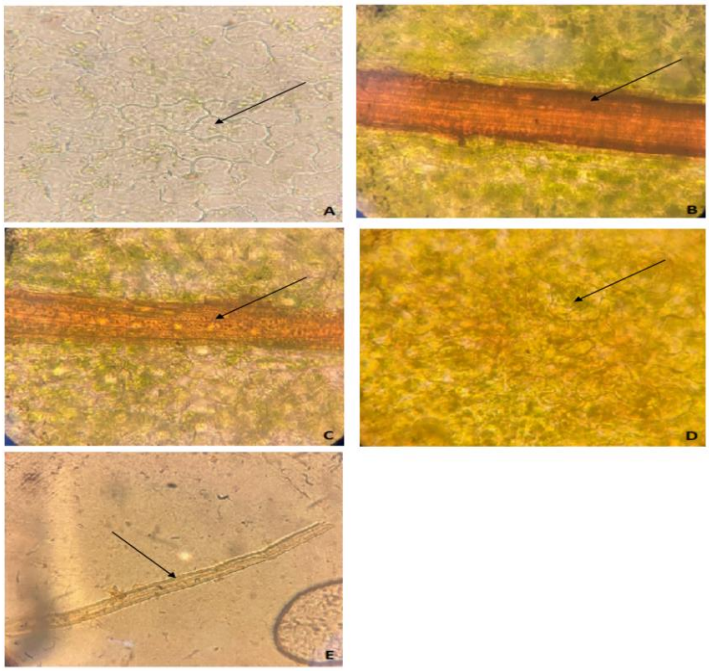

Figure 2: Anatomical features of N. biserrata showing the: (a) epidermal cells (b) midrib (c) midrib with xylem vessels (d) stomatal arrangement (e)fiber as viewed under microscope.

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Phenolic compounds have drawn significant interest due to their potential as antioxidants. When combined with the phosphotungstic and phospho-molybdic acids in the Folin-Ciocalteu reagent, phenols go through a complicated redox process.[34] Plant phenolics have increased recognition due to their substantial anti-oxidant capabilities and significant effects in the prevention of different oxidative stress-related disorders such as cancer.[35] The study indicates that there are significant levels of phenolic compounds in the ethanol extract of *N. biserrata* leaves. Flavonoids which are present in significant levels in this extract, are known to exhibit biological activities including antioxidant and anti-inflammatory. Flavonoids are powerful water-soluble super antioxidants and free radical scavengers that prevent oxidative cell damage, have substantial anticancer action, and suppress tumor growth. Tannins are also observed to be present in high levels in the extract.[36] Tannins have been shown in studies to have antibacterial, hepatoprotective, antitumor, and antiviral properties. Tannins are employed as an anti-diarrhea, hemostatic, and anti-hemorrhoid agent in medicine.[37]

Proximate Composition of N. biserrata leaves

Since many of these herbal products are consumed orally, it is essential to understand their proximate analysis to evaluate their nutritional value and health impacts. The result of proximate analysis (Table 5) shows differing percentages of bio-chemicals in *N. biserrata* leaves. From the results, it can be deduced that the crude fat content is higher than other components contained in the extract. The composition is obtained to consist of 7 % moisture, 1.16 ± 0.02 Ash value, 34.35 ± 1.63 Crude fat, 18.13 ± 0.88 crude protein, 19.55 ± 0.49 crude fiber and 19.82 ± 0.23 total carbohydrate content. Low fiber and ash content were an indication that the roughage and mineral constituents of the *N. biserrata leaves* were poor. The long shelf life of crude extract could be attributed to the low moisture content as high moisture content can hasten spoilage and enhance microbial growth.

Anti-Oxidant Assay

DPPH Radical Scavenging Activity

DPPH scavenging assay has been employed for studying the antioxidant activities of food as well as medicinal plants due to its stability.[38] DPPH is a stable nitrogen-centered free radical that can receive a proton from a suitable free radical scavenger (reducing agent) to form a non-radical DPPH-H. In solution, when the stable DPPH radical accepts an electron from an antioxidant substance, the violet color of the radical is reduced to yellow-colored diphenylpicrylhydrazine radical which is then measured colorimetrically. Compounds that undergo this reaction can be referred to as antioxidants and therefore radical scavengers. This assay determines the scavenging of stable radical species DPPH by antioxidant compounds that occur in the ethanol extract. The extract showed various levels of DPPH radical scavenging activity (Figure 3) over different concentrations from 10-250 µg/ml and the IC_{50} value of the extract was 90.3 µg/ml. The standard used was ascorbic acid over different concentrations from 10-250 µg/ml and the IC_{50} value was found to be 19.10 µg/ml. both the extract and the standard showed a dose-dependent inhibition of the DPPH radicals. The regression coefficient was found to be 0.9876 and 0.7531 for the ethanol extract and standard respectively. From the results, *N. biserrata* leaf extract demonstrated significant free radical scavenging activity when compared with the standard.

Total Antioxidant Capacity (TAC)

The total Antioxidant Capacity (TAC) assay is based on the reduction of Mo^{6+} to Mo^{5+} by antioxidants present in the extract and the formation of a green phosphate-molybdate complex subsequently at acidic pH, which is then measured spectrophotometrically. The concentration of ascorbic acid equivalent in the extract from the calibration plot (Figure 4) from the TAC assay is 79.121 ± 0.565 µg/mL. This shows that the extract has a significant antioxidant property as compared to ascorbic acid.

Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric Reducing Antioxidant Power (FRAP) assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyl triazine (Fe^{3+} -TPTZ) complex to produce a blue complex ferrous tripyridyl triazine (Fe^{2+}-TPTZ) formed by the ability of the antioxidant to donate electrons at low pH. This reaction is monitored spectrophotometrically by measuring the change in absorbance, which can be directly correlated to the ferric-reducing power of the electron-giving antioxidant present in the reaction mixture. The concentration of ascorbic acid equivalent in the extract from the calibration plot (Figure 5) from the FRAP assay was obtained to be 148.502 ± 2.242 µg/mL. This shows that the extract has a significant antioxidant property as compared to ascorbic acid.

Table 3: Phytochemical screening of the ethanol extract of N. biserrata leaves

Test	Inference
Alkaloids	+
Tannins	+
Phenols	+
Flavonoids	+
Saponin	-
Cardiac Glycosides	+
Anthraquinones	+
Terpenoids	+
Triterpenoids	+
Steroids	-

NB: + means present; - means absent

Table 4: Quantitative analysis of the ethanol extract of N. biserrata leaves

Parameters	Values
Alkaloid Content	5.3 ± 0.11 mg/g
Total Phenolic Content	58.024 ± 0.13 mgGAE/g
Tannin Content	102.28 ± 0.161 mgTAE/g
Flavonoid Content	60.56 ± 0.025 mgRE/g
Cardiac Glycoside Content	17.379 ± 0.00 mgDE/g
Steroid content	14.553 ± 0.611 mg/g

Data expressed as Mean ± Standard Deviation

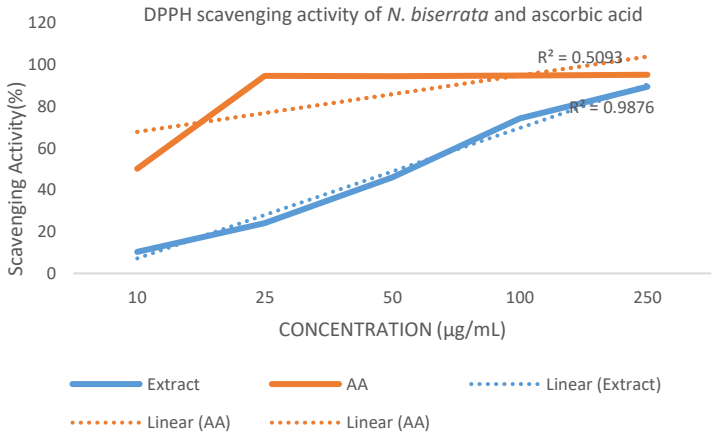

Figure 3: Graph of DPPH scavenging activity of N. biserrata and ascorbic acid versus concentration (µg/mL)

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Table 5: Proximate Values of ethanol extract of N. biserrata leaves

Parameters	Values (%)
Moisture Content	7.00 ± 0.00
Ash value	1.16 ± 0.02
Crude fat	34.35 ± 1.63
Crude protein	18.13 ± 0.88
Crude fiber	19.55 ± 0.49
Total Carbohydrate	19.82 ± 0.23

Data expressed as Mean ± Standard Deviation

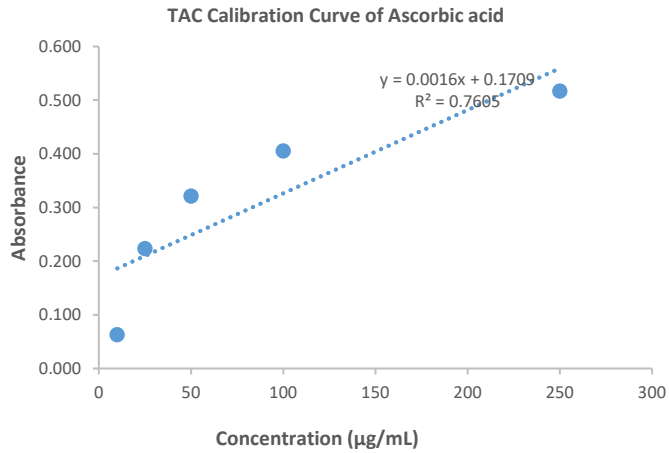

Figure 4: TAC Calibration Curve of Ascorbic Acid

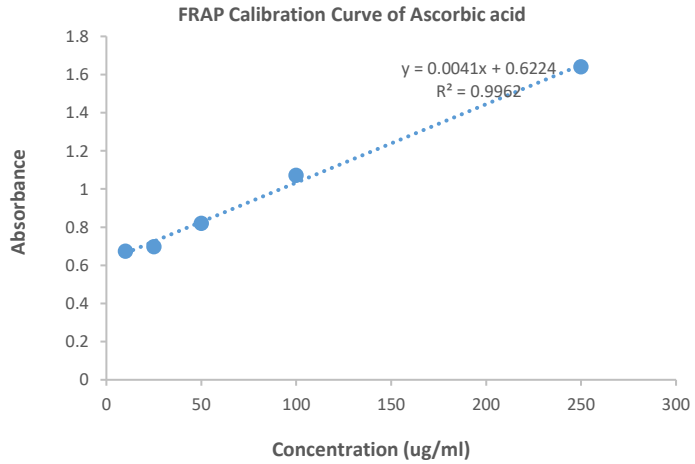

Figure 5: Ferric Reducing Antioxidant Power (FRAP) Assay Calibration Curve of Ascorbic acid as a standard

Gas Chromatography-Mass Spectrometry Analysis

N. biserrata was subjected to chemical analysis using GC-MS method and this confirmed the occurrence of phytocompounds with various biological activities It has also been noted that there is a developing understanding of the relationship between phytochemical substances and biological activity.[39]

Some bioactive compounds have been identified in the ethanol extract of *N. biserrata* leaves using GC-MS analysis (Table 6, Figure 6). The major compounds identified in this study may possess significant biological potential which may be important for future development and discovery of drugs. Among the bioactive compounds identified in this study, 14-methyl pentadecanoic acid is the most abundant compound which has been reported to possess anti-microbial activity against microorganisms. It can disrupt cell membranes, leading to their inhibition or destruction. It may also possess anti-inflammatory properties. Another important compound identified is Cyclohexasiloxane dodecamethyl which is usually employed in skincare products to facilitate absorption into the skin. Another compound that is chemically related to the previously mentioned, is Cyclotetrasiloxane and it is used in cosmetic products. 9,12-octadecanoic acid which is another major compound was identified. This is also known as linoleic acid. Linoleic acid is an essential fatty acid. It serves as a precursor for the synthesis of other important fatty acids. It also serves as a precursor for the synthesis of eicosanoids including prostaglandins, thromboxane, and leukotrienes. Linoleic acid also possesses pro-inflammatory and anti-inflammatory effects. Another compound, hexadecanoic acid, methyl ester is commonly known as palmitic acid ester. This fatty acid has been also detected in the extract of *Vitex negundo* and reported with antioxidant, and hypocholesterolemic activities.[40]

Conclusion

The pharmacognostic (macroscopy, microscopy, and physicochemical) parameters determined in this study can be utilized in establishing the identity, purity, and quality of *N. bisserata* among other closely related ferns. The ethanol extract of *N. biserrata* leaves contained different phytochemicals whose presence might be responsible for the use of the plant for the treatment of various ailments traditionally. This study showed that the ethanol extract of *N. biserrata* leaves possesses antioxidant properties and isolation of bioactive compounds might be essential to establish the pharmacological properties of its phytoconstituents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

Figure 6: Total Ion chromatogram of N. bisserrata

Table 6: Some compounds identified in the ethanol extract of *N. biserrata* leaves by GC-MS Analysis

S/N	Retention Time (min)	Name of Compound	Peak area (%)
1	3.333	Cyclotetrasiloxane, octamethyl	1.76
2	13.158	Pentadecanoic acid, 14-methyl	2.24
3	13.158	Pentadecanoic acid, 14-methyl-, methylester	2.24
4	7.344	Cyclohexasiloxane, dodecamethyl-	0.84
5	8.140	Lysergamide	0.73
6	12.42	9,12-octadecanoic acid	0.80
7	13.158	Hexadecanoic acid, methyl ester	2.24
8	4.546	Undecane, 4,6-dimethyl-	7.5
9	5.181	Cyclopentasiloxane, decamethyl	1.08
10	9.931	4-pyridinecarboxylicacid, 3-cyano	1.22
11	10.257	Diethylphthalate	50.89
12	4.626	Carbonic acid, nonyl vinyl ester	5.54

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