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Impact of Aqueous *Bryophyllum Pinnatum* Leaf Extract on Hematological Indices, Parasitemia, and Mortality in Wistar Albino Rats

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ABSTRACT

Bryophyllumpinnatum is a perennial plant, traditionally used to manage multiple health issues in folk medicine. This research investigated the hematological and parasitemic consequences of sub-acute exposure to aqueous extract of B. pinnatum leaves in Wistar rats. Five groups of six adult male rats each were randomly formed and treated with varying concentrations of the extract administered orally for 14 days. The extract elevated the red blood cells, hemoglobin, hematocrit which ranged from 7.1 to 8.3x10^6 µL, 8.7 to 12.3 g/dL, 26.3 to 37.0 %, and survival rate 33.33 to 100 % but reduced the parasitemia level from day 4 to 14 (27.3 to 14.1 and 60.8 to 29.3%). Phytochemical, proximate, and mineral compositions of the plant portions were also analyzed using the standard conventional methods. The qualitative phytochemical results revealed that the plant portions contain some secondary metabolites. Quantitative results ranged from alkaloids 69.3 to 153.3 mg/g, flavonoids 4.5 to 30.5 mgQE/g, tannin 46.0 to 113.8 mg/g, glycosides 9.30 to27.7 mg/g, saponin 21.7 to 42.2 mg/g, phenol 66.6 to 143.1 mg/g. Proximate results ranged from 2.93 to 4.04 moisture, 1.73 to 1.80 ash, 3.48 to 3.84 protein, 0.54 to 0.81 liquids, 1.83 to 3.20 fibre, and 87.28 to 89.08 % carbohydrates, respectively. Mineral results ranged from 26.46 to 43.84, 19.11 to 34.70, 31.41 to 53.24, 16.28 to 30.69, 1.38 to 2.46, 0.14 to 0.25, and 0.13 to 0.20, 0.03 to 0.09 ppm Ca, Mg, Na, K, Fe, Mn, Cu and Zn, respectively. The presence of bioactive compounds in the plant parts may underline its medicinal value, indicating its potential use in therapeutic applications.

Keywords: *Bryophyllum pinnatum, phytochemicals, proximate, minerals, anti-malaria, and parasitemia*

Introduction

Plants have long served as invaluable resources in the quest for novel therapeutic agents, largely due to their rich array of bioactive compounds such as antioxidants, hypoglycemic agents, and hypolipidemic constituents. A substantial proportion of modern pharmaceutical drugs are either directly derived from plants or modeled after plant-based compounds, highlighting the pivotal role of flora in drug discovery and development [17]. The vast chemical diversity present in botanical species continues to be a significant focus of scientific research for the identification of new pharmacological leads.

In many rural and underserved communities, medicinal plants remain the cornerstone of primary healthcare. Their widespread use is attributed to their accessibility, affordability, and perceived efficacy and safety [17]. According to the World Health Organization, approximately 80% of the global population relies on traditional medicine as their primary healthcare system [18]. The significance of medicinal plants in global health is underscored by the fact that at least 25% of contemporary drugs listed in modern pharmacopoeias are plant-derived, along with many synthetic analogues inspired by natural phytochemicals [19].

This growing interest in medicinal plants is further driven by the escalating cost of conventional pharmaceuticals, the rise in demand for personalized healthcare, and the continuous global effort to bioprospect novel plant-based therapies [20]. Medicinal plants serve as reservoirs of numerous bioactive constituents including alkaloids, glycosides, saponins, oleoresins, sesquiterpene lactones, and essential oils [21]. These phytochemicals exhibit notable prophylactic and therapeutic properties, particularly in the treatment of infectious diseases [22]. Across various cultures, traditional medicine has employed these botanicals to treat ailments such as malaria, epilepsy, diarrhea, dysentery, and microbial infections [23].

Among such medicinal plants, *Bryophyllumpinnatum*—a succulent perennial from the Crassulaceae family—is widely recognized for its ethnomedicinal importance. This erect plant, which can grow up to 1.5 meters in height, reproduces through both seeds and vegetative leaf buds [24].

Originally cultivated for ornamental purposes, *Bryophyllumpinnatum* has become naturalized and is often found as a weed near plantation crops [25]. Commonly referred to as "air plant," "miracle leaf," or "never die," it is used extensively in traditional medicine systems throughout tropical regions including Africa, the Americas, India, China, and Australia.

The plant is particularly valued for its wound healing and hemostatic properties. In Southeastern Nigeria, for example, the juice extracted from mildly heated leaves is applied topically to aid in postnatal umbilical care, and crushed leaves mixed with palm oil are used externally to treat abscesses [24, 25]. Pharmacological studies have substantiated several of its traditional uses, revealing a broad spectrum of bioactivities including anthelmintic, immunosuppressive, hepatoprotective, antinociceptive, anti-inflammatory, antidiabetic, nephroprotective, antimicrobial, analgesic, neuropharmaco logical, and antipyretic effects [26].

In Nigeria and other tropical regions, *Bryophyllumpinnatum* is traditionally employed to manage conditions such as rheumatism, arthritis, heartburn, ulcers, diabetes mellitus, microbial infections, and hypertension [54]. Its pharmacological potential is supported by numerous studies that have confirmed a range of bioactive compounds including alkaloids, flavonoids, triterpenes, glycosides, steroids, bufadienolides, lipids, and organic acids [55–57]. These constituents are believed to contribute significantly to its wide array of therapeutic effects. This study aims to provide comprehensive insights into the phytochemical profile, proximate composition, mineral content, and antimalarial activity of *Bryophyllumpinnatum*. By evaluating its chemical constituents and pharmacological potential, the study seeks to validate and expand upon the traditional uses of this versatile medicinal plant.

Materials and Methods

Source of Raw Material

Bryophyllumpinnatum plants were collected from a vegetative garden located in Emene, Enugu East Local Government Area, Enugu State, Nigeria. The collected plant parts were carefully sundried to preserve their phytochemical integrity, and then pulverized into a fine powder using a mechanical grinder. The powdered plant material was subsequently stored in airtight containers under dry conditions to prevent contamination and degradation prior to laboratory analysis.

Phytochemical Screening

Qualitative analysis of the plant extracts was carried out using the standard phytochemical method as described by [4].

Extraction of the plant material

The plant parts were extracted with the following solvents: (n-hexane, butanol, acetone, water and ethanol). Exactly 10 gram of the sample was reacted against 100 mL of each solvent (n-hexane, butanol, acetone, water, and methanol) in an Erlenmeyer flask. The mixture was placed in a vibrator shaker (IKA MS 3 digital) at room temperature for 3hrs at 400 rpm. The volume of the extract was reduced to about 50 mL using the procedure below:

Recovery of Extracted Mixture

Each plant extract was filtered using Whatman No. 1 filter paper into a clean, oven-dried, and pre-weighed 250 cm^3 round-bottom flask. The flask was mounted on a rotary evaporator (IKA RV 10), connected to a receiving flask and condenser system. The setup was immersed in a thermostatically controlled water bath (TS-1006), and solvent removal was carried out by distillation until the extract volume was reduced to approximately 50 mL. The concentrated extract was then transferred into a separate clean, oven-dried, and pre-weighed evaporation basin. Residual solvent was allowed to evaporate under ambient conditions in a fume chamber. After complete solvent evaporation, the extract was cooled to room temperature and re-weighed to determine the yield of the dried crude extract.

Phytochemical Screening

The crude extracts, separated based on the type of solvent used during extraction, were subjected to preliminary phytochemical analysis for the presence of various bioactive constituents including alkaloids, flavonoids, tannins, glycosides, steroids, polyphenols, and saponins. Standard qualitative methods were employed for each test as outlined below:

Test for Alkaloids

Exactly 1 gram of each crude extract was weighed and divided equally into two test tubes. To the first test tube, two drops of Dragendorff's reagent were added, and to the second, two drops of Mayer's reagent. The formation of an orange-red precipitate in the presence of Dragendorff's reagent or a white precipitate with Mayer's reagent was taken as indicative of the presence of alkaloids.

Phytochemical Screening

Qualitative phytochemical analysis was conducted on the crude extracts of *Bryophyllumpinnatum* using standard procedures to detect the presence of major secondary metabolites. The specific tests performed included those for alkaloids, saponins, flavonoids, steroids, tannins, and glycosides, as described below:

Test for Alkaloids

Approximately 1 g of each extract was placed into two separate test tubes. To one tube, 2 drops of Dragendorff's reagent were added, and to the other, 2 drops of Mayer's reagent. The development of an orange-red precipitate (Dragendorff's) or a white precipitate (Mayer's) confirmed the presence of alkaloids.

Test for Saponins

About 5 g of the extract was placed in a test tube, and distilled water was added. The mixture was vigorously shaken for several minutes. The formation of a stable, persistent froth lasting at least 15 minutes indicated the presence of saponins.

Test for Flavonoids

A volume of 3 mL of the extract was mixed with 10 mL of distilled water and shaken thoroughly. Then, 1 mL of 10% sodium hydroxide (NaOH) solution was added. The appearance of a yellow coloration was considered indicative of flavonoids.

Test for Steroids (Salkowski's Test)

To 1 mL of the extract in a test tube, 5 drops of concentrated sulfuric acid (H_2SO_4) were added carefully. The development of a red coloration at the interface suggested the presence of steroidal compounds.

Test for Tannins

About 2 mL of each extract was gently boiled for 2 minutes and allowed to cool. Three drops of ferric chloride ($FeCl_3$) solution were added to each test tube. The formation of an orange coloration indicated the presence of tannins.

Test for Glycosides (Keller-Killiani Test)

Approximately 0.5 g of the extract was dissolved in 5 mL of distilled water. To this solution, 2 mL of glacial acetic acid containing one drop of ferric chloride was added. Subsequently, 1 mL of concentrated sulfuric acid was carefully layered beneath the mixture. The formation of a distinct brown ring at the interface confirmed the presence of cardiac glycosides.

Quantitative Analysis

Flavonoid content

Total flavonoid content was determined spectrophotometrically using aluminum chloride ($Alcl_36H_2O$) solution and quercetin was used as a reference to produce a standard curve. The total flavonoid content was described by[5]. 5 mL of sample extract was placed in a 50mL volumetric flask containing 20mL of distilled water and 1mL of 5% sodium nitrite was added and mixed. After 5 min, 1 mL of 10 % aluminum chloride solution was added and the mixture is allowed to stand for another 5 minutes, after which 5 mL of 1 M sodium hydroxide was added and properly mixed. The absorbance of the reaction mixture was read at 510nm after 15 minute with a spectrophotometer. Quercetin (10-750µ/mL) was used to plot a standard curve. Total flavonoid content was expressed in milligram quercetin equivalent per gram of sample mg QE/mc or (mg QE/100mL or per g sample extract).

Flavonoid (mg QE/g) = C×V×/M x D.F.

Determination of Tannin and Alkaloid Content

Tannin Content

Tannin concentration in the plant extract was determined using the spectrophotometric method described by [7]. One gram (1.0 g) of the dried sample was weighed and dispersed in 50 mL of distilled water. The mixture was agitated and left to stand for 30 minutes at room temperature, with intermittent shaking every 5 minutes. After 30 minutes, the mixture was centrifuged, and the supernatant (extract) was collected.

Into a 50 mL volumetric flask, 2.5 mL of the sample extract was added. Similarly, 2.5 mL of a standard tannic acid solution was dispensed into another 50 mL volumetric flask to serve as the standard. To each flask, 1.0 mL of Folin-Denis reagent and 2.5 mL of saturated sodium carbonate solution were added. The mixtures were then diluted to volume with distilled water and incubated for 90 minutes at room temperature.

The absorbance of the sample and standard solutions was read at 250 nm using a spectrophotometer, with a reagent blank used to zero the instrument.

Alkaloids (mg/g) = M_3–M_2/M1 ×1000

Alkaloid Content

The alkaloid content was determined using the gravimetric method as described by [8]. One gram (1.0 g) of the sample was macerated in 50 mL of 10% acetic acid prepared in ethanol. The mixture was allowed to stand for 4 hours with intermittent shaking, and then filtered. The resulting filtrate was concentrated to one-quarter (¼) of its original volume using a water bath.To precipitate the alkaloids, concentrated ammonium hydroxide (NH_4OH) was added dropwise until complete precipitation occurred.

The precipitate was collected on a pre-weighed filter paper, washed with 1% NH_4OH solution, and dried in a hot air oven at 80 °C for 30 minutes. The dried residue (alkaloid) along with the filter paper was weighed.

Calculation

The weight of the alkaloid content was determined by subtracting the initial weight of the filter paper from the final weight (filter paper + alkaloid precipitate), and results were expressed in mg/g of sample.

Saponin Content

Saponin content was determined using the gravimetric method described by [8]. Five grams (5.0 g) of the sample was weighed into a 250 mL conical flask and soaked in 100 mL of 20% ethanol for 10 minutes. The mixture was then heated at 55 °C for 3 hours in a water bath. After the initial extraction, an additional 100 mL of 20% ethanol was used for re-extraction, and the pooled extracts were concentrated to one-quarter of their original volume at 90 °C.

The concentrated extract was transferred into a 500 mL separating funnel. Exactly 25 mL of diethyl ether was added, and the mixture was shaken vigorously. The two layers formed were allowed to separate, and the aqueous (upper) layer was retained while the ether layer was discarded. This purification step was repeated once. Then, 60 mL of n-butanol was added, and after mixing, the lower layer was discarded while the upper (n-butanol) layer was retained.

The combined n-butanol extract was washed twice with 10 mL of 5% aqueous NaCl solution, and the lower layer was again discarded. The final extract was evaporated to dryness in a water bath and the weight was recorded.

Saponin (mg/g) = M_2–M_1/M ×1000

Cyanogenic Glycoside Content

Cyanogenic glycoside content was determined spectrophotometrically using the method described by [5]. Exactly 5.0 g of the sample was soaked and agitated in 100 mL of distilled water for 3 hours at room temperature. The mixture was filtered, and 1 mL of the extract was transferred into a 50 mL conical flask. Then, 10 mL of 10% 2,2-dinitrosalicylic acid solution was added. The mixture was boiled in a water bath at 60 °C for 30 minutes and subsequently cooled for 15 minutes. The final volume was made up to 50 mL with distilled water in a volumetric flask. The absorbance was measured at 540 nm using a spectrophotometer.

Glycoside (mg/g) = Abs × VTe × D.F/Volume analyzed × Mass of sample ×Volume of cuvette used.

Steroid Content

Steroid content was determined according to the method described by [8].The plant extract was filtered using Whatman No. 42 filter paper. The filtrate was transferred into a separatory funnel and an equal volume of ethyl acetate was added. After thorough mixing and phase separation, the ethyl acetate (upper) layer containing the extract was recovered, and the aqueous phase was discarded.

The extract was dried at 100 °C for 5 minutes on a steam bath. It was then heated with concentrated amyl alcohol to extract the steroids, resulting in a turbid mixture. The mixture was filtered using a pre-weighed Whatman No. 42 filter paper, dried, and then cooled in a desiccator.

This process was repeated two more times, and the average yield was calculated.
Steroid (%) = M2–M1/Mass of test portion ×100/1.

Proximate Composition
The proximate composition—moisture content, crude protein, crude fat, ash, crude fiber, and total carbohydrates—was determined in triplicate using standard AOAC methods as described by [6].

Moisture Content
Moisture content of the powdered samples was determined using a Sartorius Moisture Analyzer MA-300000V3 (Göttingen, Germany) following the manufacturer's standard operating procedure.
The analyzer was preheated for at least 30 minutes before use. Approximately 2.0 g of the sample was evenly spread on a pre-weighed, tarred aluminum pan. The analysis was carried out in fully automated mode at a constant temperature of 105 °C until drying was complete. Moisture content was recorded as the percentage loss in weight.

Total Ash Content
Total ash was determined using the dry ashing method described by [6]. Two grams (2.0 g) of the sample was placed in a clean, pre-ignited, and pre-weighed silica dish. The dish was first gently heated over a low flame and then transferred to a muffle furnace (Thermotec TIC-400) at 550 °C for 3 hours until a light grey or white ash was obtained. The dish was then cooled in a desiccator and reweighed. The ash content was calculated as a percentage of the original sample weight.

Fat Content
Fat content was determined using Soxhlet extraction method as outlined in [6].A 500 mL round-bottom flask was filled with 250 mL of *n*-hexane and connected to a Soxhlet extractor. Five grams (5.0 g) of the sample was placed in a labeled extraction thimble, which was sealed with cotton wool. The apparatus was heated to allow reflux for 3 hours. After extraction, the *n*-hexane was recovered via distillation, and the flask was dried at 70 °C for 15 minutes using a heating mantle. The flask was cooled in a desiccator and reweighed. Fat content was calculated by the weight difference.

Crude Protein Content
Crude protein was determined using the Kjeldahl method as described by [6].One gram (1.0 g) of the sample was digested in a 50 mL Kjeldahl flask containing 10 g of anhydrous sodium sulfate (Na_2SO_4), 1 g of copper sulfate ($CuSO_4$) as a catalyst, and 20 mL of concentrated sulfuric acid (H_2SO_4). The mixture was digested in a fume hood until a clear bluish-green solution was obtained. The digest was allowed to cool, transferred to a 250 mL volumetric flask, and diluted to volume with distilled water.
For distillation, 100 mL of 4% boric acid solution with a few drops of methyl red indicator was placed in a receiving flask. Five milliliters (5 mL) of the digest was pipetted into the distillation unit, washed with distilled water, and treated with 40 mL of 60% sodium hydroxide (NaOH). The distillate (approximately 200 mL) was collected and titrated against 0.1 N sulfuric acid until a pink endpoint was observed. A blank determination was also carried out using filter paper only.

Crude Fiber Content
Crude fiber content was determined using the standard method described by [6].One gram (1.0 g) of the defatted sample was weighed into a 250 mL conical flask. To this, 200 mL of 1.25% sulfuric acid (H_2SO_4) and a few drops of an anti-foaming agent were added. The flask was placed on a digestion apparatus equipped with a re-adjustable hot plate and boiled for 30 minutes. During boiling, the flask was periodically rotated to prevent the solid material from adhering to the walls. After 30 minutes, the mixture was allowed to settle for 1 minute and filtered using a Büchner funnel under vacuum. Without breaking the suction, the residue was thoroughly washed with boiling water until free from acid.
The insoluble matter was then transferred back into the cleaned original flask, and 200 mL of 1.25% sodium hydroxide (NaOH) solution was added. The mixture was boiled again for 30 minutes under similar conditions. Afterward, it was allowed to stand for 1 minute and filtered immediately under vacuum. The residue was washed successively with boiling water, 1% hydrochloric acid (HCl), and again with boiling water until acid-free. It was subsequently washed twice with ethanol and three times with ether.
The final residue was transferred to a pre-weighed ashing dish and dried in an oven at 100 °C to a constant weight. It was then incinerated in a muffle furnace at 600 °C for 6 hours, cooled in a desiccator, and reweighed. The crude fiber content was calculated as the difference between the oven-dry weight and the weight after ashing and expressed as a percentage of the original sample mass.

Total Carbohydrate Content
Total carbohydrate content was determined by difference, using the proximate values obtained for moisture, crude protein, crude fat, crude fiber, and ash, as described by [6].

Determination of Mineral Composition
The mineral content of the samples was determined using a wet digestion method as described by [6]. Exactly 1 gram of the powdered sample was digested in a 100 mL beaker with 15 mL of a 2:1 mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$). The mixture was placed on a water bath at 65 °C for 1 hour. The resulting clear digest was allowed to cool at room temperature and filtered using Whatman No. 42 filter paper. The filtrate was then made up to 100 mL with deionized water in a standard volumetric flask and transferred into sterile sample bottles for analysis.
Elemental analysis was carried out using a BUCK 210 VGP Flame Atomic Absorption Spectrophotometer (FAAS). Analytical-grade reagents and deionized water were used throughout. Stock standard solutions of 1000 mg/kg for each element (Cu, Zn, Mg, Cd, Ca, K, Mn, Fe, Pb, and Na) were prepared from their respective salts, and working standards were obtained by serial dilution.
Blank determinations were carried out as per the instrument manual. The concentrations of the elements in each digested sample were calculated by comparing absorbance readings against standard calibration curves for each element. Specific hollow cathode lamps were used as radiation sources at the following wavelengths:

- **Cd** – 228.9 nm
- **Cu** – 327.4 nm
- **Mn** – 279.5 nm
- **Pb** – 283.3 nm

- **Fe** – 372.0 nm
- **Ca** – 422.7 nm
- **Mg** – 202.6 nm
- **K** – 203.0 nm
- **Na** – 589.0 nm
- **Zn** – 307.6 nm

Lamp intensities, slit widths, and band pass settings were used according to the manufacturer's specifications. Acetylene and air flow rates were maintained at 5 L/min and 20 L/min, respectively, for all elements.

Experimental Animals

This study was conducted in accordance with the OECD/OCDE Guidelines No. 425 for acute oral toxicity testing using the limit dose up-and-down method. A total of thirty (30) adult male albino Wistar rats, aged 12 weeks, were procured from the Animal House of the Enugu State University of Science and Technology (ESUT), Enugu, Nigeria. The animals were housed under standard laboratory conditions (well-ventilated environment, ambient room temperature) and allowed free access to standard rodent chow and clean drinking water ad libitum.

All experimental procedures involving animals were approved by the Animal Research Ethics Committee of the university and carried out in accordance with the guidelines for the care and use of laboratory animals.

Experimental Design

The thirty animals were randomly assigned into five (5) groups, with six (6) rats per group, as follows:

- **Group I (Normal Control):** Received normal saline (1 mL/kg) daily for 14 days.
- **Group II (Negative Control):** Inoculated with *Plasmodium chabaudi* (5 mg/kg) daily for 14 days without treatment.
- **Group III (BPE Low Dose):** Inoculated with *Plasmodium chabaudi* and treated with 100 mg/kg of *Bryophyllumpinnatum* leaf extract.
- **Group IV (BPE Medium Dose):** Inoculated and treated with 200 mg/kg of the extract.
- **Group V (BPE High Dose):** Inoculated and treated with 400 mg/kg of the extract.

Extracts were administered by oral gavage once daily for 14 consecutive days.

At the end of the treatment period, all animals were sacrificed by cervical dislocation under deep diethyl ether anesthesia. Blood samples were collected into EDTA bottles and plain tubes for analysis of:

- **Parasitemia levels**
- **Survival rate**
- **Hematological parameters, including:**
- **Hemoglobin (Hb)**
- **Hematocrit (HCT)**
- **Red blood cell (RBC) counts**

Determination of Parasitemia in Blood Samples of Wistar Rats

The determination of parasitemia was carried out following the procedure described by [10]. Blood samples were collected from the tail vein of the experimental rats into EDTA-coated tubes to prevent coagulation. Samples were properly labeled and stored at room temperature until further analysis.

A drop of blood from each sample was placed on a clean glass microscope slide and evenly spread using a sterile spreader to form a thin film (blood smear).

The smears were air-dried completely and then fixed in absolute methanol for 7 minutes.

Following fixation, the smears were stained using Giemsa stain, prepared and applied according to the manufacturer's instructions. After staining, the slides were gently rinsed with distilled water and allowed to air-dry again.

Each stained smear was examined under a light microscope using the oil immersion lens (100x objective). For each slide, parasitemia was assessed by counting the number of *Plasmodium* parasites per 100 red blood cells (RBCs). The degree of parasitemia was recorded accordingly.

Determination of Hematological parameters

According to ICSH [11], the hematological parameters were carried out usingautomated hematology analyzer (ErbaMannheim Models – H360). The blood sample was collected into EDTA tubes and it was mixed thoroughly. The samples were labeled with sample name, date and time collected. The samples were carefully stored at room temperature (25 °C) until the analysis was ready. Prior to the main analysis, the samples were checked for visible signs of hemolysis, clotting, or contamination. The automated hematology analyzer was turned on. The samples were loaded onto the analyzer and the desired parameter (Hb, Hct, and RBC) were selected). The analyzer was allowed to perform the analysis automatically. The results were displayed after the performance.

Statistical Analysis

Data obtained from the study were expressed as mean ±standard error of mean (SEM) for each group. Statistical differences among the experimental groups were evaluated using one-way analysis of variance (ANOVA), followed byDuncan's Multiple Range Test for post hoc comparisons. All analyses were conducted using IBM SPSS Statistics, version 25.0 (IBM Corp., Armonk, NY, USA). A p-value ≤ 0.05 was considered statistically significant.

Results and Discussion

The results presented in Table 1 (a–c) show the phytochemical screening of *Bryophyllumpinnatum* leaves, stems, and roots, respectively. Phytochemical analysis is essential for identifying bio active constituents in medicinal plants, which are often responsible for their therapeutic properties [48]. The qualitative evaluation of the different plant parts of *B. pinnatum* revealed the presence of important secondary metabolites such as alkaloids, glycosides,tannins, phenols, saponins, and flavonoids. These compounds are well-documented for their pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, antiplasmodial, and cyto protective effects.

Table 1(a): Phytochemical screening of Bryophllumpinnatumleaves.

Sample	Constituents	HEN	MET	BUT	ACT	WAT
BPL	Alkaloids	+++	+	++	-	++
	Flavonoid	+	+	-	-	+
	Tannin	+	+++	+	+	+++
	Glycoside	+	-	+	-	+
	Saponin	+	++	++	+	+
	Phenol	+++	+++	++	+	++
	Steroid	++	+	-	-	+++

BPL: Bryophyllumpinnatumleave; HEN-Hexane; MET-Methanol, BUT-Butanol; ACT-Acetone; WAT-Water

Table 1(b) phytochemical screening of Bryophyllumpinnatum stems

Sample	Constituents	HEN	MET	BUT	ACT	WAT
BPS	Alkaloids	++	+	+	++	+
	Flavonoid	-	-	+	+	-
	Tannin	-	-	+	+	-
	Glycoside	-	-	-	-	-
	Saponin	+	-	-	+	+
	Phenol	++	+	+	+	+
	Steroid	+	-	+	-	+

BPS: Bryophyllumpinnatumstem

Table 1(c): Phytochemical screening of Bryophyllumpinnatum root

Sample	Constituents	HEN	MET	BUT	ACT	WAT
BPR	Alkaloids	+	+	+	+	+
	Flavonoid	+	-	+	+	+
	Tannin	+	+	++	+	-
	Glycoside	-	+	-	-	-
	Saponin	+	-	-	+	+
	Phenol	++	++	+	+	-
	Steroid	++	+	+	+	-

BPR: Bryophyllumpinnatumroot

Quantitative Phytochemical Composition

Quantitative analysis revealed that alkaloids were present in notably high concentrations, ranging from 69.3 to 153.3 mg/g across different parts of *Bryophyllumpinnatum*. Alkaloids are among the most therapeutically significant bioactive constituents in medicinal plants [35]. Both naturally occurring and synthetic alkaloids are widely employed in modern medicine for their analgesic, antispasmodic, and bactericidal activities [27]. Structurally, they are nitrogen-containing organic compounds known for a wide range of physiological effects, including sedative, analgesic, and neuroactive properties. In addition, they have been shown to alleviate stress and depressive symptoms [28]. However, in high doses, alkaloids may become toxic, potentially aggravating neurological or cellular disorders due to their stimulatory effects [29]. The alkaloid levels observed in this study are consistent with previously reported values ranging from 5.70% to 18.72% [3].

The tannin content, ranging from 46.0 to 113.8 mg/g, indicates that the leaves, stems, and roots of *B. pinnatum* possess significant astringent properties. Tannins are known for their wound-healing, anti-inflammatory, and antimicrobial effects [30]. These water-soluble phenolic compounds are capable of precipitating proteins and are widely distributed in vascular plants. Through protein-binding mechanisms, tannins can interfere with nutrient absorption, which may be beneficial or detrimental depending on dosage and application [31]. The values obtained align with those reported in similar studies on medicinal plants [32].

The saponin content ranged from 2.17 to 42.2 mg/g, suggesting potential application in areas such as fertility enhancement and immune modulation. However, the saponin levels detected were relatively lower than those reported in other studies [33]. Saponins are glycosidic compounds comprising a triterpenoid or steroidala glycone linked to a carbohydrate moiety. At low concentrations (typically below 10%), they are considered safe and non-toxic. Nevertheless, at higher concentrations, saponins have been associated with gastrointestinal irritation, such as diarrhea and dysentery, primarily due to gastroenteritis induced by membrane disruption [36]. These results underscore the therapeutic potential of *Bryophyllumpinnatum*, especially given the presence of bioactive constituents in pharmacologically relevant concentrations. However, caution must be exercised in dosage formulations, especially for compounds like alkaloids and saponins, which exhibit dose-dependent toxicity.

The appreciable levels of flavonoids, ranging from 4.50 to 30.5 mg QE/g, in the *Bryophyllumpinnatum* samples indicate the plant's potential to exert a wide range of biological activities. Flavonoids are well-documented for their antioxidant properties and their ability to confer protective effects against various pathological conditions, including inflammation, allergies, microbial infections, ulcers, viral infections, free radical-induced cellular damage, platelet aggregation, and tumor development [49]. These multifunctional phytochemicals are capable of scavenging reactive oxygen species and modulating signaling pathways involved in cellular defense mechanisms.

The glycoside content was found to range between 9.30 and 27.0 mg/g, with the leaf portion exhibiting the lowest concentration and the root portion the highest. These values are consistent with previously reported ranges of 15.3 to 31.2 mg/g [1]. Glycosides, particularly cardiac glycosides, are known for their pharmacological significance, especially in the treatment of congestive heart failure and other cardiovascular conditions [37]. These compounds act by inhibiting the Na^+/K^+-ATPase pump, which leads to an increase in intracellular calcium, thereby enhancing cardiac contractility. However, despite their therapeutic potential, glycosides must be used with caution due to their narrow therapeutic index.

Table 2. Quantitative analysis of Bryophyllumpinnatum

Sample portion	Alkaloid (mg/g)	Flavonoid (mgQE/g)	Tannin (mg/g)	Glycoside (mg/g)	Saponin (mg/g)	Phenol (mg/g)	Steroid (mg/g)
Leaves	153.3±1.9	30.5±0.24	113.8±0.70	9.30±0.30	36.1±0.80	87.3±0.01	23.4±0.50
Steams	12.4±0.55	25.4±0.91	97.8±1.21	13.1±0.33	21.7±0.77	66.6±0.31	41.1±0.20
Roots	69.3±1.94	4.5±0.30	46.0±0.34	27.7±0.40	42.2±0.16	143.1±0.11	83.4±0.11

Values are means of triplicate determinations

Steroids are a class of both naturally occurring and synthetic lipid-based compounds, characterized by their fat-solubility and involvement in a wide range of physiological and biochemical functions [16]. They play key roles in hormonal regulation, immune response, and cell membrane stability, and are frequently targeted in drug development due to their diverse bioactivity.

The proximate composition of *Bryophyllumpinnatum*, as shown in Table 3, indicates a high nutritional value. The moisture content, ranging from 2.90% to 4.04%, reflects a low susceptibility to microbial spoilage, which suggests an extended shelf life for the dried plant material. These values are in agreement with previously reported findings (3.13–3.18%) [3].

The ash content (1.73%–1.90%) indicates the presence of inorganic mineral constituents such as calcium, zinc, magnesium, copper, and potassium, which are essential for various metabolic and structural functions in the body.

Crude protein levels ranged from 3.48% to 3.84%, suggesting that *B. pinnatum* can serve as a modest source of dietary protein. Proteins are crucial macronutrients involved in tissue repair, energy metabolism, and the synthesis of enzymes, hormones, and antibodies. Protein deficiency, particularly in children, is associated with growth retardation, muscle wasting, edema, and other signs of protein-energy malnutrition [38, 39].

The crude fiber content varied between 1.83% and 3.20%, with the root portion exhibiting the highest value. Dietary fiber enhances gastrointestinal health by promoting regular bowel movements, absorbing water, and increasing stool bulk—thereby preventing constipation [40]. Additionally, adequate fiber intake has been linked to weight management, reduced blood cholesterol levels, and a lower risk of colorectal cancer.

The lipid content was notably low across all plant parts, further supporting its potential role in the formulation of low-fat or weight-reducing diets. A diet low in fat is often associated with improved cardiovascular health, lower cholesterol levels, and a reduced risk of obesity [41].

The mineral composition of *Bryophyllumpinnatum*, as shown in Table 4, reveals that the plant contains appreciable levels of essential minerals. Notably, the calcium content ranged from 26.46 to 43.84 ppm, with relatively high concentrations across all plant parts. Calcium is a critical mineral involved in the regulation of muscle contraction and is essential for the formation and maintenance of bones and teeth, particularly during growth and development in infants, children, and fetuses [42]. Moreover, the maintenance of normal extracellular calcium levels is vital for blood coagulation and the structural integrity of intercellular cement substances in epithelial tissues [43].

Potassium levels ranged from 16.28 to 30.69 ppm, with the root portion exhibiting the highest concentration. These findings are consistent with prior studies, which report that potassium is among the most abundant minerals in Nigerian agricultural products [44]. Functionally, potassium plays a key role in regulating water and electrolyte balance, maintaining normal blood pressure, supporting nerve transmission, and ensuring proper muscle function [45]. Additionally, potassium contributes to body weight regulation and helps mitigate the risks associated with hypertension and cardiovascular diseases.

Table 3: Proximate composition of Bryophyllumpinnatum(%)

Plant portion	Moisture	Ash	Protein	Lipids	Fibre	CHO
Leaves	2.93±0.38	1.73±0.71	3.62±0.50	0.81±0.21	1.83±0.11	87.08±0.43
Stems	4.04±0.41	1.90±0.81	3.48±0.74	0.54±0.14	2.34±0.22	87.70±0.55
Roots	3.19±0.61	1.80±0.30	3.84±0.11	0.69±0.99	3.20±0.11	87.28±0.39

Values are means of triplicate determination

The sodium concentration in *Bryophyllumpinnatum* ranged from 31.41 to 53.24 ppm, with the root containing the highest level and the stem the lowest. This relatively high sodium content may support traditional claims regarding the plant's use in managing heart-related conditions, as sodium plays a role in cardiac function and fluid balance. However, it is important to note that excessive sodium intake has been linked to hypertension and cardiovascular risk [45].

The zinc content in the plant samples was relatively low, ranging from 0.03 to 0.09 ppm. Despite its low concentration, zinc remains a critical trace element essential for protein and nucleic acid synthesis, as well as for normal growth and development [46]. Zinc also stimulates the activity of certain vitamins, contributes to the formation of red and white blood cells, and plays a key role in male fertility. Moreover, zinc's involvement in insulin function suggests that the presence of this element in *B. pinnatum* could be beneficial in the management of diabetes linked to insulin dysregulation [49].

Iron concentrations ranged from 1.38 to 2.46 ppm, aligning closely with previously reported values of 1.489 to 2.339 ppm [3]. Iron is a fundamental component of hemoglobin, essential for oxygen transport in the blood. It is particularly crucial in the diets of pregnant women, nursing mothers, infants, convalescing patients, and the elderly, to prevent iron-deficiency anemia and other related conditions [51].

The magnesium content of the samples ranged from 19.11 to 34.70 ppm. Magnesium is vital for numerous enzymatic processes, especially those involving phosphate transfer [50]. It contributes to the structural stability of nucleic acids and plays a significant role in electrolyte absorption within the intestines. Magnesium deficiency in humans may lead to symptoms such as severe diarrhea and recurrent migraines [51].

Copper levels were found to range from 0.13 to 0.20 ppm. Copper is essential for the formation of red blood cells, synthesis of hemoglobin, and is involved in energy production, wound healing, and the maintenance of skin and hair pigmentation. It also works synergistically with zinc in the enzyme superoxide dismutase, which helps to neutralize oxygen free radicals, thereby contributing to antioxidant defense [52].

The manganese content, consistent with previously reported values (0.20–0.27 ppm) [3], supports its role in immune system function, blood sugar regulation, cellular energy production, and cell reproduction. Manganese deficiency, particularly during pregnancy, may result in birth defects if maternal intake is insufficient [46].

Table 4: FAAS mineral composition of Bryophyllumpinnatum (ppm)

Plant portion	Ca	Ma	Na	K	Fe	Mn	Cu	Zn
Leaves	34.55±0.02	20.44±0.30	31.41±0.10	16.28±0.27	2.28±0.40	0.25±0.22	0.19±0.20	0.09±0.34
Stems	26.46±0.37	19.11±0.13	31.92±0.20	18.77±0.46	1.38±0.17	0.25±0.30	0.13±0.33	0.05±0.39
Roots	43.84±0.11	34.70±0.21	53.24±0.45	30.69±0.31	2.46±0.02	0.14±0.34	0.20±0.21	0.03±0.11

The percentage parasitemia results from this study highlight the antimalarial efficacy of *Bryophyllumpinnatum* extract (BPE) in Wistar rats. The extract significantly reduced parasitemia levels, improved survival rates, and enhanced haematological parameters, indicating its potential as a therapeutic agent against malaria. The observed efficacy of BPE is comparable to that of other established antimalarial agents. For instance, [13] reported that the methanolic extract of *B. pinnatum* achieved 71.4% inhibition of parasitemia at a dose of 200 mg/kg, while [14] documented 63.2% inhibition with an aqueous extract at a dose of 300 mg/kg.

These findings are consistent with the present study, which recorded parasitemia inhibition ranging from 55.09% to 74.72% at a dose of 400 mg/kg between Day 4 and Day 14.

Notably, the optimal dose of BPE observed in this study (400 mg/kg) was higher than that reported by [12], who found 100 mg/kg to be the most effective. This variation may be attributed to differences in extraction methods, parasite strains, or host animal models used across studies.

The survival rate is a crucial endpoint in malaria studies, often used to assess the therapeutic efficacy of test compounds. It was calculated as the percentage of animals that survived until the

end of the 14-day study period relative to the initial group size. The results showed that BPE significantly increased survival rates, with the 400 mg/kg dose demonstrating the greatest protective effect in Wistar rats infected with *Plasmodium chabaudi* [57].

Additionally, the hematological parameters showed marked improvements following treatment with BPE. Specifically, hemoglobin (Hb), hematocrit (HCT), and red blood cell (RBC) counts improved significantly across the treatment groups. The hemoglobin concentration in the negative control group (infected, untreated) was 8.8 g/dL, which was significantly lower than the positive control group (10.4 g/dL), suggesting that BPE treatment contributed to restoring normal hematological values by mitigating the hematotoxic effects of malaria infection.

The BPE-treated groups demonstrated a dose-dependent increase in hemoglobin levels, with the highest dose (400 mg/kg) producing the most significant elevation (12.3 g/dL). These values were significantly higher than those of the negative control group, suggesting a protective effect of the extract on hemoglobin synthesis or preservation during *Plasmodium* infection. This hematinic effect of BPE indicates its potential in mitigating malaria-induced anemia.

Similarly, hematocrit (HCT) values followed a comparable pattern. The negative control group exhibited significantly reduced hematocrit levels (26.5%) compared to the positive control group (32.1%) as shown in Table 7. In contrast, the BPE-treated groups displayed a dose-dependent improvement, with the 400 mg/kg group reaching 37.0%, representing the highest increase among all groups. The significantly higher hematocrit values in the BPE-treated rats further confirm the erythropoietic or cytoprotective properties of the extract.

In terms of red blood cell (RBC) counts, the negative control group recorded the lowest value ($4.4 \times 10^6/\mu L$). Treatment with BPE resulted in a dose-dependent increase, with the 400 mg/kg group achieving the highest count ($8.3 \times 10^6/\mu L$). These increases in RBC counts were statistically significant compared to the negative control, implying that BPE may aid in restoring erythrocyte levels compromised by parasitic infection.

These findings collectively support the hematoprotective and antimalarial potential of *Bryophyllumpinnatum*, and are consistent with the observations reported by [15], which noted similar improvements in hematological parameters following treatment with the extract in a malaria-induced model.

Table 5: Percentage parasitemia effect of Bryophyllumpinnatum extract on Wistar rats

Group	Day4	Day7	Day10	Day14
Negative control	31.4±2.0	44.8±3.8	70.1±5.6	91.0±7.3
Positive control	11.7±1.9	1.6±0.5	0.8±0.4	0.2±0.1
BPE (100mg/kg)	27.3±2.8	39.8±3.0	54.6±4.9	60.8±5.7
BPE (200mg/kg)	19.2±2.3	26.8±2.9	37.1±3.8	43.4±4.8
BPE (400mg/kg)	14.1±1.6	17.8±2.3	23.0±2.8	29.3±3.3

Values are means of triplicate determinations.

The hematological analysis results indicate that *Bryophyllumpinnatum* extract (BPE) exerts a protective effect on hemoglobin levels, hematocrit, and red blood cell (RBC) counts in *Plasmodium chabaudi*-infected Wistar rats. The extract's efficacy in improving these hematological parameters suggests its potential as an adjunctive therapeutic agent in the management of malaria. These findings support the hypothesis that BPE not only possesses antimalarial properties but also contributes to ameliorating malaria-induced hematological abnormalities, thereby enhancing the overall health and survival of infected individuals.

Table 6: Survival race of Wistar rat after BPE administration

Group	No of surviving rats	Survival rat (%)
Negative control	2	33.33
Positive control	4	66.70
BPE (100mg/kg)	5	83.33
BPE (200mg/kg)	5	83.33
BPE (400mg/kg)	6	100.00

Table 7: Hematoelogical analysis of Byrophyllum pinnatum

Group	Hemoglobin (g/dL)	Hematocrit (%)	Red blood ($x10^6$ µ)
Negative control	8.8±0.5	26.5±1.5	4.4±0.3
Positive control	10.4±0.6	32.1±2.1	8.1±0.5
BPE (100mg/kg)	8.7±0.6	26.3±1.9	7.1±0.4
BPE (200mg/kg)	10.2±0.7	33.6±2.2	7.8±0.5
BPE (400mg/kg)	12.3±0.8	37.0±2.3	8.3±0.5

Values are means of triplicate determinations

5. CONCLUSION

This study demonstrates that the leaves, stems, and roots of *Bryophyllumpinnatum* are rich sources of bioactive phytochemicals, proximate nutrients, andessential mineral elements. The quantitative and qualitative analyses confirm the presence of pharmacologically important compounds such as alkaloids, tannins, flavonoids, saponins, glycosides, and steroids, alongside appreciable levels of proteins, fibers, and vital minerals like calcium, potassium, sodium, magnesium, and iron. These constituents collectively underscore the therapeutic potential of *B. pinnatum*, supporting its ethnomedicinal applications. The plant parts exhibited a broad range of pharmacological properties, including anti-diabetic, anti-ulcer, anthelmintic, immunosuppressive, hepatoprotective, antinociceptive, anti-inflammatory, analgesic, anticonvulsant, neuropharmacological, antipyretic, and antihy pertensive effects, the antimalarial activity of the extract was evident through its significant reduction in parasitemia, improvement in survival rates, and enhancement of hematological parameters in *Plasmodium chabaudi*-infected Wistar rats. These findings provide scientific validation for the traditional use of *Bryophyllumpinnatum* in treating malaria and related ailments, suggesting its potential as a natural therapeutic agent or as acomplementary remedy in malaria management.

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