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Treatment of Plasmodiasis: Preparation, Phytochemical Studies and Nutrient Evaluations of a New Herbal Formula

J.C. Onwusonye, C.O. Osuchukwu, C.O. Onwusonye, M.U. Kalu, I.I. Olekamma, U.S. Anukam, H.A. Ihejieto, I.C. Ndu-Osuoji, C.N. Anyanwu, N.T. Obijuru, C.R. Onyema, C.C. Onumonu

Department of Microbiology/Biochemistry, Federal Polytechnic Nekede Owerri, Nigeria.

Abstract

The high cost of standard drugs and nutraceuticals, persistence of substandard drugs and food products in many African markets as well as development of resistance to standard drugs by disease pathogens have led to continued search for alternative medications and local sources of essential food nutrients. In this study, a multi-herbal formula was made from four African medicinal plants, *Alstonia scholaris*, *Annona senegalensis*, *Pteridium aquilinum* and *Caesalpinia bonducella*. This formulation was hereby subjected to phytochemical, proximate and mineral evaluations. The herbal formula was subjected to both qualitative and quantitative analysis for phytochemical determinations, atomic absorption spectroscopy and inductively coupled Plasma-optical emission spectroscopy (ICP-OES) for mineral determinations, while proximate analysis was done by the methods described by Association of Official Analytical Chemists (AOAC). Results showed the presence of the following phytochemicals: alkaloids, flavonoids, glycosides, saponins, tannins, tarpenoids and essential oils in the sample. Quantitatively, these bioactive compounds occurred in appreciable amounts in the formula with alkaloids, tannins and glycosides contents being as high as 642, 1350 and 685mg/100g respectively. Proximate analysis showed that the sample is rich in carbohydrates (40.35±0.13%), crude protein (22.70±0.13%), and crude fibre (13.01±0.02%). Appreciable amounts of minerals were found in the sample, the highest being: Ca (1206.51 ± 8.30mg/100g), Mg (586.92 ± 5.00mg/100g, potassium (350.10 ± 6.00mg/100g) and Fe (295.68 ± 4.00mg/100g). It was concluded that the herbal formula, apart from its observed antimalarial activities, can also be used to manage other ailments, as well as contribute significantly to human/livestock nutritional needs.

Keywords: Plasmodiasis, Phytochemical, Nutritient, evaluation, Herbal formula.

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1. Introduction

Malaria has remained a serious challenge confronting humanity, especially the developing world. It has been reported to cause more energy loss, debilitation, loss of work capacity and more economic damage than any other human parasitic disease [18]. In spite of the wide range of antimalarial drugs available in the market today, the problem of malaria has continued to increase, owing to a number of factors like the prevalence of sub-standard and fake drugs in many African markets, high cost of available drugs (which makes them hardly affordable by the poor rural dwellers) as well as emergence of antimalarial drugs resistance by the causative parasites. In view of these challenges, continued and sustained search for alternative medications becomes imperative. The exploitation of plants by man for the treatment of diseases has been a long time practice in history [14-30]. The tradition of herbal medicine has continued to the present time in China and many African and South American countries [21]. Plants owe their medicinal values to their respective phytochemical constituents [11]. These bioactive compounds inherent in plants, when consumed, elicit various physiological effects on both humans and livestock. Phytochemicals are chemical compounds produced as a result of metabolic reactions during plant growth [11].

These phytochemicals are also referred to as secondary metabolites [15-24]. Among the physiological actions exhibited by these phytochemicals are antioxidant, hormonal, antibacterial, enzyme activation interference with DNA [4]. Preventive medicine has been greatly enhanced by the use of natural plants antioxidants [11]. Majority of these pharmacologically active compounds found in plants include alkaloids, sesquitarpenes, glycosides, ditarpenes, tritarpenes, saponins, flavonoids etc. [12]. *HBF-19* is a multiherbal antimalarial powder formulated from four African medicinal plants – *Alstonia scholaris, Annona senegalensis, Pteridium aquilinum and Caesalpinia bonducella.* In an earlier study

(report being prepared for publication), the herbal formula showed strong antimalarial activities very comparable to two standard drugs Chloroqquine phosphate and artesunate. Through phytochemical screening, one could detect the various important compounds which may be used as the basis of modern drugs for curing various diseases [11-28]. This present study is therefore aimed at evaluating the secondary metabolic products that could possibly give credence to the observed antimalarial activities of the herbal formula as well as to assess the possibility of the herbal powder contributing in a considerable way to human dietary needs.

2. Materials and Methods

2.1. Plant Samples Collection and Identification

The four plant samples used in the herbal formulation were collected from different location in the South-Eastern part of Nigeria and duly identified by a Taxonomist in the department of Biological Sciences, Federal University of Technology Owerri.

2.2. Sample Preparation and Herbal Formulation

The fresh stem bark samples of *Alstonia scholaris* were sorted out to rid it of unwanted materials. They were cut into pieces and air-dried under shade for two weeks and subsequently milled to fine powder. The fresh leaves of *Annona senegalensis* were air-dried under shade for ten days after which they were milled to a fine powder. The fresh leaves and young shoots of *Pteridium aquilinun* were dried under shade for ten days after which they were milled to fine powder. Fresh leaves of *Caesalpinia bonducella* were air dried under shade for two weeks and subsequently milled to fine powder. Five Hundred grams of each plant powder was measured out in different breakers. These were subsequently mixed together and homogenized in an electric blender to give 2kg of mixed herbal powder.

2.3. Qualitative Phytochemical Analysis

Phytochemical analyses were done in this work following standard methods reported by Ejikeme *et al.* [10].

2.3.1. Test for the presence of Alkaloids

This was done according to the method described by Hikino *et al.* [17]. Two grams of the polyherbal powder were measured into a 250cm^3 conical flask and 20cm^3 of a solution containing 5% tetraoxosulphate (IV) acid (H_2SO_4) in 50% ethanol. After boiling for 2 minutes, the mixture was filtered using Whatman No 42 (125mm) filter paper. The resulting filtrate was made alkaline in a separating funnel by the addition of 5cm of 28% ammonia solution (NH^3). Equal volume (5cm^3) of chloroform was used to extract the resulting alkaline solution. The chloroform solution was subsequently extracted with two 5cm^3 portions of 1.0M dilute tetraoxosulphate (vi) acid. The resulting acid extract was used to carry out a test as described below: 2cm^3 of the acid extract was mixed with 0.5cm of Dragennorff's reagent (solution of Bismuth potassium iodide). An orange coloured precipitate indicates the presence of alkaloid.

2.3.2. Test for the Presence of Flavonoids

His was done according to the method of harborne and Sofowora [16-29]. Exactly 0.3g of the polyherbal powder was placed into a beaker and 30cm^3 of distilled water was added. After 2 hours, the soaked materials was filtered through Whatman filter paper No 42 (125mm). Exactly 10cm of aqueous filtrate mixed with 5cm of 1.0M dilute ammonia solution followed by addition of 5cm^3 of concentrated tetraoxosulphate (vi) acid. A yellow colouration which becomes clear on standing shows presence of flavonoids.

2.3.3. Test for the presence of Glycosides

This was done by the method of Hikino *et al.* [17]. Two grams of the herbal formula was measured out and soaked in 20cm of distilled water and heated for 5 minutes in a water bath. The mixture was subsequently filtered using Gem filter paper (12.5cm). The resulting filtrate was used to perform the following operations:

(i) Exactly 5cm of the filtrate was mixed with 0.2cm^3 of Fehlings solutions A and B until it becomes alkaline. The alkaline solution, (confirmed with litmus paper) was heated in a water bath to produce a brick-red coloration.

(ii) The above test (i) was repeated using 15cm^3 of 1.0M tetraoxosulphate (iv) acid instead of distilled water. The amount of resulting precipitate was compared with that of (i) above. A higher amount of precipitate shows the presence of glycoside while a lower amount shows the absence of glycoside.

2.3.4. Test for the presence of Saponin

This was done by the analytical method reported by Ejikeme *et al.* [10]**.** Some 0.30g of the polyherbal powder was dispersed in 30cm^3 of distilled water, boiled for 10mins in a water bath and filtered through Whatmann No 42 (125mm) filter paper. Some 10cm^3 of filtrate mixed with 5cm of distilled water and vigorously shaken to yield a persistent froth to which added 3 drops of olive oil. When shaking vigorously again appearance of an emulsion indicates presence of saponin.

2.3.5. Test for the presence of Tannin

Analytical method reported Ejikeme *et al* [10]. An amount of the polyherbal powder (0.30g) was dispersed in 30cm^3 of distilled water and boiled for 10 minutes in a water bath. Filtration was done using Whatmann No 42 (125mm) filter paper. Three drops of 0.1% ferric chloride was mixed with 5cm^3 of the herbal filtrate. Brownish-green or blue-black coloration indicates the presence of tannin.

2.3.6. Test for the presence of phlobatanins

Analytical method reported by Ejikeme *et al.* [10]. To a beaker was placed 0.30g of herbal powder and soaked with 30cm^3 of distilled water. This was extracted after 24 hours. Some 10cm^3 of the extract was boiled with 5cm^3 of 1% aqueous hydrochloric acid (HCl). A red precipitate shows the presence of phlobatanin.

2.3.7. Test for the presence of Tarpenoids

Analytical method reported by Ejikeme *et al.* [10]. Polyherbal powder (0.30g) placed into a beaker containing 30cm^3 of distilled water. This was allowed to stand for 2 hours. Some 5cm^3 of the extract was mixed with 2cm^3 of chloroform. Some 3cm^3 of concentrated tetraoxosulphate (iv) acid was introduced to form a layer. A reddish brown coloration at the interface shows the presence of terpenoids.

2.3.8. Extraction of essential oil

Essential oil was extracted by hydro distillation of the herbal powder using the Clevenger-type apparatus as described by Jarubol *et al.* [19]. Some 500 µL of the extract was added to 2 drops of 1M alcoholic $K_2Cr_2O_7$ and 3 drops of phenolphthalein in a clean test tube. Soap formation indicates the presence of essential oil [6-8].

2.4. Quantitative determination of the phytochemical constituents of the herbal formula

2.4.1. Determination of Flavonoids

This was done by the method reported by Ejikeme *et al* [10]. Some 2.50g of the polyherbal powder was measured into a 250cm beaker, to this was added 50cm of 80% aqueous methanol. The set up was covered and allowed to stand at room temperature for a period of 24 hours. After discarding the supanatant, the residue was re extracted three times with the same volume of ethanol. The solution was filtered through the Whatman No 42 (125mm) filter paper. The filtrate subsequently transferred into a crucible & evaporated to dryness in a water bath. After cooling the crucible and its content in a desiccator, it weighed to a constant weight [5]. The percentage of the flavonoid is expressed mathematically as:

$$\% \text{ Flavonoid} = \frac{Weight\ of\ Flavonoid}{Weight\ of\ sample}\ x\ \frac{100}{1}$$

2.4.2. Determination of Cyanogenic glycoside

This was done by the method reported by Ejikeme *et al.* [10]. Exactly 1g of the polyherbal powder was measured into a 250cm^3 round bottom flask. Autolysis of the sample was achieved by adding 200cm^3 of distilled water and allowing to stand for 2 hours. Tannic acid (an antifoaming agent) was introduced and complete distillation was carried out in a 250cm^3 conical flask containing 20cm^3 of 2.5% sodium hydroxide (NaOH). Exactly 100cm^3 of the distillate containing cyanogenic glycoside was measured out, to which was added 8cm^3 of 6M ammonium hydroxide (NH_4OH) and 2cm^3 of 5% potassium iodide (KI). These were mixed and titrated with 0.02M silver nitrate ($AgNo^3$) using a micro-burette against a black background. The appearance of permanent turbidity indicates titration end point. The cyanogenic glycoside content of herbal powder calculated as:

$$\text{Cyanogenic glycoside (mg/100g)} = \frac{Titrate\ value\ (cm3) x\ 1.08\ x\ exact\ volume}{Aliquot\ volume\ (cm3)\ x\ Weight\ of\ sample}\ x\ \frac{100}{1}$$

2.4.3. Determination of Saponins

This was done by the method [10-22]. To 5g of the herbal powder contained in 250cm^3 conical flask was added 100cm^3 of 20% aqueous ethanol. The mixture was heated with continuous stirring for 4 hours in a water bath at a temperature of 55ºC. This was followed by filtration after which the residue was re-extracted using another 100cm^3 of 20% aqueous ethanol with continued heating for 4 hours at a constant temperature of 90ºC. The resulting concentrate was placed in 250cm^3 separating funnel. Some 20cm^3 of diethylether was added and shaken vigorously. The aqueous layer was recovered and the purification procedure repeated two times. Some 60cm^3 of n-butanol was added and the butanol extract was washed twice using 10cm^3 of 5% sodium chloride. After discarding the sodium chloride layer, the remaining solution was heated in a water bath for 30 minutes. The solution was subsequently transferred into a crucible and oven dried to a constant weight.

Percentage Saponin content was calculated as:

$$\% \text{ Saponin} = \frac{Weight\ of\ Saponin}{Weight\ of\ Sample}\ x\ \frac{100}{1}$$

2.4.4. Determination of Tannin

Preparation of Folin-Denis reagent was achieved by dissolving 50g of sodium tungstate (Na_2WO_4) in 37cm^3 of distilled water. Subsequently, 10g of phosphomolybdic acid ($H_3PMO_{12}O_{40}$) and 25cm^3 of orthophosphoric acid (H_3PO_4) were added. After refluxing for 2 hours, the mixture was cooled and diluted to 500cm^3 with distilled water.Tannin determination was done according to the method reported [1-10]. One gram of the polyherbal powder was dispersed into 100cm^3 of distilled water contained in a conical flask. This was boiled gently on an electric hot plate for 1 hour followed by filtration using Whatman No 42 (125mm) filter paper into a 100cm^3 volumetric flask. To achieve color development, 50cm^3 of distilled water and 10cm^3 of dilute extract (aliquot volume) were pipette into a 100cm^3 conical flask, followed by introduction of 5.0cm^3 of Folin-Denis reagent and 10cm^3 of saturated sodium carbonate (Na_2CO_3) solution.

These were thoroughly mixed and allowed to stand for 30 minutes in a water bath at a temperature of 25ºC. The absorbance of the solution was measured at 700nm using a spectrophotometer. The absorbance was compared on a standard tannic acid curve prepared as follows: Exactly 0.20g of tannic acid was dissolved in distilled water and diluted to 200cm^3 mark (now the concentration is 1mg/cm^3). Varying concentrations of the standard tannic acid solution (0.2 – 1.0mg/cm^3) were pipette into five different test tubes. Some 5cm^3 of Folin-Denis reagent and 10cm^3 of saturated sodium carbonate solution were pipette into the test tube and made up to 100cm^3 mark with distilled water. Solution was allowed to stand in a water bath for 30 minutes at a temperature of 25ºC. Absorbance taken at 700nm in a spectrophotometer. A curve of absorbance against tannic acid concentration made.

Tannic acid concentration (mg/100g)

$$= \frac{C\ X\ Extract\ Volume}{Aliquot\ volume\ x\ weight\ of\ sample}\ x\ \frac{100}{1}$$

Where C = Concentration of tannic acid read off the graph.

2.4.5. Analysis of alkaloids

This was done by the method reported Obadoni and Ochuko [22]. Alkaloid fractions were analyzed by gas chromatography. Two hundred and fifty mLs of boiling deionized water was added to 30g of the poly herbal powder and allowed to soak for 30minutes before filtration. The filtrate was later acidified to pH 4 with acetic acid. The acidified solution was extracted with 30ml of petroleum spirit and chloroform. The acidic aqueous phase was later made basic at pH 9 with 25% aqueous ammonia. This was later extracted with chloroform three times with 30ml of the extracting solvent. The chloroform extract was concentrated to 1.0ml in a water bath [15]. Gas chromatography (GC) analysis of the chloroform extract was done using Hewlett Packard (HP) 6890 powered system with HP chemstation Rev. A_o9.01 [1206] software [SEM Ltd, Istanbul, Turkey], and a DB-5MS capillary column (30m x 0.25mm x 0.25µm film thickness).Nitrogen gas was used with initial oven temperature of 60^{0}C for 20 minutes and at 15^{0}C for 4minutes. Injection temperature 250ºC while percentage composition of

individual components were obtained from electronic integration using flame ionization detector (FID, 32ºC). The volume injected was 0.2µL and split ratio was 20:1.

2.5. Proximate Composition

Procedures for the determination of moisture, crude protein, crude fat, crude fibre and ash content of the polyherbal powder were adapted by Standard AOAC methods reported reported by Olaniyi *et al.* [26].

2.5.1. Determination of Moisture

Exactly 2g of the polyherbal powder was placed in a clean dry crucible (W_1). The crucible was placed inside an oven for 12 hours at a temperature of 105ºC to achieve a constant weight. The crucible was subsequently placed in a desiccators for 30 minutes. After cooling, it was reweighed (W_2). The percentage of moisture in sample calculated as:

$$\% \text{ Moisture} = \frac{W_1 - W_2}{Weight\ of\ Sample}\ x\ \frac{100}{1}$$

Where W_1 = Initial weight of crucible + sample

W_2 = Final weight of crucible + sample

2.5.2. Determination of ash content

Clean empty crucibles were heated at in a muffle furnace at 600ºC for 1 hour. Crucibles cooled in desiccators and weighed again (W_1). Exactly 1g of herbal powder was placed into the crucible (W_2). The crucible placed in a muffle furnace for 2 hours at 550ºC. Complete oxidation of all organic materials in the sample is achieved at appearance of grey white ash. After this complete ashing, crucible cooled and weighed (W_3). The percent ash content calculated thus:

$$\% \text{ Ash} = \frac{\frac{W_3 - W_1\ X\ 100}{W_2 - W_1}}{Weight\ of\ Sample}$$

Where $W_1 - W_3$ = Difference in the weight of ash.

2.5.3. Determination of Crude Fat

Exactly 1g of herbal powder was wrapped in a filter paper. This was placed in a thimble and introduced into the extraction tube of the soxhlet apparatus. The receiving beaker was filled with petroleum ether and fitted into the apparatus. Water and heater were subsequently turned on to start the extraction process. The ether was allowed to evaporate after six rounds of siphoning, the beaker was disconnected just before one more (i.e. final) siphoning. The extract was transferred into a clean glass dish and the ether was washed and evaporated in a water bath. After this, the dish was placed in an oven at 105ºC for 2 hours and cooled in a disiccator.

The crucible fat was calculated using the expression below:

$$\% \text{ Crude fat} = \frac{Weight\ of\ ether\ extract}{Weight\ of\ Sample}\ x\ \frac{100}{1}$$

2.5.4. Determination of Crude Fibre

Exactly 0.2g of the herbal powder was placed in a porous crucible. The crucible was in turn placed into a bosi-fibre unit, keeping the valve in the OFF position. Some 150ml of pre-heated H_2SO_4 solution was added and few drops of acetone was added to each column. After opening the cooling circuit, the heating element was turned on. The power was set at 90%. When boiling began, the power was reduced to 30% and left for 30 minutes. The valves kept open (for acid to drain) were rinsed three times with distilled water. This same procedure was used for alkali digestion where potassium hydroxide (KOH) replaced sulphuric acid (H_2SO_4). The sample oven-dried at 105ºC for 1 hour to achieve a constant weight. After this sample cooled in a desiccators and re-weighed (W1). Sample crucibles ashed at 550ºC in a muffle furnace for 4 hours ate end of which samples removed from furnace, cooled in a desiccators and weighed again (W_2).

Percentage crude fibre calculated from the expression below:

$$\% \text{ Crude fibre} = \frac{W_1 - W_2}{Weight\ of\ Sample}\ x\ \frac{100}{1}$$

2.5.5. Determination of carbohydrate

The carbohydrate content of the sample was determined by difference using the following relationship: % Carbohydrate = 100 - (% Moisture +% ash + % fat + % Protein + % crude fibre

2.6. Determination of Minerals

Mineral content of herbal powder was determined according to method described by Bouba *et al* [7]. An amount of herbal powder weighing 2.5g was placed in a beaker and ashed at the temperature of 550ºC. The ash residue dissolved in 4mls of concentrated hydrochloric acid. This subsequently filtered into a 10ml volumetric flask and volume made up to mark with distilled water. Extract subsequently subjected to some mineral analysis. Sodium (Na), Potassium (K), Calcium (Ca), Zinc (Zn), Magnesium, Manganese (Mn) and Copper (Cu) determined by atomic absorption spectrophotometer (Varian Spectra A. A. 220, USA) while iron (Fe) determined by ICP – OES 710 – ES series.

2.7. Statistical analysis

Result data obtained from the research were subjected to analysis of variance (ANOVA) and least of significant difference (LSD) at $p<0.05$. Data were analyzed using SPSS 20.0 software and results presented as Mean values ± standard deviation (SD).

3. Results and discussion

Table 1 shows a list of the various herbs used in making the herbal formulation. Their families, common names as well as the various parts used were shown.

3.1. Results

3.1.1. Qualitative phytochemical screening

The results of various tests used to detect presence of the phytochemicals in herbal powder are shown in table 2. Qualitative tests showed presence of alkaloids, flavonoids, glycosides, saponins, tannins, tarpenoids and essential oils in formula. Phlobatanins however not detected in sample.

3.1.2. Quantitative phytochemical evaluations

The total flavonoids and saponins contents of the herbal powder were shown in table 3. The sample contains 11.61% flavonoid and 6.85% saponins. Table 4 shows the amounts of tannins and glycosides present in the sample. The sample was observed to contain 51.256mg/100g tannins and 596.351mg/100g cyanogenic glycosides. The various fractions of alkaloids contained in the herbal formula as obtained from gas chromatography (G. C.) separations shown in table 5. The formula was found to contain various fractions all amounting to 642.56477mg/100g. The sample found to be rich in some alkaloids, norephedrine (47.26001mg/100g), ephedrine (257.95716mg/100g), stanchdrine (97.29721mg/100g) & wilforine (237.56065mg/100g).

Table 1: Names of plants used in the composition of the sample and the parts used

S/N	Botanical Name	Common Name	Family	Parts used
1	*Alstonia scholaris*	Blackboard tree	*Apocynaceae*	Stem bark
2	*Annona senegalensis*	Wild custard apple	*Annonaceae*	Leaves
3	*Pteridium aquilinum*	Braken fern	*Dennstaedtiaceae*	young shoots and Leaves
4	*Caesalpinia bonducella*	Fever nut	*Ceasalpiniaceae*	Leaves

Table 2: Qualitative phytochemical screening of *HBF-19*

S/N	Parameter (Phytochemical)	
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Saponins	+
5	Tannins	+
6	Tarpenoids	+
7	Phlobatanins	-
8	Essential oil	+

+: present, -: absent.

Table 3: Flavonoids and Saponin Constituents of the herbal mixture

Parameter	Quantity (%)
Flavonoids	11.61
Saponins	6.85

Table 4: Tannin and Cyanogenic glycoside Constituents of the herbal mixture

Parameter	Composition (mg/100g)
Tannin (as tannic acid)	51.256
Cyanogenic glycosides	596.351

Table 5: GC Alkaloid Fractions of the herbal mixture

Name of Compound	Retention Time (mins.)	Amount (mg/100g)
Augustifoline	5.498	0.00477
Sparteine	5.797	0.00374
Norephedrine	6.956	47.26001
Nimbol	8.158	0.00019
1,3-Alphahydrorhombifoline	8.770	0.14059
Sugiol	8.978	0.00013
9-octa Decenamide	10.642	0.05492
Ephedrine	11.377	257.95716
Dihydro-demethoxyhaemnathamine	12.262	0.89654
Augustamine	13.398	0.01797
Stanchdrine	13.845	97.29721
Oxoassoanine	14.517	0.00421
Nimbrin	15.398	0.00305
Crinane-3-alpha-ol	16.466	0.00253
Buphanidrine	17.393	0.00044
Wilforine	17.714	237.56065
Powelline	18.788	1.06849
Undulatine	19.264	0.28784
Ambelline	20.462	0.00081
6-hydroxybuphanidrine	20.958	0.00083
6-hydroxypowelline	22.171	0.00150
Crinamidine	23.095	0.00447
Total alkaloid constituent		**642.56477**

Table 6: Proximate Composition of the herbal mixture

Parameters	Quantity (%)
Moisture	6.95 ± 0.02
Crude fat	3.58 ± 0.01
Crude protein	22.70 ± 0.13
Ash	13.43 ± 0.03
Crude fibre	13.01 ± 0.02
Carbohydrate	40.35 ± 0.13

Values represent Mean ± SD (n = 3)

Table 7: Mineral Composition of the herbal mixture

Mineral	Composition (mg/100g)
Na	215.2 ± 11.05
K	350.10 ± 6.00
Ca	1206.51 ± 8.30
Mg	586.92 ± 5.00
Zn	8.30 ± 0.20
Mn	26.50 ± 1.13
Cu	1.89 ± 0.01
Fe	295.68 ± 4.00

Values represent Mean ± SD (n = 3)

3.1.3. Proximate Composition

The proximate composition of *HBF-19* is shown in table 6. The result shows high amounts of carbohydrate (40.35 ± 0.13%) and the crude protein (22.70 ± 0.13%) in the sample. Also observed in considerable amounts in the sample are the crude fibre (13.01 ±0.02%)) and the ash (13.43 ± 0.03%).

3.1.4. Mineral Composition

The composition of the minerals evaluated in the sample is shown in table 7. The mineral, calcium was found to be in highest amounts (1206.51 ± 8.30mg/100mg). Also found in appreciable amounts are magnesium (586.92 ± 5.00mg/100g) potassium (350.10 ± 6.00mg/100g and iron (295.68 ± 4.00mg/100g).

3.2. Discussion

Plants have medicinal values due to their phytochemical (bioactive) constituents which show various physiological effects on human body [11]. Also responsible for the nutritional values of plants are presence of the various compounds found in plant cells. Among such compounds are carbohydrates, proteins, lipids, fibre, vitamins and minerals. Thus the value of any herbal product for use as food or drug depends on the quantity and quality of these constituent compounds in plant samples from which product is made. Phytochemicals are compounds associated with plants, which are not produced for growth and development of plants [23]. The poly-herbal formula *HBF-19* was observed to contain significant amounts of alkaloids, tannins and glycosides. The percentage contents of saponins and flavonoids in formula are also appreciable. Alkaloids consist of chemical compounds containing mostly basic nitrogen atoms occurring naturally in plants [11]. Alkaloids have a wide range of pharmacological activities prominent among which are antimalarial, anticancer, antibacterial and antihyperglycemic [9]. Alkaloids almost uniformly evoke a bitter task [13]. Flavonoids have shown to have antioxidant activities and also protect against development of platelet aggregation, tumors and ulcers. [3-20]. Saponins elicit antifungal activities by disruption of cell membranes of fungal pathogens [13]. Tannins have stringent properties and encourage healing of wounds & inflamed mucus membranes [25].

The high amounts of these phytochemicals occurring in the polyherbal powder suggest that it will exhibit antioxidant, anticancer, antimicrobial, and antihyperglycemic activities among others. The high alkaloid content also lays credence to the earlier observed antimalarial activities of the herbal powder. Results of the proximate evaluation show that the herbal powder has low moisture content. This makes for good storage quality. The sample has appreciable amounts of carbohydrate, protein and crude fibre. Proteins are known to repair worn out tissues, carbohydrates are good sources of ATP. Crude fibres encourage proper digestion of foods. Results of the mineral evaluations have shown the following minerals Na, K, Ca, Mg, Mn and Fe present in the sample in appreciable amounts. Calcium is needed to maintain strong bones and teeth. Among the metabolic functions of magnesium is its role as a co-factor for some metabolic enzymes. It also helps decrease blood pressure [33]. Sodium and potassium play important roles in trans-membrane transport and transmission of neural impulses. Iron plays an important role in the cellular transport of oxygen, while Manganese functions in the metabolism of amino acids, cholesterol and carbohydrates. The result of this study shows that the herbal powder is highly enriched with many bioactive compounds with a wide array of pharmacological actions as well as appreciable amounts of various food nutrients.

4. Conclusions

This study showed that the poly herbal formula contains appreciable amounts of bioactive compounds documented to have a wide array of medicinal values. The significant amounts of phytochemicals with antimalarial properties observed in the formula lays a scientific credence to our earlier work in which the sample significantly reduced

parasiteaemia in malaria parasite infected mice. Results of the nutritional (proximate and mineral) evaluations also suggest that the herbal formula can contribute significantly to human /livestock nutritional needs.

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