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Phytochemical and Mineral Composition of Triherbal Formulation

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Abstract

Herbal combinations are widely used for the treatment of various diseases in the developing countries. Their ability in boosting the immune system is a function of Mineral constituents present. Currently available drugs have been associated with number of side effects. Herbal Combinations have often been used in traditional medicine systems like Ayurveda, unani, African, Chinese Medicine, and Western herbalism. Combination of herbs is superior with increased therapeutic efficacy to individual herb. Triherbal combinations consisting of (*Mucuna pruriens, Phyllantus amarus and Securidaca longipedunculata*) 1:1:1 ratio W/W. The study was aimed at investigating the phytochemical profile, *in-vivo* antioxidant activities, The quantitative Phytochemical result revealed present of Alkaloids, Flavonoids, Terpenoids, Saponins and glycosides with Total phenol having the highest values (111.67±0.23mg/GAE/g) followed by (92.50±0.14mg/GAE/g). Chemical profiling analysis showed two prominent compounds chlorogenic acid (78.2 %) and Caffeic acid (21.8%.).The results of Mineral compositions clearly indicated that triherbal fromulation is a rich sources of minerals indicating the presence of the following: Sodium(Na), Potassium(k), Magnesium(Mg), Calcium(Ca), Iron(Fe),

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Phosphporus (P), and Zinc(Zn) with Magnesium having the highest value of (830.5±0.0) and the least value zinc (9.8±0.14). Conclusively, These findings indicate that these triherbal formulations contain the essential minerals and bioactive compounds consequently exhibiting diverse biochemical activities, through multiple mechanism that could probably boost the immune system, making this formulation a potential therapeutic interventions for preventing liver damage and maintaining overall health.

Keywords: Triherbal Formulation; Mineral; Antioxidant; Phytochemicals, Caffein acid, Chlorogenic acid

INTRODUCTION

Medicinal plants have been used to manage various illness since ancient times. They composed of some bioactive compounds influencing the physiological functions of the animals and human body, They contained active phyto-constituents (Kennedy and Wightman 2011), biosynthesized by the pathways of acetyl coenzyme A, shikimic acid, mevalonic acid and 1-deoxylulose 5-phosphate in the plant (Kabera *et al*., 2014)

The Phytochemicals are being broadly examined for their ability to provide health benefits due to their bioactivities as substrates for Biochemical reactions, cofactors of enzymatic reactions, and inhibitors of enzymatic reactions (Dillard and German, 2000).

Minerals are those elements on the earth and in foods that our bodies need to develop and function normally. The minerals work together for tissue regeneration and proper body functioning. Minerals are essential nutrients needed for normal growth and maintenance of body functions via boosting of immune system. Famewo *et al.,*(2018). They are biomolecules that maintain the physiology wellbeing, boast immune system and are responsible for a spectrum of vital functions in the body due to their anti-oxidant, pro-oxidant, anti-inflammatory effects and metabolic functions (Islam, *et al*., 2021). Triherbal formulations, which combine three medicinal plants, have been used in traditional medicine systems across various cultures for centuries. These formulations are often valued for their synergistic therapeutic effects, where the combined action of the three plants is believed to be greater than the sum of their individual effects. The selection of specific plants for triherbal formulations often stems from a deep understanding of their individual medicinal properties and how they may complement each other to address complex health

conditions. Traditional healers carefully combine plants with known actions, such as anti-inflammatories, antimicrobials, and antioxidants, to create a holistic treatment approach.

Literature Review

Phytochemicals are bioactive compounds found in plants that contribute to their color, flavor, and aroma, and also provide health benefits to humans. These compounds are not essential for plant survival but play a crucial role in their interaction with the environment, defending against pathogens, herbivores, and environmental stressors. Triherbal formulations are known to contain a wide variety of phytochemicals, including:

Alkaloids: These nitrogen-containing compounds have diverse pharmacological activities, including analgesic, antispasmodic, and antimicrobial effects (Bhambhani et al., 2021). Alkaloids such as morphine and codeine from the opium poppy, for instance, are well-known for their potent analgesic properties, while others like quinine from cinchona bark, have been historically important in the treatment of malaria. The structural diversity of alkaloids contributes to their wide range of therapeutic applications.

Flavonoids: These polyphenolic compounds are known for their antioxidant, anti-inflammatory, and anticancer properties (Al-Khayri et al., 2022). Flavonoids are abundant in fruits, vegetables, and certain herbs, and are responsible for many of the vibrant colors observed in the plant kingdom. Their antioxidant activity helps to protect cells against damage caused by free radicals, while their anti-inflammatory properties contribute to the management of chronic diseases.

Phenols: This large group of compounds exhibits strong antioxidant activity, protecting against oxidative stress and related diseases (Pisoschi & Pop, 2015; Chaudhary et al., 2023). Phenolic compounds are characterized by the presence of one or more hydroxyl groups attached directly to an aromatic ring. This structural feature enables them to effectively scavenge free radicals, thereby preventing cellular damage and reducing the risk of chronic diseases such as heart disease, cancer, and neurodegenerative disorders.

Saponins: These glycosides have been shown to possess various biological activities, including anti-inflammatory, antimicrobial, and antitumor effects. Saponins are characterized by their soap-like foaming properties when dissolved in water. Their diverse biological activities make them valuable components of traditional medicine systems.

Terpenoids: These compounds have diverse functions, including anti-inflammatory, antimicrobial, and anticancer properties. Terpenoids, also known as isoprenoids, are a large and diverse class of organic compounds produced by plants. They often contribute to the aroma of plants and are used in essential oils and perfumes.

The specific phytochemical composition of a triherbal formulation will vary depending on the plant species used, the plant parts included (e.g., leaves, roots, bark), and the preparation methods. Different plant parts accumulate different phytochemicals, and extraction techniques can also influence the final composition of the formulation.

Mineral Content

Minerals are essential nutrients that play crucial roles in various physiological processes, including:

- Enzyme function
- Nerve transmission
- Bone health
- Immune function

These inorganic substances are required for maintaining proper physiological function, and their deficiency can lead to a variety of health problems.

Triherbal formulations can be a significant source of essential minerals, including:

Sodium (Na) and Potassium (K): These electrolytes are vital for maintaining fluid balance, nerve function, and muscle contraction (Jomova et al., 2022). Sodium and potassium ions create electrochemical gradients across cell membranes, which are essential for nerve impulse transmission, muscle contraction, and the regulation of blood pressure.

Magnesium (Mg) and Calcium (Ca): These minerals are essential for bone health, muscle function, and enzyme activity. Calcium is the most abundant mineral in the human body and is a key component of bones and teeth, while magnesium plays a crucial role in over 300 enzymatic reactions.

Iron (Fe): A crucial component of hemoglobin, iron is essential for oxygen transport (Jomova et al., 2022). Hemoglobin, a protein found in red blood cells, binds to oxygen in the lungs and transports it to various tissues throughout the body. Iron deficiency is a

common cause of anemia, a condition characterized by a reduced number of red blood cells or a decreased amount of hemoglobin.

Zinc (Zn): This trace element plays a vital role in immune function, wound healing, and cell growth. Zinc is involved in numerous biochemical pathways and is essential for the activity of many enzymes.

Phosphorus (P): Important for bone health, energy metabolism, and cell signaling. Phosphorus is a key component of adenosine triphosphate (ATP), the primary energy carrier in cells.

The mineral content of triherbal formulations can contribute to their therapeutic effects, particularly in addressing mineral deficiencies and supporting overall physiological function (Alghamdi et al., 2022; Razzaque & Wimalawansa, 2025). The bioavailability of minerals from herbal formulations, however, can be influenced by various factors, including the presence of other compounds that may enhance or inhibit their absorption.

MATERIALS AND METHODS

The leaves of *Mucuna pruriens, Phyllantus amarus and Securidaca longipeduculata* were collected from vicinity of Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria.

The chemicals and equipment used were of analytical grade and obtained known vendors.

Preparation of the sample

Fresh leaves of *M. pruriens, P. amarus and S. longipeduculata* were properly cleaned, air dried then Pulverized to fine powder using electric blender.

Cold extraction method

Powdered sample was weighed into glass jar and methanol was added in about a ratio of 1:3. The glass jar was tightly covered with aluminum foil, for 72 hours, It was then filtered using vacuum filter, the filtrate was evaporated to dryness.

Quantitative determination of Secondary metabolites was carried out according to the methods described by AOAC (1995), adopted by (Ushie *et al.,* 2019)

Quantitative Phytochemical Analysis

Qantitative determination of some secondary metabolites (Ushie *et al.,* 2019) was carried out to know their percentage in the T. danielli leaves by the methods described by AOAC (1995), Ushie *et al*., (2019).

Determination of tannins

The sample (1g) was macerated with 50 ml of methanol and filtered. To the filtrate (5 ml), 0.30 ml of 0.1N ferric chloride in 0.1N HCl and 0.3 ml of 0.0008M of potassium Ferro cyanide were added and the absorbance read at 720nm.

Determination of phenols

The sample (1g) was macerated with 20 ml of 80% ethanol and then filtered. 5ml of the filtrated was added to 0.5 ml of folicio calteus reagent (FCR) and allowed to stand for 30minutes. 2 ml of 20% sodium carbonate (Na_2CO_3) was added and the absorbance read at 650nm.

Determination of Saponnins

The sample (1g) was macerated with 10ml of PET ether and decanted into a beaker. Another 10ml of PET ether was added into the beaker, filtered and the filtrate was evaporated to dryness, the residue was dissolved in 6ml 0f ethanol. 2 ml of the solution was put into in a test tube, and 2 ml of chromagen solution was added into it and was left to stand for 30 minutes. The absorbance was read at 550nm.

Determination of flavonoid

The sample (1g) was macerated with 20ml of ethyl acetate for 5minutes and filtered. To 5ml of the filtrate, 5ml of the dilute ammonia (NH3) was added and shaken for another 5minutes. The upper layer was collected and the absorbance was read at 490nm.

Determination of alkaloids

The sample (1g) was macerated with 20ml of ethanol and 20% sulphuric acid (H_2SO_4) (I.e. 1:1 v/v) and then filtered. 1 ml of the filtrate was added to 5 ml of 60% sulphuric acid. After 5 minutes, 5 ml of 0.5% formaldehyde in 60% H_2SO_4 (sulphuric acid) was mixed with the mixture (i.e. filtrate (1 ml) + 5 ml 60% H_2SO_4) and allowed to stand for 3hours. The absorbance was read at 565nm.

HPLC Chemical profiling (Kotásková *et al.*, 2016).

Procedure

Sample digestion done using solvent method and filtration and then diluted with the same solvent used for extraction. 2ml of the diluted sample Measured and filtered with 0.45µm syringe filter into the vial. Mobile phase solvent was filtered with 0.45µm filter in a vacuum flask; the vial was labeled appropriately with the sample ID. The vial is placed in the auto sampler of the HPLC machine and the vial number noted. Phytochemicals analysis methods stored in the System Application is activated and calibrated, analysis sequence created, wavelength at 214nm to run for 30minutes using the Reprosil 100, C8, 5um, 4,6 x 150mm column, flow rate of solvents was set at 1.0 mL/min, and the column temperature was maintained at 25°C. HPLC chromatograms of MLEs were recorded at 280 nm and 350 nm, retention time and peaks were observed in the chromatograph on the monitor finally, chromatogram result is printed. The detection method used was modified from the procedures reported by Kotásková *et al.*, 2016.

Mineral Analysis (Bvenura and Afolayan 2012)

Procedure

The method described by Bvenura and Afolayan (2012) in which chemicals are used for the digestion of the samples. Chemicals used include Selenium powder, sulfuric acid and salicylic acid were the reagents used for digestion. 0.3 g of sample was weighed into dry and clean digestion tubes. A volume of 2.5 ml of the digestion mixture was added to each tube and allowed to react at room temperature for 120 min. The tubes were heated in a block digester at 110◦C for 60 min., Allowed to cool and three successive portions of 1.0 ml hydrogen peroxide were added, allowing at least 10 sec, heated to temperature of 330◦C and removed from the block digester when colorless to cool. Then transferred into 50ml volumetric flasks and made up to 50 ml volumes with deionized water. Standards were prepared for all the elements. The samples were then analyzed for the following nutrients: calcium, magnesium, sodium, potassium, nitrogen, phosphorus, iron, zinc, aluminum, manganese and copper using an inductively coupled plasma optical emission spectrometer (ICP OES; Varian 710–ES series, SMM Instruments, Cape Town, South Africa). All analyses were performed in duplicates. The macro and micro nutrient contents were expressed as mg/100 g.

Data Analysis

Analysis of variance (ANOVA) of statistical Package for the social Sciences (SPSS) version 26.0 will be used. Results of different treatment groups will be expressed as mean ± Standard deviation. The difference between various treatments groups will be compare using the Duncan Multiple Range Test. P values less that 0.05 ($p< 0.05$) were taken as significant. Results will be presented in Tables and charts.

RESULTS

Considering the quality of mineral constituents, it give credence to the extensively usage of this Herbal formulation (*M. pruriens, P. amarus and S. longipediculata*) in the treatment and management of various diseases in Igala folk medicine.

Table 1: Mineral composition in triherbal formulation

MINERALS	Na	k	Mg	Ca	Fe	P	Zn
Means (mg/100 g)	420.6±0.14	640.15±0.07	830.5±0.0	673.35±0.07	13.75±0.07	611.5±0.14	9.8±0.14

Phytochemical Composition of Triherbal Formulation

Triherbal formulation reveal presence of saponin, alkaloids, tannin, flavonoids, ,Cardiac glycosides, Total phenol with Total phenol exhibiting the highest values.

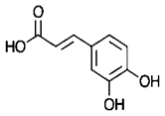
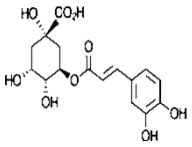
Table 2: phytochemical composition of Triherbal formulation

PHYTOCHEMICALS	QUANTITIES
Alkaloids (%)	8.75±0.35
Cardiac glycoside (mg/sec/g)	2.40±0.00
Tannins (mg/GAE/g)	92.50±0.14
Sapponin (mg/DE/g)	5.88±0.00
Total Phenol (mg/GAE/g)	111.67±0.23
Flavonoids (mg/QE/g)	20.06±0.08

HPLC Result

HPLC Result reveal two important phenolic compounds Caffein acid and Chlorogenic acid produced by plants through the shikimic acid pathway during aerobic respiration and possessed diverse biological activities such as anti-oxidant, liver and kidney protection, anti-bacterial, anti-tumor, regulation of sugar metabolism and lipid metabolism, anti-inflammatory, protection of the nervous system and anticarcinogenic activity

Table 3: HPLC profiling of triherbal combination

S/N	R. Time (min)	Amount(ppm)	%Composition	C. Name	S. Fmla
1	2.24	2.15	21.8	Caffeic acid	
2	2.52	7.69	78.2	Chlorogenic acid	

Triherbal formulations combine herbs leveraging on their rich source of phytochemicals and essential minerals that can provide various health benefits resulting from their synergistic effects to enhance their therapeutic potentials.

DISCUSSION

From the results, the triherbal formulation is characterized by a high content of essential minerals, including sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), phosphorus (P), and zinc (Zn). This mineral richness supports its traditional use in Igala folk medicine, as these minerals are vital for various physiological processes (Alghamdi *et al.,* 2022; Razzaque & Wimalawansa, 2025). Notably, potassium and magnesium are important for cardiovascular health, and iron for oxygen transport (Jomova *et al*., 2022). The presence of these minerals likely contributes to the formulation's therapeutic efficacy, a finding consistent with research on other medicinal plants (Radha e*t al.,* 2021).

Phytochemicals are the key components of herbal formulations, responsible for their diverse pharmacological activities, non-nutritive plant chemicals that have protective or disease preventive properties" (Liu, 2004),These compounds, naturally present in plants, work through various mechanisms and are essential for the use of herbs in traditional and modern medicine for treating various ailments" (Wink, 2015). The triherbal formulation contains a diverse array of bioactive phytochemicals, including alkaloids, cardiac glycosides, tannins, saponins, flavonoids, and total phenols. Notably, total phenols are present at the highest concentration, indicating strong antioxidant potential. Phenols are known to scavenge free radicals and protect against oxidative stress (Pisoschi & Pop, 2015). Each phytochemical contributes to the formulation's potential therapeutic effects; for example, alkaloids can have diverse pharmacological activities, while flavonoids possess antioxidant and anti-inflammatory properties (Bhambhani *et al.*, 2021).The presence of these phytochemicals suggests a broad spectrum of potential therapeutic activities (Omojate Godstime *et al.,* 2014). The high concentration of total phenols highlights their role as antioxidants, which are crucial in preventing oxidative stress-related diseases (Chaudhary *et al.,* 2023). Flavonoids, another component of the formulation, have been extensively studied for their anti-inflammatory, antioxidant, and anticancer properties (Al-Khayri *et al.*, 2022).the variety of phytochemicals, particularly the high total phenol content, indicates the formulation's significant therapeutic potential (Akter *et al.*, 2022). The identification of caffeic acid and chlorogenic acid as key phenolic compounds in the triherbal formulation, using high-performance liquid chromatography (HPLC), is significant. These compounds are associated with a wide range of biological activities, including antioxidant, hepatoprotective, nephroprotective, antibacterial, antitumor, hypoglycemic, lipid-regulating, anti-inflammatory, neuroprotective, and anticarcinogenic effects (Jurel *et al.*, 2024; Sun & Shahrajabian, 2023). Their presence supports the observed therapeutic effects of the formulation and offers a foundation for elucidating their mechanisms of action. The finding that chlorogenic acid has a substantially higher percentage composition than caffeic acid suggests it may be a primary active component (Tajik *et al.*, 2017). Notably, chlorogenic acid has demonstrated antioxidant, anti-inflammatory, and hypoglycemic properties (Yan et al., 2020). The well-documented beneficial effects of these compounds (Mucha *et al.,* 2021; Singh *et al.,* 2024) lend credence to the traditional uses of the herbs within the formulation. Furthermore, the potential for synergistic interactions among these compounds within the triherbal formulation warrants consideration, as it may enhance

their overall therapeutic activity. The abundance of these bioactive compounds in the triherbal formulation supports its traditional medicinal use.

The presence of a diverse array of phytochemicals and essential minerals in triherbal formulations contributes to their broad spectrum of therapeutic activities. The synergistic interactions between these compounds can enhance their overall efficacy and reduce the risk of adverse effects. Synergism refers to the phenomenon where the combined effect of multiple compounds is greater than the sum of their individual effects. This complex interplay between phytochemicals and minerals is what makes triherbal formulations so valuable in traditional medicine. Furthermore, the mineral content of these formulations can help to address mineral deficiencies, which are a common underlying factor in many diseases.

CONCLUSION

In conclusion, the studies indicated that triherbal formulation of ethanolic leaf extract (*Mucuna pruriens* (velvet beans), *Phyllantus amaru* (stone breaker), *Securidaca longipeduculata* (violet tree) represent a rich source of bioactive phytochemicals and essential minerals The presence of these compounds supports the traditional use of these formulations for treating various ailments through multiple mechanisms of actions. That alleviate oxidative stress, inflammation and immune dysregulation. Triherbal formulations. Further research is needed to fully elucidate the complex interactions between the various components of triherbal formulations and to determine their optimal composition and dosage for specific therapeutic applications. This includes the investigation of optimal extraction methods, the identification of specific synergistic interactions, and well-designed clinical trials to validate their efficacy and safety.

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