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Assessment of *in-vitro* proximate composition and mineral analysis of different combinations of moringa (*Moringa oleifera*) leaves and ginger (*Zingiber officinale*) rhizomes as herbal supplements in the possible prevention and management of hypertension

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Abstract

Hypertension is one of the most common causes of cardiovascular diseases (CVDs) and is among the leading causes of death globally. Recent reports revealed that hypertension and related cardiovascular complications are gaining more importance in sub-Saharan Africa, especially in Nigeria. Plant herbs such as moringa (*Moringa oleifera*) leaves and ginger (*Zingiber officinale*) rhizomes are popular folklore for the management of hypertension, but limited scientific information is available on their possible synergistic effect in militating against the scourge of high blood pressure. This study sought to evaluate the effect of combination of moringa (*Moringa oleifera*) leaves and ginger (*Zingiber officinale*) root in different proportions. The proximate composition and mineral analysis of these combinations was assessed. Sample A contained 100% powdered moringa leaves, sample B contained 100% powdered ginger rhizomes, sample C contained 50% powdered moringa leaves and 50% powdered ginger rhizomes, sample D contained 25% powdered moringa leaves and 75% powdered ginger rhizomes, while sample E contained 75% powdered moringa leaves and 25% powdered ginger rhizomes. The proximate composition of moringa leaves and ginger rhizome showed that moringa had a higher percentage of moisture, fat and carbohydrate than ginger while ginger had a higher percentage of protein, fibre and ash. Upon combination at different proportions, a number of positive synergy was observed. The different combinations of the two herbal plants show increased percentage of the proximate compositions and minerals compared to the lower individual percentage in nearly all parameters assessed. This can be harnessed to achieve the desired nutrient and nutraceuticals aimed at managing hypertension.

Keywords: *Moringa oleifera; Zingiber officinale*; Hypertension; Minerals; Proximate Composition, Synergy

1. Introduction

Hypertension (HTN) or high blood pressure (BP) is a chronic medical condition in which the BP in the arteries is elevated. It is classified as *either primary* (essential) or secondary. About 90 to 95% of cases are termed primary HTN, which refers to high BP for which no medical cause can be found (1). The remaining 5 to 10% of cases, called *secondary* HTN, are caused by other conditions that affect the kidneys, arteries, heart, or endocrine system (2). Hypertension is the main risk factor in the development of cardiovascular disease (CVD) (3). Persistent HTN is one of the risk factors for strokes, heart attacks, heart failure, and arterial aneurysm, and is a leading cause of chronic kidney failure (4). A large body of evidence links high intake of sodium with hypertension and cardiovascular diseases (5). *Moringa oleifera* Lam commonly called 'drumstick' belongs to family Moringaceae, is a highly valued plant, distributed

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in many countries of the tropics and subtropics. The tree is found almost all over the Asian and African countries and its fruit and leaves are consumed as food by the people. It has an impressive range of medicinal uses with high nutritional value. The leaf of this plant has been shown to have anti-inflammatory and hypotensive effect (6; 7; 8). Ginger (*Zingiber officinale*) root is commonly used in Asian cooking. It acts to improve blood circulation and relaxes muscles surrounding blood vessels. The crude extract of ginger has been reported to induce a dose-dependent (0.3-3 mg/kg) fall in the arterial BP of anesthetized rats. In guinea pig paired atria, crude extract of ginger exhibited a cardiodepressant activity on the rate and force of spontaneous contractions. The renewed interest in the search for new drugs from natural sources, especially from plant sources, has gained global attention during the last two decades. The tropical rain forests have become an important point of this activity, primarily due to the rich biodiversity they harbour, which promises a high diversity of chemicals with the potential novel structures. However, of this rich biodiversity, only a small portion has been studied for its medicinal potential. Thus, natural plants and herbs can be our source of drugs, with fewer side effects and better bioavailability for treatment of HTN in future.

1.1 Justification for the research

Recently, work related to understanding the pathophysiology and mechanism of action of essential HTN and related cardiovascular complications are gaining more importance in sub-Saharan Africa especially Nigeria due to the high prevalence of hypertension (9; 10). In Nigeria, the last two decades has seen a rise in the number of prevalence of hypertension because of the increasing adult population estimated to be about 170 million and changing lifestyle of Nigerians (9; 10). However, the inhibition of Angiotensin I-converting enzyme (ACE), key enzyme of rennin-angiotensin system (RAS) has been suggested to be a useful approach for the management/prevention of hypertension; drugs that serve as inhibitors of this enzyme have been suggested to be a practical approach for the management of hypertension but are expensive and come with side-effects. Both dietary and lifestyle changes as well as medicines can improve BP control and decrease the risk of associated health complications.Plant herbs such as moringa (*Moringa oleifera*) leaves and ginger (*Zingiber officinale*) rhizomes are popular folklore for the management of hypertension, but limited scientific information is available on their possible positive synergistic effect against high blood pressure. An increased intake of minerals such as potassium, magnesium, and calcium by dietary means has been shown in some but not all studies to reduce blood pressure in patients with hypertension. (11). Hence, this study sought to assess possible synergistic effect of combination of different proportions of moringa (*Moringa oleifera*) leaves and ginger (*Zingiber officinale*) rhizome by looking at their proximate composition and mineral analysis, thus assessing the properties of the combined extracts in the possible prevention and management of hypertension.

2. Material and methods

2.1 Plant Materials and Sample Preparation

Fresh leaves of *Moringa oliefera* were collected from the Federal Polytechnic, Ado Ekiti farm; and ginger rhizomes were purchased from Shasha Market, along Ikere road in Ekiti State. The ginger rhizomes were washed and cut into smaller pieces to allow for faster drying. Both the Leaves and rhizomes of the plant samples were allowed to air dry at room temperature for six weeks. The dried samples were pulverized into powder using a mechanical grinder and weighed. The total weight of both samples was 960g each; both samples were characterized in a mixture of different proportions as shown in the table below.

Table 1 Composition of different proportion of samples

Samples	% Proportions	Weight in grams (g)
Sample A	100% Moringa leaves	240 g
Sample B	100% Ginger rhizomes	240 g
Sample C	50% Moringa leaves +50% Ginger rhizomes	120 g+120 g
Sample D	75% Moringa leaves +25% Ginger rhizomes	180 g+60 g
Sample E	25% Moringa leaves +75% Ginger rhizomes	60 g+180 g

2.2 Proximate Analysis

2.2.1 Moisture Content

2.0g of the sample(s) were placed in an oven maintained at 100 – 103 °C for 16 hours with the weight of the wet sample and the weight after drying noted. The drying was repeated until a constant weight was obtained. The moisture content was expressed in terms of loss in weight of the wet sample (12).

2.2.2 Ash Content

2.0g of each of the oven-dried samples in powder form were accurately weighed and placed in crucible of known weight. These were ignited in a muffle furnace and ashed for 8 hours at 550 °C. The crucible containing the ash was then removed, cooled in a dessicator and weighed and the ash content expressed in term of the oven-dried weight of the sample (12).

2.2.3 Protein

The protein nitrogen in 1g of the dried samples were converted to ammonium sulphate by digestion with concentrated H2SO4 and in the presence of CuSO4 and Na2SO4. These were heated and the ammonia evolved was steam distilled into boric acid solution. The nitrogen from ammonia was deduced from the titration of the trapped ammonia with 0.1M HCl with Tashirus indicator (double indicator) until a purplish pink color was obtained. Crude protein was calculated by multiplying the value of the deduced nitrogen by the factor 6.25mg (12).

2.2.4 Crude Fibre

2.0g of each sample was weighed into separate beakers, the samples were then extracted with petroleum ether by stirring, settling and decanting 3 times. The samples were then air dried and transferred into a dried 100ml conical flask. 200cm3 of 0.127M sulphuric acid solution was added at room temperature to the samples. The first 40cm3 of the acid was used to disperse the sample. This was heated gently to boiling point and boiled for 30 minutes. The contents were filtered to remove insoluble materials, which was then washed with distilled water, then with 1% HCI, next with twice ethanol and finally with diethyl ether. Finally, the oven-dried residue was ignited in a furnace at 550^{o}C. The fibre contents were measured by the weight left after ignition and were expressed in term of the weight of the sample before ignition (12).

2.2.5 Lipid Content

The lipid content was determined by extracting the fat from 10g of the samples using petroleum ether in a soxhlet apparatus. The weight of the lipid obtained after evaporating off the petroleum ether from the extract gave the weight of the crude fat in the sample (12).

2.2.6 Carbohydrate

The carbohydrate content of the samples was determined as the difference obtained after subtracting the values of protein, lipid, ash and fibre from the total dry matter (12).

2.3 Mineral analysis

2.3.1 Calcium, potassium and sodium determination

Apparatus: Heating mantle, Crucible, Glass rod, Flame photometer, 100ml volumetric flask, what man No. 1 Filter paper, Wash bottle, 10 ml pipette and funnel. Reagents: 2 MHCL.

Determination: The ash of each sample obtained was digested by adding 5ml of 2 MHCL to the ash in the crucible and heated to dryness on a heating mantle. 5ml of 2 MHCL was added again, heated to boil and filtered through what man No. 1 filter paper into a 100ml volumetric flask. The filtrate was made up to mark with distilled water stoppered and made ready for reading of concentration of Calcium, Potassium and Sodium on the Jenway Digital Flame Photometer(PFP7 Model) using the filter corresponding to each mineral element.

2.3.2 Phosphorus determination

Apparatus: Spectrophotometer or colorimeter, 50ml volumetric flask, 10ml pipette, filter paper, funnel, wash bottle, glass rod, heating mantle, crucibles.

Reagents: Vanadate – Molybdate yellow solution, 2 MHCL.

Determination: The ash of each sample obtained was treated 2 MHCL solution as described for calcium determination above. 10ml of the filtrate solution was pipetted into 50ml standard flask and 10ml of vanadate yellow solution was added and the flask was made up to mark with distilled water, stoppered and left for 10 minutes for full yellow development. The concentration of phosphorus was obtained by taking the optical density (OD) or absorbance of the solution on a Spectronic 20 spectrophotometer or colorimeter at a wavelength of 470nm.

The percentage phosphorus was calculated from using the formula:

$$\%Phosphorus = \frac{Absorbance\ x\ Slope\ x\ Dilution\ factor}{1000}$$

2.3.3 Determination of Mn, Cu, Cr, Fe, S, and I using AAS Buck Scientifi 211 AAS VGP

The digest of the ash of each sample above as obtained in calcium and potassium determination was washed into 100ml volumetric flask with deionized or distilled water and made up to mark. These diluents were aspirated into the Buck 211 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements was read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combination.

3. Results

Table 2 Proximate Composition of different composition of Moringa leaves and Ginger rhizomes

S/N	Parameter	Sample A	Sample B	Sample C	Sample D	Sample E
1	Moisture (%)	10.40	9.40	9.80	9.45	10.40
2	Protein (%)	9.50	19.50	14.80	16.80	10.90
3	Fat (%)	6.20	2.20	4.10	3.10	5.20
4	Fibre (%)	5.80	16.10	11.40	13.90	8.20
5	Ash (%)	5.50	10.80	8.50	9.60	6.80
6	CHO (%)	62.60	42.00	51.40	47.15	58.50

Table 3 Analysis of macrominerals present in different composition of Moringa leaves and Ginger rhizomes

Macroelement mineral analysis					
Samples	**Calcium**	**Magnesium**	**Sodium**	**Potassium**	**Phosphorus**
A	1.2166	2.1164	0.2816	12.3614	2108.55
B	1.2295	2.2967	0.0427	4.9855	4218.10
C	1.2851	2.2597	0.3224	8.8640	3118.18
D	1.3965	2.2597	0.0912	6.4601	3963.68
E	1.2486	2.1365	0.2328	10.1765	2529.20

*All values in Conc (ppm). Conc = Concentration, * ppm= part per million

Table 4 Analysis of microminerals present in different composition of Moringa leaves and Ginger rhizomes

Microelement mineral analysis							
Samples	**Manganese**	**Copper**	**Chromium**	**Iron**	**Sulphur**	**Selenium**	**Iodine**
A	0.3166	4.7963	0.9154	0.1861	9417.44	2108.55	0.4179
B	0.0389	5.2118	0.3631	0.0629	4173.09	4218.10	0.4462
C	0.1829	4.8859	0.6638	0.1388	6115.15	3118.10	0.4094
D	0.0944	5.1238	0.4711	0.0869	5221.40	3793.68	0.4406
E	0.2596	4.8692	0.8012	0.1601	8367.64	2529.20	0.4235

*All values in Conc (ppm). Conc = Concentration, * ppm= part per million

4. Discussion

The proximate composition of moringa leaves and ginger rhizome showed that moringa had a higher percentage of moisture, fat and carbohydrate than ginger while ginger had a higher percentage of protein, fibre and ash. Upon combination at different proportions, a number of positive synergies was observed. Synergy is defined as the combined interaction of several system elements which produces an entirely different or greater effect compared to what they produce by their separate effects. In many cases, a synergistic relationship between certain elements can produce long-term benefits (13). From the results observed in the table 2, the different combinations of the two herbal plants show increased percentage of the proximate compositions compared to the lower individual percentage in nearly all parameters assessed. For example, the Moringa leaves had a lower ash percentage of 5.50% while the three different combinations were at 8.5%, 9.60% and 6.80%. This provides a possible route to make up for observed nutrient deficiencies in particular disease conditions, thereby helping with the needed nutrient to counter observed negative effect of its deficiency. From the results observed in the table 3 and 4, the different combinations of the two herbal plants show increased macro and micro mineral element levels compared to the lower individual element level in nearly all the minerals assessed.

The mineral elements sodium, potassium, calcium and magnesium have been observed to play a central function in the normal regulation of blood pressure. Particularly, these mineral elements have important interrelationships in the control of arterial resistance. These elements, especially sodium and potassium, have been shown to also regulate the fluid balance of the body and, thus, influence the cardiac output. Various recent research shows that the present levels of intake of mineral elements are not optimum for maintaining normal blood pressure but predispose to the development of arterial hypertension. Some research results suggest that without the addition of sodium chloride (common salt) and other sodium compounds to diet, arterial hypertension would almost be nonexistent. In places with a relatively high consumption of added sodium, a high intake of potassium and, possibly, magnesium seems to protect against the development of arterial hypertension and the rise of blood pressure with age. For the prevention and basic treatment of elevated blood pressure (arterial hypertension), various methods to decrease the intake of sodium and to increase the intakes of potassium, calcium, and magnesium should be sought (14).

The mineral constituents from the different combinations of moringa leaves and ginger rhizome from the observed results provides for a higher level of potassium, phosphorus, calcium and magnesium, all important minerals in the management of hypertension. This again stems from positive synergy from the different combinations. For example, ginger had a lower potassium level at 4.9855ppm while the different combinations of moringa leaves and ginger rhizome were 8.86, 6.47 and 10.17, all higher than that of ginger alone. The positive synergy observed in the combinations tends to increase the Na/k ratio which is indicative of a positive tendency towards the management of hypertension.

The combination of increased intake of magnesium and potassium coupled with reduced sodium intake is has been seen to be more effective in reducing BP than single mineral intake and is often as effective as one antihypertensive drug in treating hypertension and this is without the associated side effects from those drugs. The overall effect of diet or herbal supplements on BP is determined by the net contribution of various nutrients on cytosolic-free minerals such as potassium, calcium magnesium, and sodium (15). Minerals and Proximate compositions in a food or herbal supplement is important for the proper growth and development of a healthy body and secondary metabolites included in diet act as a nutraceutical and thereby helps in fighting various health problems and diseases. Dietary therapies known to lower

BP include reduced sodium intake, increased potassium and magnesium intake, possibly an increase in calcium intake, and a diet rich in fruits and vegetables (16).

5. Conclusion

The combination of the different proportions of moringa leaves and ginger rhizome have showed promising synergistic effect that can be harnessed to achieve the required metabolite in diet aimed at managing hypertension.

Compliance with ethical standards

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Disclosure of conflict of interest

This is an independently prepared paper. The authors have not declared any conflict of interests either commercially or otherwise in the publication of this paper.

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