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Nutritional Evaluation and Phytochemical Analysis of *Ageratum conyzoides* Leaves in Relation to Hypertension Management

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Abstract

Plants remain one of the most valuable sources of therapeutic agents for managing diseases and maintaining health. *Ageratum conyzoides* (AC) belongs to the family Asteraceae and has been known since antiquity for its medicinal properties and effectiveness in the treatment of various ailments ranging from skin disease, fever, to relief colic, flatulence, dysentery, and diarrhea. Despite its extensive ethnomedicinal use, limited scientific evidence exists to substantiate its pharmacological properties. This study was therefore designed to determine its nutrition and phytochemical component as herbal remedy for hypertension. Proximate analysis (%) shows that the plant has a low moisture content (6.57 ± 0.053), a low crude protein content (8.15 ± 0.041), low concentration of fat (3.10 ± 0.000), a moderate amount of fibre (16.58 ± 0.088), low Ash content (4. 29 ± 0.010) and high amount of carbohydrate (61.21 ± 0.010). This confirms AC as a good source of energy and fibre that aids digestion and improve the intestinal tract. The phytochemical constituents (Tannins, Phenols, Alkaloids, Flavonoids and Saponins) were found to be 2.79%, 49.03mg/g, 0.94%, 38.22mg/g and 3.80% respectively. This confirms the plant's richness in phytochemicals and antioxidant compounds. The concentration of essential amino acids (mg/g protein) (Leucine, Lysine, Phenylalanine, Isoleucine, Threonine, and Methionine) were found to be 66.00, 38.05, 48.00, 36.00, 38.00 and 16.05 showing the nutritional quality of AC. Mineral compositions (mg/100g) (magnesium, calcium, iron, potassium, phosphorus, sulphur, manganese, cobalt, selenium and zinc) were found to be 27.45, 65.00, 8.16, 720.07, 100.42, 25.00, 5.15, 0.05, 0.72 and 2.24 confirming the nutritional and medicinal richness of *Ageratum conyzoides* as a herbal remedy. This study demonstrated that dietary supplementation of *Ageratum conyzoides* in local delicacy (black soup) has the potential to improve the nutritional status vis a vis the overall health benefit with regards to hypertension and other traditional use.

Keywords: *Ageratum conyzoides*, Phytochemicals, Proximate analysis, Hypertension, Herbal remedy, Essential amino acids, Mineral composition, Asteraceae.

INTRODUCTION

Medicinal plants

Plant is an important source of medicine and plays a key role in world health (Sandberg and Corrigan, 2001). Medicinal herbs or plants have been known to be an important potential source of therapeutics or curative aids. The use of medicinal plants has attained a commanding role in health system all over the world. This involves the use of medicinal plants not only for the treatment of diseases but also as potential material for maintaining good health and conditions. From records, most of the used drugs contain plant extracts. Some contain active ingredients (bioactive components or substances) obtained from plants. Through recent researches, plant-derived drugs were discovered from the study of curative, therapeutic, traditional cures and most especially the folk knowledge of indigenous people and some of these claims and believe of people are irreplaceable despite the recent advancement in science and technology.

People who use traditional remedies may not understand the scientific rationale behind their medicines, but they know from personal experience that some medicinal plants can be highly effective if used at therapeutic doses. Since we have a better understanding today of how the body functions, we are thus in a better position to understand the healing powers of plants and their potential for their potential as multi-functional chemical entities for treating complicated health conditions. Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health. A single plant may, for example, contain bitter substances that stimulate digestion, anti-inflammatory compounds that reduce swellings and pain, phenolic compounds that can act as an antioxidant and venotonics, anti-bacterial and anti-fungal tannins that act as natural antibiotics, diuretic substances that enhance the elimination of waste products and toxins and alkaloids that enhance mood and give a sense of well-being.

Medicinal plants possess essential food components such as carbohydrates, protein, and fat. These components are important for the human body's requirements and they are used in a different physiological, metabolic, and morphological activities (Cheeke, 2009; Radha, *et al.,* 2021). Natural plant-derived products are used in medications, nutritional supplements, and in different healthcare products. Medicinal plants include a variety of physiologically active components, including minerals and phytochemicals that show numerous physiological effects on humans (Petrovska, 2012; Dagli *et al.,* 2015). Plants, including many presently used as spices and culinary herbs, have been used as medicines, not essentially effectively, from prehistoric times. Spices are partly used to counter food spoilage microorganism (bacteria), especially in hot climates (Asfaw, 2019), and especially in meat dishes that spoil more readily (Macwan *et al.,* 2016), most of the extracts including sugar alcohols do not contribute to tooth day (Awuchi, 2017).

The quality of food can be judged from their nutritive values, which, in turn, can be assessed from the composition of food with respect to the contents of carbohydrates, proteins, fats, minerals and vitamins. The caloric value of food depends on the amount of these components. The deficiency of one or more components may result in the signs and symptoms of various diseases. Wild green leafy vegetables hold an important place in providing a balanced diet. They contain sufficient amount of carbohydrates, proteins, fats, minerals, vitamins, antioxidants and other biologically active constituents (Imran *et al.,*2007, 2010) and provide metabolic needs and protection against various diseases. Some plants contain antioxidants (notably flavonoids and other polyphenols). The consumption of such food is advantageous for health as they protect the body from free radicals and retard the progress of various chronic diseases. There are many reports that support the use of antioxidant supplementation for reducing the level of oxidative stress and preventing the development of complications associated with diseases (Ordonez *et al.,*2006; Hasnat *et al.,*2014; Janu *et al.,*2014).

Phytochemicals, are non-nutritive plant chemicals that have either defensive or disease protective properties. They are nonessential nutrients and mainly produced by plants to provide them protection. Dietary intake of phytochemicals may promote health benefits, protecting against chronic degenerative disorders, such as cancer, cardiovascular and neurodegenerative diseases. Majority of foods, such as whole grains, beans, fruits, vegetables and herbs contain phytonutrients/ phytochemicals. These phytochemicals, either alone and/or in combination, have tremendous therapeutic potential in curing various ailments. Phytochemicals with nutraceutical properties present in food are of enormous significance due to their beneficial effects on human health since they offer protection against numerous diseases or disorders such as cancers, coronary heart disease, diabetes, high blood pressure, inflammation, microbial, viral and parasitic infections, psychotic diseases, spasmodic conditions, ulcers, osteoporosis and associated disorders.

Hypertension is the leading preventable risk factor for cardiovascular disease (CVD) and all-cause mortality worldwide (Stanaway *et al.,* 2018; Roth *et al.,* 2018). In 2010, 31.1% of the global adult population (1.39 billion people) had hypertension, defined as systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg (Mills *et al.,* 2016). The prevalence of hypertension is rising globally owing to ageing of the population and increases in exposure to lifestyle risk factors including unhealthy diets (i.e. high sodium and low potassium intake and lack of physical activity (Mills *et al.,* 2016). However, changes in hypertension prevalence are not uniform worldwide. In the past two decades, high-income countries (HICs) experienced a modest decrease in hypertension prevalence, while low- and middle-income countries (LMICs) experienced significant increases (Mills *et al.,* 2016 These disparities in hypertension prevalence trends suggest that health care systems in LMICs could be facing a rapidly increasing burden of hypertension and BP-related cardiovascular diseases, in some cases in addition to a substantial burden of infectious diseases.

Ageratum conyzoides L. (Asteraceae) is an aromatic, annual herb, native to Central America, Southeast Asia, South China, India, West Africa, e t c (Prince and Prabakaram, 2011). It belongs to the family and tribe of Asteraceae and Eupatoriae respectively. It is an erect, annual, branched, slender, hairy and aromatic plant, which grows to approximately 1 m in height. It is a weed, commonly called Billy goat-weed, Goat weed etc., and generally found in cultivated fields and other ecosystems such as pastures, grasslands, wastelands and even forest areas (Nasrin, 2013). *Ageratum conyzoides* has been known since ancient times for its curative properties and has been utilized for the treatment of various ailments, such as burns and wounds, headaches pneumonia, analgesic, inflammation, asthma, spasmodic and haemostatic effects, stomach ailments, gynaecological diseases, leprosy and other skin diseases (Kambo and Saluja, 2008). The plant is scientifically reported to possess anti-oxidant (Nasrin 2013), wound healing, analgesic, anti-inflammatory (Vikasari *et al.,* 2022), anti-malarial (Ukwe *et al.,* 2010), anti-cancer (Adebayo *et al.,* 2010), anti-hyperglycaemic (Nyunai *et al.,* 2010), anti-ulcerogenic (Thorat *et al.,* 2018) anti-microbial and insecticidal (Yadav *et al.,* 2019) properties. Flavonoids (eupalestin, kaempferol, sinensetin, quercetin etc), triterpenes and sterols (β-sitosterol, stigmasterol, brassicasterol etc), alkaloids (echinaline, lycopsamine etc), mono and sesquiterpenes (linalool, limonene, eugenol e t c) and amino acids are reported as the phytochemical constituents from this plant (Puro *et al.,* 2018). Amongst these phytochemical constituents, eugenol has been scientifically reported to be useful for the eradication of head lice infestation (Shailajan *et al.,* 2013). The ethnomedicinal claim by the local tribes in India uncovered that A. conyzoides is a potential source for controlling pediculosis. Therefore, presence of these functional ingredients in *Agerantum conyzoides* makes it a strong candidate to ameliorate hypertensive stress.

MATERIALS AND METHODS

Materials

Chemicals and Reagents

Petroleum ether, Ammonia, boric acid, tricholoroacetic acid, copper sulphate, potassium sulphate selenium, sulphuric acid, follin's reagent, tannin, paraffin, sodium carbonate, potassium ferrocyanide, hydrogen chloride, ethyl acetate magnesium carbonate, isobutyl alcohol, iron III chloride, sodium hydroxide, nitric acid, perchloric, hydrogen peroxide.

Plant Material

Leaves of *Ageratum conyzoides* were collected from Okitipupa, ($6^0 25'$ and $6^0 25'$ N latitude and $4^0 35'$ and $4^0 50'$ E longitude), Nigeria.

Methods

Proximate Analysis

The proximate analyses were determined following standard methods (AOAC, 2005).

Moisture content and Total Ash

Moisture content was determined by the drying/indirect method. Total ash was determined by weighing 2g each of the leaf samples into the crucible. The samples were ashed in a muffle furnace at a temperature of 550^0C, cooled in a desiccator and weighed. The total ash was then calculated.

Crude fat

Crude fat was obtained using Soxhlet direct (petroleum ether) solvent extraction method (Reinik *et al*., 2007). This process involved weighing 3g each of the leaf samples into a thimble and plugged into the soxhlet extractor with cotton wool. The solvent was poured into a round bottom flask which later fitted to the soxhlet and placed on a heating mantle. The oil was extracted as the solvent was refluxed several times. The extraction lasted for about 7 hours after which the flask was cooled and disconnected. The final weight was recorded and the fat content was calculated.

Crude fibre

To 1g of defatted sample in a digestion beaker was added 100 mL of trichloroacetic acid (TCA) digestion reagent. This was placed on the heating unit of digester and water supply was opened to reflux condenser. The mixture was brought to boiling and refluxed for exactly 40 minutes counting from the time boiling commenced. The beaker was removed from the heater, cooled a little bit and filtered through Whatman No.4 (15 cm in diameter) filter paper. The residue was washed six times with hot water, once with industrial spirit and transferred to a previously ignited and pre-weighed dish. The residue was dried overnight in an oven at 105^0C, transferred to a desiccator and weighed after cooling. It was ashed in a muffle furnace at 600^0C for 6 hours, allowed to cool and re-weighed. The crude fiber content was calculated as follows:

% Crude fiber = Loss in weight on ashing X 100

Protein

This was determined according to the method of Pearson, (1976). The nitrogen was determined by Kjeldahl's digestion-distillation method as described by Jackson (1973). In this method, leaves of the sample were dried to 105^0C until their weight was stable and they were finely ground to homogenize the material and to assure good digestion. 0.5 g of powdered sample was digested with Conc. H_2SO_4 in presence of digestion mixture (K_2SO_4:$CuSO_4.5H_2O$:Se) in the proportion of (100:20:1) and distilled under alkaline medium. The liberated NH_3 was trapped in 25.0 mL of 4.0% (w/v) boric acid containing mixed indicator and titrated against standard H_2SO_4. Crude protein was determined using Macro kjeldahl method from the nitrogen content by the protein conversion factor 6.25 to give the crude protein content (Pearson, 1976).

Carbohydrate: The percentage carbohydrate was obtained using the below equation

100 – (% Protein + % Ash + %Crude fibre + % Moisture + % Fat)

Determination of phytochemical constituents

Total Tannin

The method of Swain (1979) was used for the determination of tannin contents. Grinded powder of 0.20g of the *Ageratum conyzoides* accessions was measured into a 50 mL beaker 20 mL of 50% methanol was added and covered with paraffin and placed in a water bath at 77- 80^0C for 1 h. It was shaking thoroughly to ensure a uniform mixing. The extract was quantitatively filtered using a double layered Whatman's No. 41 filter paper into a 100 mL volumetric flask, 20ml water added, 2.5 mL follin-Denis reagent and 10 mL of

17% Sodium carbonate (Na_2CO_3) were added and mixed properly. The mixture was made up to mark with water mixed well and allow standing for 20 mins. The bluish-green color developed at the end of range 0-10 ppm were treated similarly as 1 mL sample above. The absorbance of the Tannic acid standard solutions and those of the samples were read after color development on a spectronic 21 D spectrophotometer at a wavelength of 760 nm. Percentage Tannin was calculated using the formula:

$$\% \text{ Tannin} = \frac{\text{absorbance of sample X average gradient factor X Dilution factor}}{\text{Wt. of Sample X 10,000}}$$

Alkaloid

Distillation and titrimetric procedure was used. Two grams (2 g) of finely ground sample was weighed into a 100 mL beaker and 20 mL of 80% absolute alcohol added to give a smooth paste. The mixture was transferred to a 250 mL flask and more alcohol added to make up to 100 mL and 1 g magnesium oxide added. The mixture was digested in a boiling water bath for 1h 30 mins under a reflux air condenser with occasional shaking. The mixture was filtered while hot through a small bucher funnel. The residue was returned to the flask and redigested for 30min with 50 mL alcohol after which the alcohol will be evaporated, adding hot water to replace the alcohol lost. When all the alcohol has been removed, 3 drops of 10% HCl was added. The whole solution was later transferred into a 250 mL volumetric flask 5 mL of zinc acetate solution and 5 mL of potassium ferrocyanide solution was added, thoroughly mixed to give a homogenous solution. The flask was allowed to stand for a few minutes, filtered through a dry filter paper and 10 mL of the filtrate was transferred into a separatory funnel and the alkaloids present were extracted vigorously by shaking with five successive portions of chloroform. The residue obtained was dissolved in 10 mL hot distilled water and transferred into a kjeldahl tube with the addition of 0.2 g sucrose and 10ml Concentrated H_2SO_4 and 0.02 g selenium for digestion to a colorless solution to determine %N by Kjeldahl distillation method. % Nitrogen got is converted to % total alkaloid by multiplying by a factor of 3.26.
% Total alkaloid = % N X 3.26

Phenols

Accurately weigh about 100 mg test material and transfer into a 100 mL volumetric flask. Add about 75 mL water and sonicate in an ultrasonic bath containing about 3 cm water for up to 10 min until solids are dissolved. Dilute to volume with water and mix well. Quantitatively pipet 5.0 mL of this solution into a 100 mL volumetric flask. Dilute to volume with water and mix well. Weight of test material can be adjusted based on total phenolic content in order to achieve an absorbance within the calibration curve range. Calculate total phenolic content of the test material, in % w/w as is, as follows:

Total phenols (% w/w) = $[(A - b)/m] \times [(V \times D)/(W \times 1000)] \times 100$
where A = absorbance of the sample test solution at 765 nm; b = y-intercept of the calibration curve; m = slope of the calibration curve; W = weight of the test material (mg); V = volume of the sample test solution (100 mL); D = dilution factor (20); and 1000 = conversion from mL to L. Note that values are expressed as Gallic acid equivalents

Flavonoids

This was determined according to the method of Harborne (1973). Five gram (5 g) each of the grinded powder of the accessions was boiled in 50 mL of 2 M Hydrochloric acid (HCl) solution for 30 minutes under reflux. It was allowed to cool and then filtered through Whatman's No. 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

Saponin

The spectrophotometric method of Brunner (1984) was used for Saponin Analysis. One gram (1 g) of finely ground sample was weighed into a 250 mL beaker and 100 mL of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5h to ensure uniform mixing. Thereafter the mixture was filtered through a Whatman's No. 1 filter paper into a 100 mL beaker and 20 mL of 40% saturated solution of magnesium carbonate was added. The mixture obtained with saturated Magnesium carbonate ($MgCO_3$) was again filtered through a Whatman No 1 filter paper to obtain a clear colorless solution. One mL of the colorless solution was pipetted into 50 mL volumetric flask and 2 mL of 5% $FeCl_3$ solution was added and made up to mark with distilled water. It was allowed to stand for 30 mins for blood red color to develop. Standard Saponin solutions of 0-10 ppm were prepared from Saponin stock solution. The standard solutions were treated similarly with 2ml of 5% $FeCl_3$ solution as done for 1ml sample above. The absorbance of the sample as well as standard Saponin solutions were read after color development in a Jenway V 6300 Spectrophotometer at a wavelength of 380nm.

$$\% \text{ Saponin} = \frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{\text{Wt. of sample} \times 10000}$$

Determination of Essential Amino Acids

The protein content of each of sample was determined according to the Association of Official Agricultural Chemists (AOAC) International method 954.01. The results are presented as grams of protein per hundred grams of sample dry weight (g/100 g dw). For amino acids quantifications, the sample were hydrolyzed in 6 M HCl for 24 h at 110^{0}C in the presence of Phenol, 3,3′-dithiodipropionic acid (DDP), and Norvaline (Nva). Phenol was added to prevent halogenation of tyrosine. Nva was added as a major internal standard. Cystine and cysteine derivatives were first converted to S-2-carboxyethylthiocysteine (XCys) by DDP. After hydrolysis and neutralization, amino acids and XCys are derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). Derivatized AAs are separated using reversed phase UHPLC with UV detection at 260 nm. The same thing is repeated for leucine, isoleucine and lysine at 100^{0}C and 90^{0}C respectively before passing through the same final process using changing excitation, 202 nm; emission, 296 nm for 5.0 min (for tramadol with retention). During acid hydrolysis, asparagine is converted to arginine and phenylalanine respectively. Thus, the values represent the combined values of the two at different temperatures 10^{0}C. Tryptophan is degraded by acid hydrolysis and can be analyzed by addition of NaOH before taking the spectrophotometric reading.

Mineral iron analysis

Determination of iron, magnesium, manganese and zinc

Iron, Magnesium, Manganese and Zinc in *Ageratum conyzoides* were determined using atomic absorption Spectrophotometer (AAS) described by Jaleel *et al.,* (2009). Two grams of the dried sample were digested using 10 mL of concentrated H_2SO_4 and 5 mL of concentrated HNO_3. The sample was cautiously heated at 250^{0}C until vigorous reaction subsided. Heating was continued to 450^{0}C for three hours. The digested sample were filtered through a Whatmann's No. 40 filter into a 100 mL flat-bottom flask and contents were topped up to the mark with distilled water. One milliliter of 10% w/v of chloride salt was added to each sample and then run into an Atomic Absorption Spectrophotometer (AAS). Concentrations of the minerals were obtained from calibration curves made from standards of the elements. Sodium, Iron, Magnesium, Manganese and Zinc were measured using Atomic colometric Assay.

Determination of Calcium and Potassium

Determination of Calcium and Potassium was done using the methods described by Jaleel *et al.,* (2009). Dried and ground samples weighing 0.5 g were digested into 100 mL of Kjeldahl flask using 15 mL of concentrated nitric acid, 0.5 mL of 60 % perchloric acid and 0.5 mL of concentrated sulphuric acid. Digestion continued until the nitric and perchloric acids were dried. The ionic residue was cooled, diluted with 15 mL of distilled water and then filtered through Whatmann Number 42 filter paper. The filtrate was made up to 50 mL with distilled water. It will then be used for calcium and potassium estimation by flame photometer.

Determination of Sulphur

Weigh 300 mg of plant material into a Whatman No. 54 filter paper. Wrap the sample carefully and secure it by means of a 2 in. piece of platinum wire. Add 5 ml of reagent sodium hydroxide solution and 1 ml of hydrogen peroxide (sulphate-free) to the combustion flask. Flush the flask with oxygen for 10 sec, then stopper with an ordinary Quickfit stopper. Wet the inside of the flask thoroughly with the reagent solutions. Moisten the ground-glass joint of the ignition head with distilled water, ignite the taper, remove the Quickfit stopper and insert the ignition head firmly into the flask. Rotate the flask carefully during burning to prevent localized heating of the glass walls, until ignition is completed. Allow the flask to stand 45-60 min. Into a 100 ml beaker add 2·5 ml of reagent barium chloride and 3.5 ml of reagent hydrochloric acid solution. Wash the contents of the flask and the ash on the combustion cone into the beaker. Use Cover the beaker with a cover glass and boil the barium sulphate suspension for 10 min. Allow the beaker and contents to cool and transfer to a 50 ml volumetric flask. Allow 10 ml of reagent strontium chloride and make to volume. Stopper the flask, shake thoroughly, and filter a portion through a Whatman No. 40 filter paper. Prepare a calibration curve using the 0-300 p.p.m. barium standards, plot peak height v. p.p.m. barium. Read the peak height of the sample filtrate against calibration curve and convert this reading to percent sulphur in the original sample, using the expressions:

% sulphur = (300 - p.p.m. barium observed) x 1 · 17
Sample weight (mg)

Determination of Selenium

0.50 g of plant material were placed in Kjeldahl flasks to which were added 5 ml of concentrated HNO_3 few glass beads. After 60 min at room temperature, 2 ml of perchloric acid (727^{0}C) were added; the flasks were put on the electro thermal furnace, and kept at low heat for 20 min. Then, the heat was increased so that maximum oxidation conditions were reached some 10 min later, when fumes of HCIO, started to evolve. The acid boiled strongly enough to condense on the upper part of the flask neck after15 min. In order to ensure uniform conditions, the gradual rising of the condensation was considered to be complete when this ring reached the top of the neck. Acidified samples were diluted according to calibration range with ultrapure water and measured for selenium concentration by atomic absorption spectrophotometer (AAS) (Varian, Spectr AA 220 FS) with hydride vapour generation accessory (VGA, Make: Agilent, Model: 77).

Determination of Cobalt

0.45 g of the sample was acidified to pH ≤ 4.0 with concentrated supra pure nitric acid. Samples were stored in polypropylene bottles until extraction at room temperature. Acidified samples were extracted by vigorous shaking with APDC-MIBK in separating funnel using automated Recipro shaker (Make: Recipro Model: R1). Extracted cobalt in MIBK solvent was back extracted using suprapure nitric acid in ultrapure water and made volume up to 25 ml. Total dissolved concentration of cobalt was measured in extracted samples after appropriate dilution in atomic absorption spectrophotometer with flame mode against calibration standard of cobalt.

Statistical Analysis

All analyses were carried out in triplicates. Mean and standard deviations were taken.

RESULTS AND DISCUSSION

Results

The results are shown in Tables 1 - 5. Table 1 shows the proximate composition, Table 2 shows the nutritional evaluation, Table 3 shows the anti-nutrients/phytochemical contents, Table 4 shows essential amino acids and Table 5 show the Mineral constituent of *Ageratum conyzoides.*

Table 3.1: Proximate (%)

Analysis	Percentage
Moisture content	6.57 ± 0.053
Crude protein	8.15 ± 0.041
Fat	3.10 ± 0.000
Fibre content	16.58 ± 0.088
Ash	4.29 ± 0.010
Carbohydrate	61.21 ± 0.010

Each value represented Mean ± SD of n=3

Table 3.2: Nutritional Evaluation

Moisture content	Low in moisture content
Crude protein	Low in crude protein
Fat	Low in fat content
Fibre content	Has moderate fibre content
Ash	Low in ash content
Carbohydrate	Rich in carbohydrate and minerals

Table 3.3: Phytochemical composition

Analysis	Concentration
Total tannin (%)	2.79 ± 0.010
Phenol (mg/g)	49.03 ± 0.047
Alkaloid (%)	0.94 ± 0.000
Flavonoids (mg/g)	38.22 ± 0.024
Saponins (%)	3.80 ± 0.000

Each value represented Mean ± SD of n=3

Table 3.4: Essential amino acids (mg/g protein)

Leucine	66.00
Lysine	38.05
Phenylalanine	48.00
Isoleucine	36.00
Threonine	32.00
Methionine	16.05

Table 3.5: The Mineral composition (mg/100g)

Mineral	Content
Magnesium	27.45 ± 0.041
Calcium	65.00 ± 0.000
Iron	8.16 ± 0.010
Potassium	720.07 ± 0.047
Phosphorus	100.42 ± 0.455
Sulphur	25.00 ± 0.000
Manganese	5.15 ± 0.041
Cobalt	0.05 ± 0.000
Selenium	0.72 ± 0.020
Zinc	2.24 ± 0.010

Each value represented Mean ± SD of n=3

DISCUSSION

Plant products play a great significant role in meeting the nutritional and therapeutic needs of man and animal. This has attracted a sense of concern in search of plants materials which could meet these needs. Many indigenous communities worldwide rely on wild edible plants as part of their diet (Orech *et al.,* 2007; Rankoana, 2021), and as a potential source of bioactive compounds, colors, and flavors (Sanchez-Mata 2012). The therapeutic usage of indigenous plant products for ethnomedicinal and nutritional objectives has attracted scientists' curiosity, motivating them to look for bioactive compounds. Medicinal plants possess essential food components such as carbohydrates, protein, and fat. These components are important for the human body's requirements and they are used in a different physiological, metabolic, and morphological activities (Radha, *et al.,* 2012; Cheeke, 2009). Natural plant-derived products are used in medications, nutritional supplements, and in different healthcare products.

Results of the proximate composition of the *Ageratum conyzoides* investigated are shown in Tables one and two. It shows that the plant has a low moisture content (6.57 ± 0.053), a low crude protein content (8.15 ± 0.041), low concentration of fat (3.10 ± 0.000), a moderate amount of fibre (16.58 ± 0.088), low Ash content (4. 29 ± 0.010) and high amount of carbohydrate (61.21 ± 0.010).

As reported by Emebu and Anyika (2011), micro-organisms that encourage food spoilage flourish well in foods with high moisture contents, thereby reducing the shelf life. Therefore, the lower moisture content of *Ageratum conyzoides* will definitely prolong the shelf life and help its storage.

The protein content of the leaves was found to be low (8.15 ± 0.041). Protein is vital for various body functions such as body development, maintenance of fluid balance, formation of hormones, enzymes and sustaining strong immune function (Emebu and Anyika, 2011). Fresh green leafy vegetables with low protein content have been reported (Oboh and Masodje, 2009; Achi *et al.,* 2017) and the protein in these leaves are in the form of enzymes, instead of being a storage pool as found in grains and nuts. The protein content of *Ageratum conyzoides* in this study is lower than the percentage recommended by the Food and Agriculture Organization (FAO, 2017) which is in the range of 12-15%. This suggests the need to supplement a diet base on *Ageratum conyzoides* with a complementary protein source to make it more nutritious.

The low content of fat (3.10%) is below the range (8.3%-27.0%) reported for some leafy vegetables consumed in Nigeria. Leafy vegetables are poor sources of lipids (Achi *et al.,* 2017), therefore the increase in the consumption of vegetables would naturally lower fat intake. Dietary fat increases the palatability of food by absorbing and retaining flavor (Antia *et al.,* 2006). A diet providing 1.20% of its caloric of energy as fat is said to be deficient for human being as excess fat consumption is implicated in certain cardiovascular disorders (Antia *et al.,* 2006). However, since excess consumption of fats in humans leads to cardiovascular diseases, aging, and cancer (Kris-Etherton *et al.,* 2002), the low concentration of lipids in *Ageratum conyzoides* is advantageous.

Moderate Crude fiber content of this plant (16.58%) could aid in the absorption of trace elements in the gut and therefore increases intestinal bowel movement (Abolaji *et al.,* 2007). Consuming vegetables in our diet could aid in managing constipation problems (Olowokudejo *et al.,* 2008). Dietary fibers also lower cholesterol, triglycerides and protect against cancer and digestive disorders (Achi *et al.,* 2017). The crude fibre in this result indicates the ability of goat weed to maintain internal distention for a normal peristaltic movement of the intestinal tract; a physiological role which crude fibre plays. Diets low in crude fibre is undesirable as it could cause constipation and such diets have associated with diseases of colon like piles appendicitis and cancer (Atasie *et al.,* 2009). The plant is good source of crude fibre when consumed because adequate intake of dietary fibre can lower the serum cholesterol level, heart disease, hypertension, constipation, diabetes and breast cancer (Ishida *et al.,* 2000).

Since ash content is a measure of mineral content of the plant, it indicates the vegetables would not be very good sources of minerals. The ash content indicates the degree of the inorganic matter content of the samples. The percentage ash content *Ageratum conyzoides* is (4.29%). This can be attributed to the mineral composition of the leaves and is a reflection of the mineral preserved in the plant which represents the total mineral content. They are essential for the proper functioning of tissues and act as second messengers in some biological cascade mechanisms (Antia *et al.,* 2006).

The estimated carbohydrate content in the leaves of *Ageratum conyzoides* (61.21%) was high and carbohydrates are known to produce energy required for the body because they are essential nutrient required for adequate diet (Emebu and Anyika, 2011) and supplies energy to cells such as brain, muscle and blood (Ejelonu *et al*., 2011). Carbohydrate constitutes a major class of naturally occurring organic compounds that are essential for the maintenance and sustenance of life in plants and animals and also provide raw materials for many industries (Ebun-Oluwa and Alade, 2007). The leaves of *Ageratum conyzoides* is a good source of carbohydrate when consumed because it meets the Recommended Dietary Allowance (RDA) values (F. N. D. 2002). This value indicates that fresh - leaves of *Ageratum conyzoides* can serve as good source of energy because carbohydrate and fat are known to be the main source of energy for organisms which include human, animal, or microorganism. Adequate carbohydrate is also required for optimum function of the brain, heart, nervous, digestive and immune system while carbohydrate deficiency causes depletion of body tissue (Offor *et al*., 2014).

The results of the phytochemical constituents in *Ageratum conyzoides* investigated are shown in Tables three. The phytochemical constituents (Tannins, Phenols, Alkaloids, Flavonoids and Saponins) were found to be 2.79%, 49.03mg/g, 0.94%, 38.22mg/g and 3.80% respectively.

Phytochemicals play an important role in human health as antioxidants, antibacterial, antifungal, anti-inflammatory, anti-allergic, antispasmodic, chemopreventive, hepatoprotective, hypolipidemic, neuroprotective, hypotensive, prevent aging, diabetes, osteoporosis, cancer and heart diseases, induce apoptosis, diuretic, CNS stimulant, analgesic, protects from UVB-induced carcinogenesis, immuno-modulator and carminative (Packer and Weber, 2001; Nichenametla, *et al.,* 2006; Prakash and Kumar, 2011). The presence of phytochemical ingredients that have a specific physiological action on the human body make these plants effective for both treating and curing human ailments.

Ageratum conyzoides contain little amount of Tannins from the result of this research. Tannin and its products are an important class of secondary metabolites that have been found to exhibit many important pharmacological and physiological activities including antioxidant, anti-aging, anti-inflammatory, anticancer, mutagenic, antiatherosclerosis, cardioprotective, antiulcerogenic, hepatoprotective, antimicrobial, antiviral, vasodilator, and hypolipidemic activities.

Medicinal preparations containing tannins are now considered as an indispensable component with external and internal effects in a variety of preparations. The external treatments are the treatment of skin inflammation and injuries and may prevent the inception of many chronic diseases. Tannin and its congeners inhibit lipid peroxidation and able to hunt cellular and molecular free radicals, especially in pro-oxidant states (Hossain *et al.,* 2021). However the concentration of Tannins in this plant may not be enough to offer a strong therapeutic effect.

Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Nwozo *et al.,* 2023). Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc (Asif, 2015). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Babenko *et al.,* 2015). They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Adeleye *et al.*. 2018). *Ageratum conyzoides* has high quantity of phenols as revealed in the phytochemical results. This shows why it might be one of the therapeutical plants for hypertension.

Since ancient times, alkaloids have shown great effects on animal and human lives and are an inclusive part of the food and beverages consumed in daily life. Besides, these compounds are used in medicinal and stimulant drugs with core biological activities. Alkaloids with anticancerous activities, such as vincristine, vinblastine, and taxol, are being effectively used as chemotherapeutic drugs (Bhambhani, *et al.,* 2021). Piperine is an alkaloid from *Piper nigrum* and *P. longum* which is shown to exhibit chemopreventive effects. This dietary phytochemical can act against several kinds of carcinogen, such as 7,12-dimethyl benz(a)anthracene and benzo(a)pyrene (Rather and Bhagat, 2018). The quantity of alkaloids in not enough to proffer any therapeutic effects.

Flavonoids are phenolic substances widely distributed in all vascular plants. They are a diverse group of phytonutrients (plant chemicals) ubiquitous in many fruits, vegetables and medicinal plants in which they occur as the free forms, glycosides, as well as methylated derivatives. Cohesive evidence shows an association between consuming flavonoid-rich foods and lower risk of cardiometabolic events (Cassidy *et al.,* 2013; Jacques *et al.,* 2013; Goetz *et al.,* 2016). For example, intake of anthocyanins was associated with a lower risk of MI (Cassidy *et al.,* 2013), flavanones with a lower risk of ischemic stroke (Goetz *et al.,* 2016), and flavonols with a lower risk of type 2 diabetes mellitus (Jacques *et al.,* 2013). The antihypertensive effect of quercetin (a flavonoid) is due to its ability to improve endothelial function, to modulate the Renin Angiotensin-Aldosterone system (RAAS), and to modulate VSMC contractility (Larson *et al.,* 2012). Indeed, quercetin reduces BP in humans with diabetes or metabolic syndrome (Egert *et al.,* 2009; Zahedi *et al.,* 2013) and in different animal models (Rivera *et al.,* 2008; Olaleye *et al.,* 2014). Large concentration of flavonoids in *Ageratum conyzoides* shows that it has the potential as a therapeutic source of hypertension.

It was revealed that *Ageratum conyzoides* contain saponins which are known to produce inhibitory effect on inflammation (Hassan *et al.,* 2012). Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness. Numerous papers have been published reporting the activities of saponins, among the most impressive anitidiabetic, antifungal, antitumor activity, antiviral, antiparasitic, immunomodulatory, synthesis of hormones, acting on the cardiovascular system, acting on the central nervous and endocrine systems (Barbosa, 2014). Therefore, Saponin presence in the plant make it potential natural remedy for hypertension.

The results of the essential amino acids (mg/g protein) present in *Ageratum conyzoides* investigated are shown in Table four. The concentration of essential amino acids (mg/g protein) (Leucine, Lysine, Phenylalanine, Isoleucine, Threonine, and Methionine) were found to be 66.00, 38.05, 48.00, 36.00, 38.00 and 16.05 respectively. Adequate amounts of amino acids are necessary to produce neurotransmitters and hormones, muscle growth, and other cellular processes (Kumar *et al.,* 2011). The relationship between dietary amino acids and hypertension is complex and contradictory. Different studies have reported varying associations between specific amino acids and hypertension risk. Some studies have shown positive associations, while others have shown negative or no significant associations (Hu *et al.,* 2016; Jennings *et al.,* 2016; Teymoor *et al.,* 2018).

Methionine is an essential amino acid; among its metabolic byproducts, homocysteine, when elevated, may induce ADMA production, impair endothelial function, and increase BP (Van Guldener *et al.,* 2003). Another study showed that plasma phenylalanine, together with branched chain amino acids (BCAAs), has a positive association with both systolic and diastolic BP (Siomkajło *et al.,* 2018). In this study, Lysine in mg/g protein is 38 mg/ g protein. An average value below the adult male requirement of 45 mg/g protein indicates health risk. However, study revealed that adequate lysine intake could significantly reduce the BP of hypertensive patients especially those on low lysine diets. Glutamate serves as excitatory and gamma-aminobutyricacid (GABA) functions as inhibitory neurotransmitters (Guyton and Hall, 2000). Lysine is directly involved in the synthesis of GABA and which reduces stress and anxiety (Guyton and Hall, 2000). The deficiency of lysine reduces the availability of GABA. It stands to reason that there is a strong relationship between diets low in lysine and high blood pressure which may be due to excess stress/anxiety as a result of low levels of lysine intake. Study discovered that the combination of isoleucine– tryptophan with whey protein hydrolysate effectively inhibits plasma angiotensin-1-converting enzyme, leading to antihypertensive effects (Martin *et al.,* 2015). These findings highlight the importance of isoleucine as an essential amino acid in managing hypertension.

The results of the mineral composition (mg/100g) present in *Ageratum conyzoides* investigated are shown in Table five. The concentrations of the mineral compositions (mg/100g) (magnesium, calcium, iron, potassium, phosphorus, sulphur, manganese, cobalt, selenium and zinc) were found to be 27.45, 65.00, 8.16, 720.07, 100.42, 25.00, 5.15, 0.05, 0.72 and 2.24 respectively.

Minerals are the essential micronutrients (chemical elements or ion/electrolytes) that exhibit a broad range of biochemical functions including body development (structurally), cellular activities like metabolism (chemically), and thus maintain the overall health status. Generally, minerals (ionized form) work alone or along with macronutrients to exhibits their biological function like acid–base balance, fluid osmotic pressure (equilibrium), muscle contraction, and relaxation (Kim, *et al.,* 2016). A copious investigation reported that various minerals like sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), zinc (Zn^{2+}), selenium (Se^{2-}), copper (Cu^{2+}), and calcium (Ca^{2+}) could directly or indirectly influence the blood pressure (Kim *et al.,* 2016; Iqbal *et al.,* 2019).

Magnesium (Mg^{2+}) is a trace element and crucial for metabolism (enzymatic reaction), energy production, and cofactor for some enzymes involved in prostaglandins. Some researchers have highlighted that deficiency of Mg^{2+} might result in elevated blood pressure and thus conferring that Mg^{2+} has an inverse association with blood pressure (BP) (Zhang *et al.,* 2016). Nevertheless, no or weak BP regulating (hypotensive) effect of Mg^{2+} was observed in some of the experiments (Jee *et al.,* 2002), and also some studies indicate that Mg^{2+} has a positive association with hypertension (HT) (Taneja and Mandal, 2007). However, currently, Mg^{2+} supplementation is recommended as a DASH diet (RDA: 350–420 mg/day) for treating hypertensive subjects as it might indirectly contribute to improving overall vascular quality (Houston, 2011). Magnesium is reported to enhance the production of NO and prostaglandin E1 (vasodilator) and thus regulate the excitability (contraction and relaxation) of smooth muscle cells in endothelium and thereby maintain blood pressure by lower the risk of endothelial dysfunction (Romani, 2018).

Calcium (Ca^{2+}) is one of the main structural minerals, playing a central role in bone/teeth formation, muscle contraction, and cell signaling (secondary messenger). The relationship between Ca^{2+} intake and BP regulation (HT) are poorly understood. However, the majority of the investigations indicate an inverse connection between calcium intake (RDA: 1000–1300 mg/day) and BP, similar to potassium (Imdad *et al.,* 2011; Cormick *et al.,* 2015). Some scientists have reported the moderate or no significant hypotensive effect of Ca^{2+} in normo and hypertensive subjects (Van Mierlo *et al.,* 2006). Hence, few DASH (diet formula) included Ca^{2+} (1300 mg/day) for treating hypertensive subjects. A few studies also indicated that Ca^{2+} showed better hypotensive activity against gestation HT than essential or primary HT and thus Ca^{2+} is highly recommended to lower the risk of gestational HT (Imdad *et al.,* 2011; Khanam *et al.,* 2018)

Potassium (K^+) is also a key mineral, which plays a pivotal function in cellular metabolism and electrolytic/fluid balance, and glucose homeostasis. Potassium role in hypertension is opposite to the effect of Na^+ as the levels of K^+ increases the risk of HT reduced, since the Na^+/K^+ pump (Na^+ moves to extracellular and K^+ moves intracellular) plays an important role in the maintenance of overall Na^+ and K^+ levels. Therefore, the increase in K^+/Na^+ ratio would favor a hypotensive property (lowering sodium and higher potassium level) and thereby hampers the risk of CVD and cerebrovascular diseases (Adrogué, 2007).

Selenium (Se^{2-}) is a trace element that plays a central role in cellular function and growth. Most of the nutritionist recommended (based on RDA/RDI) to consume Se^{2-} at a dose of 50–55 µg/day (RDA) to maintain normal blood Se^{2-} level. In addition to its antioxidant (enhancing Gpx activity and selenoprotein synthesis) and pro-oxidant activities, it also exhibits a broad spectrum of biological functions including neuroprotection, immunomodulatory, anti-cancer (Drake, 2006). Nevertheless, Se^{2-} overexposure (Se^{2-} toxicity) is also associated with various deleterious diseases like diabetic Mellitus, CVD, HT, neurological disorders (Vinceti *et al.,* 2019). A growing body of evidence suggests a strong relationship exists between serum Se^{2-} concentration and hypertension. However, the exact role of Se^{2-} on the prevention or development (cause) of HT is still contradictory. Deficiency of Se^{2-} is reported to increase the risk of HT (Nawrot *et al.,* 2007; Afridi *et al.,* 2014), some reported that high Se- is associated with HT (Berthold *et al.,* 2012; Bastola *et al.,* 2020) and others have no impact on blood pressure (Silva, 2016).

Manganese and zinc are commonly referred to as antioxidant minerals that are required for the activity of some antioxidant enzymes. Hiroyuki *et al.,* (2001), reported that zinc deficiency might play a crucial role in the development of genetic hypertension presumably through the oxidative stress caused by hypertension. The blood pressure lowering effect of manganese, and zinc could be attributed to their free radical scavenging properties which decrease nitric oxide quenching by superoxide anion, decrease NADPH oxidase activity, or increase superoxide dismutase activity since these minerals form an essential and integral part of superoxide dismutase (Muhammad, *et al.,* 2012).

Zinc (Zn^{2+}) is a ubiquitous trace transition bio-metal, which is involved in various cellular functions including regulation of various gene/protein expressions, enzymatic reaction as a cofactor (metabolism), cell proliferation, cell membrane stability, and cell signaling process. Hence, deficiency of Zn^{2+} leads to various health-related problems like HT, CVD, diabetes mellitus (DM), neurological disorders, malignancies, etc. (Chasapis *et al.,* 2012). Low Zn^{2+} levels were associated with elevated blood pressure as it alters vascular tone through modulating the expression of various inflammatory markers and thus increase the prevalence of CVD and DM (Ranasinghe *et al.,* 2018; Nevárez-López *et al.,* 2019). In addition, a few clinical trials hinted that hypertensive subjects have low levels of Zn^{2+} as compared to normal healthy subjects (Canatan *et al.,* 2004; Afridi *et al.,* 2014; Çıkım *et al.,* 2017). The above statement suggests that Zn^{2+} levels have an inverse (negative) association with blood pressure. Nevertheless, few studies showed also a positive association with blood pressure (Taneja and Mandal, 2007; Kunutsor and Laukkanen, 2016) whereas other experiments showed no association with blood pressure (Kim, 2013; Kunutsor and Laukkanen, 2016; Yao *et al.,* 2018).

Iron deficiency, on the other hand, may activate chronic inflammation and produce reactive oxygen species (Ames *et al.,*2005; Von Haehling *et al.,* 2019), contribute to endothelial dysfunction, which is the initial phase in the development of hypertension (Chen *et al.,* 2015). Therefore, moderate iron intake may be significantly associated with a reduced risk of hypertension. The plausible mechanism by which higher iron increased hypertension risk is possibly based on its ability to catalyze the generation of reactive oxygen species, lipid peroxidation and LDL-oxidation, which attack and damage cellular macromolecules, promote endothelial injury and atherosclerotic plaque formation (Sullivan, 2009; Rajapurkar *et al.,* 2012; Spasojevic-Kalimanovska *et al.,* 2014).

In prospective studies, a high-P intake has been found to be associated with lower blood pressure (BP), but only when the diet is rich in calcium-containing low-fat dairy products (Alonso *et al.,* 2005; Engberink *et al.*, 2009; Alonso *et al.*, 2010). Placebo-controlled studies of the dietary approaches to stop hypertension (DASH) diet that contains low P and high Ca amounts in low-fat foods result in reduced BP of adult men and women (Appel *et al.*, 2006). A meta-analysis showed that routine dairy consumption contributed to a 13% lower risk of developing hypertension (Ralston *et al.*, 2012). In another prospective population-based investigation, however, high-P intakes derived mainly from *nondairy* foods were associated with an increase in serum PTH and an increased risk of CVDs (Tonelli *et al.*, 2005). Calcium-rich dairy foods, therefore, have a preventive effect against hypertension despite being high in P, and the Ca:P intake ratio appears to be beneficial in reducing the development of high BP. High-serum PTH concentration has been shown to be associated with hypertension in still another report.

Manganese and zinc are commonly referred to as antioxidant minerals that are required for the activity of some antioxidant enzymes. Manganese and selenium exerted lowering effects on blood pressure in 343 contrasts to the increases associated with greater lead exposure. Manganese and selenium function through enzymes (manganese metalloenzymes and 355 selenoproteins, respectively), of which several possess antioxidant properties; thus, these essential 356 micronutrients may confer protection from oxidative stress (Institute of Medicine 2000, 2001).

Cobalt concentration in *Ageratum conyzoides* is very infinitesimal. Therefore, it may not be able to exert any meaningful biological/ nutritional impact.

CONCLUSION

This study demonstrated that dietary supplementation of *Ageratum conyzoides* in local delicacy (black soup) has the potential to improve the nutritional status vis a vis the overall health benefit with regards to hypertension and the other traditional use. This was shown in the abundance of phytochemicals (e.g. Flavonoids, phenols), mineral constituents (e.g. K, Zn), amino acids (e.g. methionine, lysine) and proximate compositions (e.g. crude fibre, carbohydrate) in it. This buttresses its overall health beneficial effects especially when taken as food.

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