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Proximate and phytochemical screening of some selected herbs and spices commonly used in Nigeria

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Abstract

Background and Objective: Many common herbs and spices contain powerful phytochemical substances that can improve the quality of our health. This study provided comparative baseline data on the proximate and phytochemical components of *Allium sativum*, *Garcinia kola*, *Curcuma longa* and *Zingiber officinale*.

Materials and Methods: Proximate composition and phytochemical analyses were carried out on the selected spices powder using standard methods.

Results: The five samples showed varying percentages of moisture, crude protein, fibre, fat and ash. Meanwhile, the percentages for the moisture content, crude protein, crude fibre, crude fat and ash for the mixture were 7.65%, 16.99%, 8.60, 2.30% and 9.05% respectively. The phytochemicals detected in the samples were flavonoids, alkaloids, cardiac glycoside, tannins, saponin and steroid while anthoquinones were absent. The FTIR spectrum showed that the extract of the combined mixture has bands and wave numbers of between 3291 cm-1 to 2922 cm-1 as the prominent peaks.

Conclusion: The findings indicate that the herbs and the spices are potential source of highly nutritious feed stuff and phytochemicals. The combinatorial usage of these spices and herbs will be of nutritional, clinical and veterinary relevance considering the diverse presence of different phytochemical functional groups as demonstrated by the FTIR analysis of the mixture.

Keywords: FTIR; Proximate; Phytochemicals; *Allium sativum*; *Garcinia kola*; *Curcuma longa*; *Zingiber officinale*; *Vernonia amygdalina*

1. Introduction

Herbal remedies or medicines consist of portions of plants or unpurified plant extracts containing several constituents, which often work together synergistically for their therapeutic or medicinal value[1]. They may come from any part of the plant but are most commonly made from leaves, roots, bark seeds, and flowers. They are eaten, swallowed, drunk, inhaled, or applied topically to the skin. Herbal products often contain a variety of naturally-occurring biochemical components, many of which contribute to the plant's medicinal benefits[2]. Chemicals known to have medicinal benefits are referred to as "active ingredients" and their presence depends on a number of factors including the plant species, the time and season of harvest, the type of soil, the way the herb is prepared, etc3. During the past decade, there has been increasing public interest and acceptance of natural therapies in both developing and developed countries. Due to

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poverty and limited access to modern medicine, about 80% of the world's population, especially in the developing countries uses herbal medicine as their source of primary healthcare[4].

Ginger is a member of a plant family that includes cardamom and turmeric. Its spicy aroma is mainly due to presence of ketones, especially the gingerols, which appear to be the primary component of ginger studied in much of the health-related scientific research[5]. The rhizome, which is the horizontal stem from which the roots grow, is the main portion of ginger that is consumed.

In folk medicine, turmeric has been used in therapeutic preparations over the centuries in different parts of the world. In Ayurvedic practices, turmeric is thought to have many medicinal properties including strengthening the overall energy of the body, relieving gas, dispelling worms, improving digestion, regulating menstruation, dissolving gallstones, and relieving arthritis[6].

Garcinia kola (Guttiferae) is a widely cultivated tree that is highly valued in West and Central Africa for its edible nuts. The extracts of the seed, commonly known as "bitter kola have been employed in African herbal medicine for the treatment of illnesses such as laryngitis, liver diseases, cough, hoarseness of voice, and diabetes and its associated complications[7,8].

Vernonia amygdalina, commonly known as bitter leaf is a shrub of 2-5 m tall with petiolate green leaves of about 6mm diameter. It belongs to the family Astaraceae and it is the most widely cultivated species of the genus Vernonia which has about 1,000 species of shrubs**.** It is vegetatively cultivated by stem cutting at an angle of 450^0 and popular in most of West Africa countries including Nigeria, Cameroon, Gabon and Congo Democratic Republic. The plant is frequently found in gardens[9]**.**

Allium sativum L. is an herbaceous plant from the Liliace family species with characteristics taste and odor. *Allium sativum* is a perennial flowering plant growing from a bulb. It has a tall, erect flowering stem that grows up to 1 m (3 ft). The leaf blade is flat, linear, solid, and approximately 1.25–2.5 cm (0.5–1.0 in) wide, with an acute apex[10]. The bulb is odoriferous and contains outer layers of thin sheathing leaves surrounding an inner sheath that encloses the clove. Often the bulb contains 10 to 20 cloves that are asymmetric in shape, except for those closest to the center

About 85% of our foreign exchange is lost through importation of drugs hence we are looking for local substitute[1]. *Allium sativum* (garlic)*, Garcinia kola* (bitter cola)*, Curcuma longa* (turmeric), *Vernonia amygdalina* (bitter leaf) and *Zingiber officinale* (ginger) seem to have the potentials for utilization in drug production. Again, there is an increase in the price of drugs, and therefore, there is a need to look for cheaper and cost effective alternative. This study provided comparative baseline data on the proximate and phytochemical components of herbs and spices such as ginger, bitter cola, bitter leaf, turmeric, and garlic as well as the functional groups present in their mixture which can be used to harness their synergistic effect as herbal mixtures.

2. Material and methods

2.1. Study area

This was carried out in the Biochemistry Department of Federal University Wukari, Taraba State, Nigeria between August, 2021 and May, 2022.

2.2. Collection and preparation of plant materials

Bitter leaf (*Vernonia amygdalina*)*,* zinger (*Zingiber officinale Rosc*)*,* turmeric (*Curcuma longa*)*,* bitter kola (*Garcinia kola*)*,* garlic (*Allium sativum*) were sourced within Wukari L.G.A, Taraba State, Nigeria. They were authenticated in the Department of Biochemistry, Federal University Wukari. The freshly obtained plant materials were thoroughly washed, and sun dried for five days before pulverization. The dried samples were finely pounded using pestle and mortar and stored in a tightly covered glass jars for further use.

2.3. Determination of crude fat by gravimetric method

Exactly 2 g of the dried sample was weighed unto a previously weighed filter paper (AOAC, 2015). The extraction flask were weighed, the wrapped samples were put into the extraction thimble and were fixed unto the Soxhlet extractor with 100-150 ml of solvent in the flask and was extracted for about 4 hours at condensation rate of about 5-6 drops per second or 16hrs at 2-3 drops per second. The thimble with sample was removed and distilled off solvent leaving the

extracted fat with about 10 ml of solvent in the flask. The solvent was gently evaporated in an oven. It was transferred to the desiccator, cooled and weighed. The weight of extracted fat can be given by either:

$$\text{Crude fat (\%) by weight (\%/w)} = \frac{weight\ loss\ of\ sample}{weight\ of\ sample} \times 100$$

$$= \frac{W2-W3}{W2-W1} \times 100$$

W1= Weight of empty thimble
W2= Weight of thimble + sample
W3= Weight of thimble + exhausted sample

2.4. Moisture content determination

Accurately 2 g of the sample was weighed into an aluminum dish with a cover having a diameter of at least 50 mm and a depth of about 40 mm or any other appropriate diameter[11]. The dish was shaken until the content was evenly distributed. The dish was placed in an oven with cover kept by side at 105± 2 °C and was dried for 18 hours. The dish was transferred to a desiccator to cool. The weight loss was weighed and calculated as moisture.

$$\text{Moisture (\%)} = \frac{Loss\ in\ weight\ due\ to\ drying}{weight\ of\ sample} \times 100$$

$$= \frac{W2-W3}{W2-W1} \times 100$$

Where
W1= Weight of dish + sample before drying
W2= Weight of dish + Dried sample

2.5. Determination of total ash (incineration method)

The crucible was ignited in a muffle furnace for 1 minute. It was transferred to a desiccator to cooled and weighed[11]. 2 g of the sample was accurately into a porcelain crucible. The crucible was placed in a temperature controlled furnace pre-heated to 600°C hold for 2 hours. The crucible was transferred directly into a desiccator to cool and was weighed immediately.

$$\text{Ash (\%)} = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

$$= \frac{W3-W1=}{W2-W1} \times 100$$

Where
W1= Weight in grams of empty dish
W2= Weight in grams of dish + test sample
W3 = Weight in grams of dish + crude ash

2.6. Determination of crude fibre (modified official method)

Exactly 1 g of the sample was weighed into 250 ml conical flask and 50 ml digestion mixture was added[11]. The flask was placed on a source of heat in a fume cupboard. It was heated until it boils and the boiling was allowed for 45 minutes while been shook occasionally to keep solids from adhering to sides. The flask was removed and filtered through a previously dried and weighed ashless filter paper. The conical flask was rinsed with 50-75 ml of boiling distilled water and was washed through filter paper. The procedure was repeated with three 50 ml portions of water until the washing is free from acid. The residue was rinsed with 50 ml acetone. The residue was removed with filter paper and it was dried at 105 ± 2°C in hot air oven for 2 hours. It was transferred into previously weighed crucible and ignited for 3 hours at 600 ± 15°C in a muffle furnace, it was cooled and weighed.

$$\text{\%wt of crude fibre} = \frac{(B-A)-(D-C)}{E} \times 100$$

Where
A= Weight of ashless filter paper only

B= Weight of ashes filter paper + residue
C= Weight of empty crucible
D= Weight of crucible + ash
E= Weight of sample taken

2.7. Determination of crude protein

Accurately 2 g of the sample was weighed and transferred into kjeldahl flask[11]. Two tablets or 1g of powdered of kjeldahl catalyst was added followed by 25 cm^3 of conc. H_2SO_4. The flask was placed in an inclined position on an electric coil heater (or gas burner) in a fume chamber. The mixture was heated gently, at first the heat was increased until the solution was cleared. It was allowed to cool and the neck of the flask was rinsed with distilled water and heated again until specks disappear. The contents were transferred with several washings in to a 250 ml volumetric flask and made up to the mark and was thoroughly mixed.

2.8. Distillation

Steam was passed through the distillation apparatus for about 10 minutes. Five cm^3 of boric acid indicator was pipetted into a 100 cm^3 conical flask and placed under the condenser such that the condenser tip was under the liquid. Five cm^3 of diluted digest was pipetted into the distillation apparatus and was rinsed down with distilled water. The cup was closed with a glass rod and 5 cm^3 of 50% NaOH was slowly put in.

W= Weight of test material used.
T= Titre volume of N/100 HCl
1 Liter of NHCl = 14.01g of nitrogen.
1 Liter of N/100HCl = 0.00011401g of Nitrogen.
T × 0.0001401g N = g of N in 5cm^3 of diluted digest
$\underline{T} \times 0.0001401\frac{250}{5}$ = g of N in total digest
$\frac{T \times 0.0001401 \times 250 \times 100}{W \times 5}$ = g of N in 100g of test material

2.9. Methods for Phytochemical Screening

Phytochemical screening was performed using standard procedures[12.]

2.9.1. Test for saponins

Exactly 0.5 g of the powder was added to 5 ml of distilled water in a test tube and the solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

2.9.2. Test for tannins

About 0.5 g of the powder was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and the solution observed for brownish green or a blue-black colouration

2.9.3. Test for anthraquinones

Exactly 0.5 g of the powder was boiled with 10 ml of H2SO4 and filtered while hot. The filtrate was shaken with 5ml of chloroform, the chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes.

2.9.4. Test for steroids

About 0.5 g of the powder was dissolved in 10ml of chloroform and equal volume of concentrated H2SO4 was added by the sides of the test tubes. Reddish upper layer and yellowish sulphuric acid layer with green fluorescence indicate the presence of steroids.

2.9.5. Test for cardiac glycosides (Keller-Killiani Test)

To 0.5 g of the powder dissolved in 5 ml water was added 2ml of glacial acetic acid solution containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

2.9.6. Test for flavonoids

A portion of the powder was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes, the mixture was filtered and 4 ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration indicated the presence of flavonoids.

2.9.7. Test for alkaloids

Samples were dissolved individually in dilute HCl and filtered.

- Filtrates were treated with Mayer`s reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
- Filtrate was treated with Dragendroff`s reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloid. Filtrate was treated with Hager`s reagent (saturated picric acid solution). Presence of alkaloid is confirmed by the formation of yellow coloured precipitate

2.10. Fourier-transform infrared spectrophotometer (FT-IR)

Dried powder of the plants extract of *Vernonia amygdalina* leaves*, Allium sativum, Garcinia kola, Curcuma longa, and Zingiber officinale* will be used for FT-IR analysis. 10 mg of the powder will be encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of the extracts will be loaded in FT-IR spectroscope, with a Scan range from 400 to 4000 cm-1 with a resolution of 4 cm-1[13].

3. Results

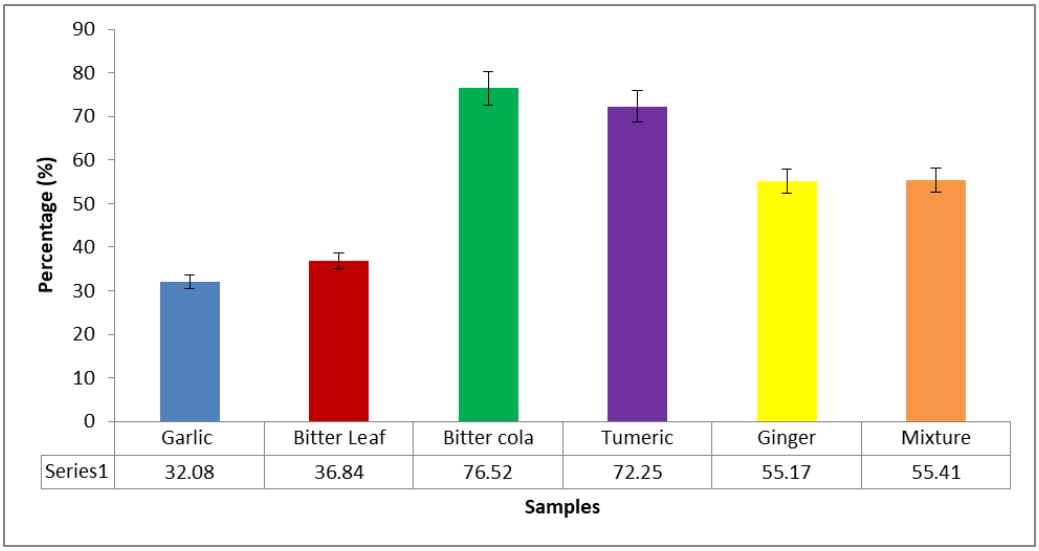
3.1. Proximate Compositions

The proximate composition of *Allium sativum*, *Vernonia amygdalina*, *Garcinia kola, Curcuma longa*, *Zingiber officinale* and the mixture of all the five samples is shown in Table 1. The individual samples showed that *Curcuma longa* has the highest moisture content (8.90%) as compared with *Garcinia kola*, *Zingiber officinale* and *Allium sativum*, whereas, *Vernonia amygdalina* has the lowest moisture content (6.50%) among the individual samples. There was a difference in the protein level across the component samples. The crude protein level was highest in *Vernonia amygdalina* with (26.56%) while *Curcuma longa* showed the least crude protein content (8.05%). For the crude fibre content, *Allium sativum* (25.40 %) was the highest with that of *Curcuma longa* being the lowest with value (3.60 %). There was variation in the crude fat content among the samples screened. *Zingiber officinale* has the highest percentage (5.80%), while the Sample of *Allium sativum* contained the lowest (0.60%). The Ash content shows little variation in the sample which ranges from 21.45% and 1.30% with the *Allium sativum* containing the highest (21.45%) while the *Garcinia kola* showed the lowest (1.30%). Meanwhile, the percentages for the moisture content, crude protein, crude fibre, crude fat and ash for the mixture were 7.65%, 16.99%, 8.60, 2.30% and 9.05% respectively. The mixture showed the crude protein is the highest while the crude fat showed the lowest value. Figure 1 demonstrated that *Garcinia kola* has the highest percentage of carbohydrate (76.52%) as compared with the other samples and the mixture.

Table 1 Proximate composition of some selected herbs and spices

Sample	Moisture (%)	Crude Protein (%)	Crude Fibre (%)	Crude Fat (%)	Ash (%)
Allium sativum	8.5±0.12	11.97±0.19	25.4±2.77	0.6±0.01	21.45±1.2
Vernonia amygdalina	6.5±0.07	26.56±0.45	15.6±0.10	1.35±0.0	13.15±1.1
Garcinia kola	7.6±0.08	8.98±0.89	4.2±0.59	1.40±0.1	1.3±0.2
Curcuma longa	8.9±0.13	8.05±0.11	3.6±0.33	0.9±0.2	6.3±0.8
Zingiber officinale	7.15±0.18	16.38±0.99	8.6±0.78	5.8±0.5	6.9±0.4
Mixture of all	7.65±0.66	16.99±0.15	8.6±0.41	2.3±0.2	9.05±0.13

Values are mean±STD of the three independent determinations



Values are mean±STD of the three independent determinations

Figure 1 Percentage crude carbohydrate of some selected herbs and spices

3.2. Qualitative phytochemical analysis

Table 2 shows the presence of saponins, tannins, steroids, alkaloids, flavonoids and cardiac glycosides while anthraquinones was absent through all the samples as evaluated.

Table 2 Qualitative phytochemical analysis

Parameters	Bitter kola	Garlic	Turmeric	Bitter Leaves	Ginger	Mixture
Saponins	+	+	-	+	+	+
Tannins	+	-	-	-	-	-
Steroids	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-

+ = presence; - = absence

3.3. FTIR analysis of the plant mixture

The FTIR spectrum in Figure 2 showed that the extract of the combined mixture has bands and wave numbers of between 3291 cm-1 to 2922 cm-1 as the prominent peaks. The peaks between the frequencies of 3291 cm-1 to 1002 cm^{-1} were strong, broad, and medium. The present FTIR results confirmed the presence of alkanes, alkenes, amines, carboxylic acids and alcohols in the extract of the combined mixture in Table 3.

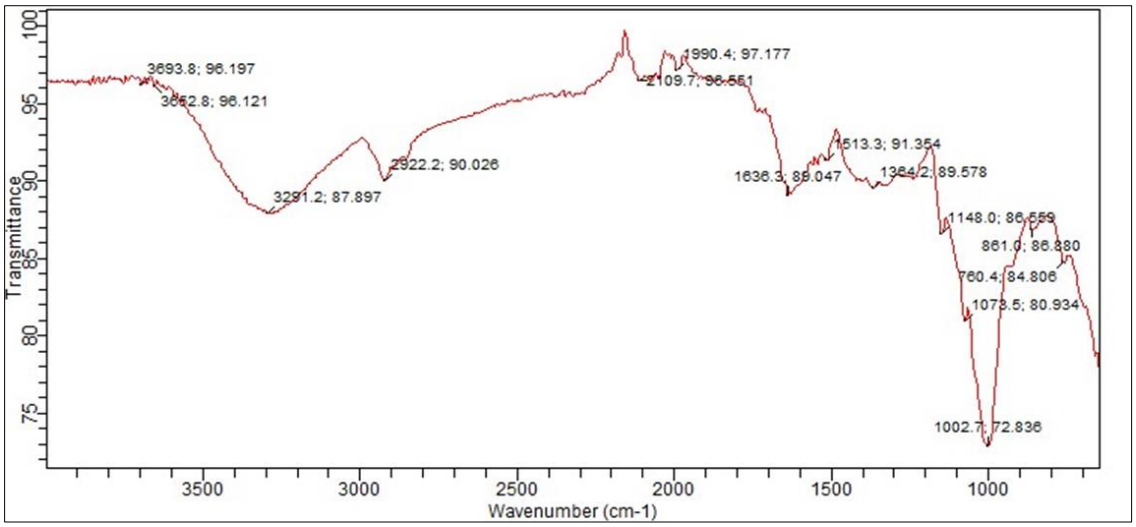


Figure 2 FTIR spectrum of the plant mixture

Table 3 Functional groups of bioactive components from the FTIR spectrum of the mixture of the five plants

S/N	Wavelength Number (cm^{-1})	Functional group	Inference
1	3693	O–H stretch, free hydroxyl	alcohols, phenols
2	3291	N–H stretch	1˚, 2˚ amines, amides
3	2922	C–H stretch	Alkanes
4	2109	–C ≡ C– stretch	Alkynes
5	1990	C-O stretch	Alcohols
6	1636	N–H bend	1˚ amines
7	1513	N–O asymmetric stretch	nitro compounds
8	1364	C–H rock	Alkanes
9	1148	C-O-C stretch	Ethers
10	1002	C–N stretch	aliphatic amines
11	760	C–Cl stretch	alkyl halides

4. Discussion

The proximate and phytochemical compositions of *Allium sativum*, *Vernonia amygdalina*, *Garcinia kola, Curcuma longa*, *Zingiber officinale* and the mixture of all the five samples were reported in this study. The individual samples showed that *Curcuma longa* has the highest moisture content (8.90%) as compared with *Garcinia kola*, *Zingiber officinale* and *Allium sativum*, whereas, *Vernonia amygdalina* has the lowest moisture content (6.50%) among the individual samples. The moisture content value of these spices and herbs was relatively low. The low moisture content would therefore hinder the growth of spoilage microorganisms and enhance shelf life[14]. The present result is in tandem with[15] that found such low moisture content between the ranges of 5-15% in *Dracaena reflexa.* Similarly, Olanipekun et al[16] reported such low level (13.42± 0.05 and 9.19± 0.52%) of moisture contents for *Morinda lucida* and *Saraca indica* respectively. However, the moisture contents of the plants is very low when compared with some leafy vegetables consumed in Nigeria such as *Colosia argenta* (80%) and *Amaranthus cruentus* (86%)[17].

There was a difference in the protein level across the component samples. The crude protein level was highest in *Vernonia amygdalina* with (26.56%) while *Curcuma longa* showed the least crude protein content (8.05%). The crude protein contents of all the studied plants are higher when compared to 1.98% as reported for *Securinea virosa* leaves, 2.7% for *G. hirsutum* and 2.46% for *M. charantia* respectively[18]**.** Plant proteins are a source of food nutrient especially

for the less privileged population in developing countries including Nigeria. Proteins are one of the macromolecule and it is an alternate energy source when other energy sources are in short supply. They are building block units and food protein is needed to make vital hormones, important brain chemicals, antibodies, digestive enzymes, and necessary elements for the manufacture of DNA. Some proteins are involved in structural support, while others are involved in bodily movement, or in defense against germs**.** Some of the spices can thus be considered a good source of protein because they provide more than 12% of caloric value from protein. Therefore, the protein content of the spices will go a long way in meeting the protein requirement of the local people.

For the crude fibre content, *Allium sativum* (25.40 %) was the highest with that of *Curcuma longa* being the lowest with value (3.60 %). Nutritionally this is important because fibre aids absorption and digestion of trace element in the gut, it also responsible for the reduction of cholesterol in the body[19,20]**.** Also, the crude fibre contents in the plants were within the range of the reported values for some Nigerian vegetables[21]**.**

There was variation in the crude fat content among the samples screened. *Zingiber officinale* has the highest percentage (5.80%), while the sample of *Allium sativum* contained the lowest (0.60%). The crude fat contents of the plants were lower when compared with 18% in *Psophocarpus tetragonolobus as* reported by Jacob et al[22]. However, the crude fat obtained from *Zingiber officinale* in the present study is relatively close to the values of *Gossypium hissuhum* 6.57± 0.04 and *Momordica charantia* 5.83± 0.1 respectively Olanipekun et al[23]. The fat contents of the studied plants were low and it can be recommended as part of weight reducing diets. The low fat food reduces level of cholesterol and thereby reduces obesity and every degenerated disease related to fat intake.

The Ash content shows little variation in the samples which ranges from 1.30% to 21.45% with the *Allium sativum* having the highest (21.45%) percentage while the *Garcinia kola* showed the lowest (1.30%) value. The ash content of the *Allium sativum* (21.45%) is higher compared to the ash contents reported for *Gnetum africanum* (6.7%) by Dike et al[24]. However, the values of the other plants except for *Allium sativum* are comparable with values of *Urera trinervis* (5.54%) and *Hippocratea myriantha* 6.14% respectively[25]. The presence of ash contents is an indication of the level of minerals and organic matter present in the plant thereby justify the traditional importance of the plants.

The study revealed that *Garcinia kola* has the highest percentage of carbohydrate (76.52%) as compared with the other samples and the mixture. The mean value of the carbohydrates of all the plants are relatively high, though not as high as the value of carbohydrate (80%) in *B. falcatum*[26], but it is still preferred when compared with plant like *Croton tiglium* with the low yield of carbohydrates of 15.51%[27]. Therefore the plants could be used as sources of energy.

There were presence of saponins, tannins, steroids, alkaloids, flavonoids and cardiac glycosides while anthraquinones was absent through all the samples as evaluated. The FTIR spectrum showed that the extract of the combined mixture has bands and wave numbers of between 3291 cm^{-1} to 2922 cm^{-1} as the prominent peaks. The peaks between the frequencies of 3291 cm^{-1} to 1002 cm^{-1} were strong, broad, and medium. The present FTIR results confirmed the presence of alkanes, alkenes, amines, carboxylic acids and alcohols in the extract of the combined mixture. The detection of phytochemicals in these plants is in line with the findings of Abu et al[28] and Umaru et al[29] that made such discovery in *Ficus glumosa* and *Senna alata* respectively. Further evaluation of both plants (*Ficus glumosa* and *Senna alata*) resulted in antioxidant and ant-diabetic activities respectively[28,29]. This implies that, the presence of these compounds in the present study would comfer protective effect on the evaluated plants against free radicals.

Significance statement

This study discovered the functional groups of some important phytochemicals in the mixture of spices and herbs which can be beneficial for nutritional, clinical and veterinary purposes. This study will help the researchers to uncover the critical areas of the combinatorial usage of these plants that many researchers were not able to explore. Thus a new theory on synergetic effects of these plants may be arrived at.

5. Conclusion

This study shows that the spices are rich in phytochemicals and that their utilization should be strongly recommended for good health. These spices are reservoirs for free radical scavenging molecules such as alkaloids, tannins, phenolic acids, flavonoids and other metabolites, which are basically rich in antioxidant activities.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest

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