

Research Article

Evaluation of Phytochemical Composition of Ginger Extracts

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Abstract

This study involved the extraction of the bioactive phytochemicals from the ethanolic and water extract of ginger (*Zingiber officinale*). Further extractions were carried out using petroleum ether, ethanol and water. Phytochemical screening revealed the presence of phytochemicals except phlobatannins. A total of ten characterised compounds were isolated from ginger. In conclusion, the ethanolic extract of ginger showed higher extraction ability than water extract in alkaloid, flavonoids, oxalate, phytate, phenols, and anthraquinone with the corresponding values of 9.02, 3.51, 1.27, 0.77, 1.81, and 1.33 mg/g respectively. Therefore, ginger contains a wide range of bioactive which could be beneficial and possesses good inhibitory activities against varying diseases in aquaculture.

Keywords: Phytochemical, Ginger, Extracts, Composition

Introduction

Ginger (*Zingiber officinale*) belongs to *Zingiberaceae* family. The used part of the plant is rhizome. This plant produces an orchid like flower with greenish yellow petals streaked with purple color. Ginger is cultivated in areas characterized by abundant rainfall. Even though it is native to southern Asia, ginger is also cultivated in tropical areas such as Jamaica, China, Nigeria and Haiti and it is an important spice crop in India [1]. Ginger, *Zingiber officinalis*, is a perennial herbaceous plant that is a part of the *Zingiberaceae* family. Ginger is an important plant with several medicinal, ethno medicinal and nutritional values (Kumar *et al.*, 2011). Ginger is the underground rhizome of the ginger plant with a firm, striated texture. *Zingiber officinale* R., commonly known as ginger belongs to family *Zingiberaceae* [1].

Ginger extracts contain polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity. Antioxidant activity is due to the presence of phytochemicals such as flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins [1]. Antioxidant property of ginger is an extremely significant activity which can be used as a preventive agent against a number of diseases. Many bioactive compounds in ginger have been identified, such as phenolic and terpene compounds. The phenolic compounds are mainly gingerols, shogaols, and paradols, which account for the various bioactivities of ginger [3].

Ginger is abundant in active constituents, such as phenolic and terpene compounds [11]. The phenolic compounds in ginger are

mainly gingerols, shogaols, and paradols. In fresh ginger, gingerols are the major polyphenols, such as 6-gingerol, 8-gingerol, and 10-gingerol. With heat treatment or long-time storage, gingerols can be transformed into corresponding shogaols. After hydrogenation, shogaols can be transformed into paradols [2]. There are also many other phenolic compounds in ginger, such as quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione [11]. Moreover, there are several terpene components in ginger, such as β -bisabolene, α -curcumene, zingiberene, α -farnesene, and β -sesquiphellandrene, which are considered to be the main constituents of ginger essential oils [11]. Besides these, polysaccharides, lipids, organic acids, and raw fibers are also present in ginger [11]. Therefore, the aim of this research study is to determine the phytoconstituent of ginger.

Materials and Methods

Study Site

The experiment was carried out in the laboratory of Biological Science Department, Gombe State University, Gombe, Gombe State. The university is located about 37 km from Gombe town of Gombe State. Gombe state university is located between latitudes 10° 18' 00"N to 10° 18' 35"N and longitudes 11° 10' 10"E to 11° 10' 52"E.

Collection and Processing of Ginger

Fresh gingers (*Zingiber officinale*) were purchased from a market in Gombe, Gombe State. They were prepared for the experiment by rinsing in distilled water.

Ginger

The rhizomes were purchased from Gombe main market. Washed with distilled water, sun-dried, and cleaned of its dirt by hand picking. The rhizomes size were reduced with pestle and mortar first, then air dried at ambient temperature before milling with hammer machine after which it was sieved using a sieving material (house hold sieve 0.2 mm) and kept in polythene bag until when needed.

Phytochemical Screening of the Active Ingredients

The qualitative and quantitative phytochemicals present in Ginger rhizomes were analysed as follows:

Determination of Qualitative Phytochemical Analysis

The qualitative phytochemical analysis of active ingredients was carried out in the Department of Biochemistry, Gombe State University, Gombe, Gombe State. [7] method was used for the qualitative determination of the phytochemicals.

Alkaloids

A few drops of Wagner's reagent were added to few ml of plant extract along the sides of test tube. A reddish-brown precipitate confirms the present of Alkaloids.

Flavonoids

0.5 g ginger was mixed with water in a test tube and shaken. Few drops of sodium hydroxide was added, formation of intense yellow colour which becomes colourless on further addition of dilute Hydrochloric acid indicate the presence of flavonoids.

Tannins

0.5 g of ginger powder was mixed with 20 ml of water in a test tube and heated. The mixture was filtered and 0.1% of ferric chloride was added. Appearance of brownish green colouration indicate the presence of tannins.

Saponins

0.5 g of ginger was mixed with water in a test tube and heat. Few drops of olive oil were added and shaken. Formation of soluble emulsion indicated the presence of Saponins.

Glycosides

Total of 100 mg of the extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution, it was then under layered with 1 ml of concentrated sulphuric acid, a brown ring obtained at the interface indicate the presence of de-oxy sugar characteristic of cardenolides.

Steroid

Analytical method was used to determined 0.5 g of additives and was dissolved in 2 ml of Chloroform and few drops of Sulphuric acid was added to form a lower layer. A reddish brown color at the interface indicates the presence of steroid.

Anthraquinones

0.5 g of the extract was collected in a dry test tube and 5 ml of chloroform was added and shaken for 5 minutes it was then filtered and the filtrate was shaken with an equal volume of 100% ammonia solution. A pink violet or red colour in ammonia lower layer indicate the presence of free Anthraquinones.

Phenols

The extract (50 mg) was dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl was added to it. White precipitate indicates the presence of phenol compounds.

Oxalates

5 ml of the extract was treated with 1 ml of concentrated sulphuric acid, this was allowed to stand for an hour and two drops of potassium permanganate was added, the formation of steady red colour indicate the presence of oxalate.

Determination of Quantitative Phytochemical Composition

The fine powder of ginger was taken to the Analytical laboratory of Department of Biochemistry, Gombe State University. The quantitative phytochemical analysis was carried out in the laboratory.

Determination of Alkaloid

Determination of Alkaloid was carried out by the method described by [7]. The alkaloid content was determined gravimetrically. 5 g of the sample was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4 hours at 28°C and it was filtered using filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drops of additional of concentrated aqueous NH_4OH until the Alkaloid is precipitated. The alkaloid precipitated in a weighed filter paper was washed with 1% ammonia solution, and dried in the oven at 80°C. Alkaloid content was calculated and expressed as a percentage of the weight sample analysed.

Determination of Flavonoids

This was determined according to the method outlined by [7]. 5 g of the sample was boiled in 50 mL of 2 mol/L HCl solution for 30 min under reflux. The content was allowed to cool and then filtered through a filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

Determination of Tannins

Tannin content of the flour samples was determined using the methods described by [7]. The sample (0.2 g) was measured in a 50-mL beaker, 20 ml of 50% methanol was added, covered with homogenizer, placed in a water bath at 77-80°C for 1 hour, and the contents stirred with a glass rod to prevent lumping. The mixture was filtered using a double-layered 1 filter paper into a 100-ml volumetric flask using 50% methanol rinse to made up the mark with distilled water and thoroughly mixed. One millilitre of the sample extract

was homogenized into a 50-ml volumetric flask, and 20 ml distilled water, 2.5 ml Folin-Denis reagent, and 10 mL of 17% Na₂CO₃ were added, thoroughly mixed and allowed to stand for 20 min when a bluish-green coloration developed. Standard tannic acid solutions in the range of 0-10 ppm were treated similarly as the 1 mL sample above. The absorbances of the tannic acid standard solutions as well as samples were read after colour development on a Spectronic 21D spectrophotometer at a wave length of 760 nm. Percentage tannin was then calculated.

Determination of Saponins

The spectrophotometric method was used to determine Saponins as described by [7]. One gram of the flour sample was put into a 250-mL beaker and 100 mL iso-butyl alcohol was added. The mixture was shaken to ensure uniform mixing. The mixture was then filtered through filter paper into a 100-mL beaker and 20 mL of 40% saturated solution of magnesium carbonate was added. The mixture obtained was further filtered through a filter paper to obtain a clear colourless solution. One millilitre of the colourless solution was homogenized into a 50-mL volumetric flask and 2 mL of 5% FeCl₃ solution was added and made up to mark with distilled water and allowed to stand for 30 min for blood red colour to develop. Standard Saponins solutions (0-10 ppm) was then prepared from Saponins stock solution and treated with 2 mL of 5% FeCl₃ solution as done for experimental samples. The absorbance of the sample as well as standard Saponins solutions were read after colour development on a Spectronic 21D spectrophotometer at a wavelength of 380 nm. The percentage of Saponins was calculated.

Determination of Steroids

Sample of fine powder of additives was weighed and transferred into 10 ml volumetric flasks. Sulphuric acid and iron (III) chloride were added, followed by potassium hexacyanoferrate (III) solution. The mixture was heated in a water-bath maintained at 70°C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank [7].

Determination of Phenols

The sample (100 g) was extracted, by stirring with methanol 250 mL for 3 h. The extracted sample was then filtered through a filter paper, the residue was washed with 100 ml methanol, and the extract was allowed to cool. The extract was then allowed to evaporate to dryness under vacuum, using a rotary evaporator. The residue was dissolved with 10 ml of methanol and used for determination of total phenolic compounds. This determination was performed as gallic acid equivalents (mg/100 g), by using Folin-Ciocalteu phenol reagent. The diluted methanol extract (0.2 ml) was added, with 0.8 ml of Folin-Ciocalteu phenol reagent and 2.0 ml of sodium carbonate (7.5%), in the given order. The mixture was vigorously vortex-mixed and diluted to 7 mL of deionized water. The reaction was allowed to complete for 2 hours in the dark, at room temperature, prior to being centrifuged for 5 min at 125 g. The supernatant was measured at 756 nm on a spectrophotometer. Methanol was applied as a control, by replacing the sample. Gallic acid was used as a standard and the result was calculated as Gallic acid equivalents (mg/100 g) of the sample [7].

Determination of Phytates

An indirect colorimetric method of [7] was used in Phytate determination. This method depends on an iron to phosphorus ratio of 4:6. A quantity of 100 g of the test sample was extracted with 3% trichloroacetic acid. The solution was precipitated as ferric Phytate and converted to ferric hydroxide and soluble sodium Phytate by adding sodium hydroxide. The precipitate was dissolved in hot 3.2 N HNO₃ and the colour read immediately at 480 nm. The standard solution was prepared from Fe(NO₃)₃ and the iron content was extrapolated from a Fe(NO₃)₃ standard curve. The Phytate concentration was calculated from the iron results assuming a 4:6 iron: phosphorus molecular ratio.

Determination of Oxalate

Oxalate was determined by [7] method. 100 g of the sample was weighed in a conical flask. Seventy-five millilitres of 3 mol/l H₂SO₄ was added and the solution was then stirred intermittently with a magnetic stirrer for about 1 h and then filtered with a filter paper. The sample filtrate (extract) (25 mL) was collected and titrated against hot (80-90°C) 0.1 N KMnO₄ solution to the point when a faint pink colour appeared that persisted for at least 30 s. The concentration of oxalate in each sample was obtained from the calculation: 1 ml 0.1 permanganate = 0.006303 g oxalate.

Results

Phytochemical Screening of Ginger

Qualitative Phytochemicals

Table 1 presents the qualitative screening of ginger (*Zingiber officinale*). Similarly, Table 1 contained information on the screened from ginger. Alkaloid and flavonoid were in excess in ginger ethanol extract while in water extract there was no bioactive compound that was in excess. Phenol and phytate were moderate in ginger ethanol extract while flavonoid, saponin, and alkaloid were moderate in ginger water extract. Steroid, anthraquinone, tannin, and saponin were extracted in trace amount in ginger ethanol extract while phenol and tannin were in trace amount in ginger water extract. Glycosides were rare in ginger ethanol extract while glycosides, steroids, anthraquinone, phytate, and oxalate were rare in ginger water extract.

Table 1: Qualitative Phytochemical Screening of the Studied Herbs as Fish Feed Additives.

Phytochemicals	Ginger Ethanol	Ginger Water
Alkaloid	+++	++
Flavonoid	+++	++
Tannins	+	+
Saponins	+	++
Glycosides	-	-
Steroid	+	-
Anthraquinone	+	-
Phenolics	++	+
Phytate	++	-
Oxalate	++	-

Keys: - Rare; + Trace; ++ Moderate; +++ Excess.

Table 2: Quantitative Phytochemical Screening from Ginger.

Phytochemicals (mg/g)	Ginger Ethanol	Ginger Water
Alkaloid	9.02a	6.52b
Flavonoid	3.51a	2.92b
Tannin	1.05bc	1.41b
Saponin	0.51d	1.07c
Glycoside	0.05d	0.09d
Oxalate	1.27b	0.09c
Phytate	0.77a	0.03c
Phenolics	1.81a	0.22b
Steroids	0.04e	0.55b
Anthraquinone	1.33b	0.99d

Means of data on the same row with different alphabets are significantly different ($p < 0.05$).

Quantitative Phytochemicals of Studies Herbs as Bio-additives

Table 2 shows variations in the quantitative values of ginger phytochemicals analysed and that ethanol extracts recorded higher values than water extract. Alkaloid was highest in ginger ethanol extract with the value of 9.02 mg/g. Flavonoids was 3.5 mg/g as the highest value screened in ginger ethanol extract. Tannins shows 1.41 mg/g as higher in ginger water extract. Oxalates, phytate, phenols, and anthraquinone were higher in ginger ethanol extract than in ginger water extract and vice versa in ginger water extract for saponin, glycosides, and steroids with the value of 1.07 mg/g, 0.09 and 0.55 mg/g respectively.

Discussion

Phytochemical Screening of Ginger

These results on the potency of ethanol extract agreed with that of [8] who screened four medicinal plants as immune stimulants against bacterial infection using water and ethanol extracts but those extracts from ethanol showed presence of more phytochemicals. The quantitative analysis results on the potency of the ethanol and water extract corroborate the findings of [8]. It should be noted that the plants are rich in medicinal and immune-stimulating phytochemicals which will be beneficial to fish's health.

Plants generally contain chemical compounds (such as saponins, tannins, oxalates, phytates, trypsin inhibitors, flavonoids and cyanogenic glycosides) known as secondary metabolites, which are biologically active [11]. Secondary metabolites may be applied in nutrition and as pharmacologically-active agents [11]. Plants are also known to have high amounts of essential nutrients, vitamins, minerals, fatty acids and fibre [11]. Flavonoids (quercetin) have inhibitory activity against disease-causing organisms in animals. Preliminary research indicates that flavonoids may modify allergens, viruses and carcinogens and so may be biological response modifiers. In vitro studies show that flavonoids also have anti allergic, anti-inflammatory, antimicrobial, anti-cancer and anti-diarrheal activities [10]. Tannins are plant polyphenols, which have ability to form complexes with metal ions and with macro-molecules such as proteins and polysaccharides [10]. Dietary tannins are said to reduce feed efficiency and weight gain in animal [11]. Environmental factors and

the method of preparation of samples may influence the concentration of tannins present. Tannin presence influences protein utilization and build defense mechanism against micro-organism [10]. Saponins are glycosides, which include steroid saponins and triterpenoid saponins. High levels of saponins in feed affect feed intake and growth rate in animal [10]. Saponins, causes hypocholestraemia because it binds cholesterol making it unavailable for absorption (Soetan and Oyewole, 2009). Saponins also have haemolytic activity against red blood cell (RBC) [10]. Saponin-protein complex formation can reduce protein digestibility (Ogbe and Affiku, 2011). Saponins reduced cholesterol by preventing its reabsorption after it has been excreted in the bile. Proper food processing would reduce antinutrients [9].

The results obtained in this study showed the presence of alkaloids, cyanogenic glycosides, saponins, tannins, flavonoids etc. The concentrations of these metabolites in the additives were moderately available. Although, [10] described that these secondary metabolites were present in higher concentration. These variations can be explained by differences in agro-climatic conditions, age of plant, genotype, environmental factors, post-harvest treatments, the season of harvesting and maturation stage of the leaves have a strong influence on the phytochemical content of plants. [11] also ascribed the antimicrobial properties to the presence of flavonoid in onion bulb. [10] reported that the phytochemical screening of some medicinal plants revealed the presence of alkaloids, carbohydrates, flavonoids, saponins and phenolic compounds which are associated with antimicrobial activities and curative properties against pathogen which are similar to the findings of this study.

Conclusion

In conclusion, the phytochemical assessment of ginger, ten known phytochemicals were discovered which are alkaloids, flavonoids, tannin, saponin, glycosides, oxalates, phytates, phenols, steroids, and anthraquinone. It has been found that ginger contains diverse bioactive compounds, such as gingerols, shogaols, and paradols, and possesses multiple bioactivities, such as antioxidant, anti-inflammatory, and antimicrobial properties. Additionally, ginger has the potential to be the ingredient for functional foods or nutraceuticals in aquaculture (Tables 1 and 2).

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