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International Digital Organization for Scientific Research ISSN: 2550-7931
IDOSR JOURNAL OF APPLIED SCIENCES 5(1) 1-8, 2020.

Qualitative and Quantitative Phytochemical Analyses of the Ethanolic Extract of *Arachis Hypogaea* L. seed. (Groundnut)

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ABSTRACT

Quantitative and qualitative analyses of phytochemical constituents namely alkaloid flavonoid, saponin, steroid, tannin, glycoside, terpenoids, carotenoids and phenol of ethanolic extracts *Arachis hvpogaea* 1. (groundnut) were performed using the method described by Harbone (1973) and Trease and Evans (1989). The species *Arachis hypogaea L.* (groundnut) also known as peanut, monkeynut, carthnut, goober and wild bean belong to the leguminous family rich in proteins and oils. Presence of alkaloids found in various seed extracts of *A.hypogaea* are useful in the prolonging of the action of several hormones and acting as stimulants. Flavonoids present in the seeds enable food to be tasty which means that they promote peculiar taste in prepared foods. Flavonoids are capable of treating certain physical disorders and diseases They are potent water soluble, super antioxidant and free radical scavengers which prevents oxidative cell damage and have strong anticancer activity which adds protection against all stages of carcinogenesis. Presence of glycosides in agricultural seeds *of Arachis hypogaea* as potential precursors of defensive metabolites could lead to a new appreciation of their roles in crop resistant to pests. Saponins are present in the seeds of the *A.hypogaea* studied and are contained in appreciable quantities, which means it has cholesterol binding properties and helps in hemolytic activities. The high values of tannins in *A.hypogaea* seeds serve as astringent properties in the healing of wounds and inflaming mucous membrane. The phenols presence indicates that these legumes have ability to block specific enzymes that causes inflammation. They also modify the prostaglandin pathways thereby protecting platelets from dumping. Steroids have anti inflammatory agents, growth stimulating agents and oral contraceptive properties. Terpenoids arc major constituents of plant resin and essential oil are extracted from such plants. Terpenoids have the ability to reduce heartburn and gastric acid reflux. The percentage yield of the ethanolic extract of *Arachis hypogaea* was found to be 2.67%. The results of the study showed maximum presence of phytochemicals in ethanolic extract of groundnut. Qualitative phytochemical analysis of the extract revealed that flavonoid and tannin were strongly present, alkaloid, saponin, glycoside and phenol were moderately present and steroid and terpenoids were present in trace. The quantitative analysis showed presence of Alkaloid (0.79 1 0.10). Flavonoid (2.49 ± 0.14), Saponin (1.46 ± 0.24), Tannin (1.34 ± 0.03), Glycoside (0.78 ± 0.06), Terpenoids (0.27 ± 0.07), Steroid (0.44 ± 0.11) and Phenol (0.76 ± 0.16). Hence, the present

investigation has been undertaken to find out the various phytochemicals present in *A.hypogaea* for the purpose of using these bioactive substances in different food items as supplements to reduce the anti-nutritional effects.

Keywords: Qualitative, quantitative, phytochemicals, analysis, ethanolic.

INTRODUCTION

Consistent consumption of foods that contain significant levels of phytochemical correlates with tangible health benefits. Legumes are rich in protein, calories, certain minerals and vitamins. Among the legumes, nuts are a good source of oil containing higher amounts of unsaturated fatty acids as compared to saturated fatty acids. Legumes also contain some of the phytochemicals which have dual effect on human health. Groundnuts *(Arachis hypogaea)* or peanuts are the world major oil seed crops. The other different names by which groundnut are called includes peanut, monkey nut, earth nut, goober, wild bean, manila nuts, jack nuts and truffle [1]. The only nut that grows on the ground is the groundnut, often called as "The king of oilseeds", and it is the important inexpensive source of protein, fat, minerals and vitamins in the diets of rural populations, especially children [2]. Groundnut is commonly called a poor man's nut. Major proportion of the groundnut produce is processed for direct consumption as peanuts butter, salted groundnuts, and confectionery; whereas in Africa, particularly in Nigeria, groundnuts are utilized mainly for oil production [3]. Groundnuts grow' best in light, sandy loam soil. They require five months of warm weather, and an annual rainfall or the equivalent in irrigation water. Poor storage of groundnuts can lead to an infection by the mold fungus Aspergillus flavus, releasing the toxic substance aflatoxin.

During the 1980s, groundnut production began to increase greatly. China leads in production of groundnut having share 37.5% of overall world production followed by India (19%) and Nigeria (1 1%).Nigeria is one of the largest producers of groundnut. The chemo preventive action of plant foods has been attributed to the presence of some biological active phytochemicals. Groundnut seed contains 44 to 56% oil and 22 to30% protein on a dry seed basis and is rich sources of minerals (P, Ca, Mg. and K) and vitamins (E. k and B group). Groundnuts are one of these plant foods that are dietary source of phytochemicals. Array of nutrients and phytochemicals play an important role in mechanism responsible for its putative health benefits. The present study is focused on screening for the detection of the Alkaloid, Flavonoid. Saponin. Steroid, Tannin, Glycoside, Terpenoids and phenols in Arachis hypogaea in raw form [4].

Aim and Objective

The overall aim of this study is to obtain knowledge about the phyto constituents of ethanolic extract of *Arachis hypogaea.* The objective of this research is to determine both qualitatively and quantitatively the phytochemical composition of the ethanolic extract of groundnut seed in raw form.

MATERIALS AND METHODS

Source of Plant Material

The seeds of *Arachis hypogaea* were used in this study. Raw *Arachis hypogaea* seeds were bought from Ogbete market, Enugu. The groundnut seeds were taken to Prof Eze. of the Department of Applied Biology and Biotechnology who authenticated and confirmed that they were seeds *of Arachis hypogaea L.* the seeds were air dried.

Extraction Of Plant Material

The dried seeds of *Arachis hypogaea* were pulverized into fine powder

using a domestic grinder. The groundnut seeds, 300g was soaked in 1200ml of ethanol and was left for 24 hours to macerate. Then the solution was filtered with Whatman filter paper NO 1. The residue was evaporated to dryness using rotary evaporator and was soaked in 900ml of ethanol and was left for another 24 hours. And then was filtered and dried. The crude extract was dissolved in distilled water.

Phytochemical Analysis

Qualitative Phytochemical Analysis of the Extract

The methods described by [5], [6] were used in carrying out chemical tests

Test for Tannins

Extract (0.lg) was stirred with 10ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2ml of each filtrate. The presence of a blue-black or blue green precipitate indicated the presence of tannins.

Test for the Alkaloids

A quantity of the extract (0.lg) was boiled with 5ml of 1% aqueous Hcl on a steam bath. The mixture was filtered and 1ml of the filtrate was treated with 2 drops of Dragendorff's reagent, an orange red precipitate indicated the presence of alkaloids.

Test for Saponins

A quantity of the extract (0.lg) was boiled with 5ml of distilled water and filtered. To the filtrate, about 3ml of distilled water was further added and shake vigorously for about 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins.

Test for Glycosides

Extract (0.lg) was mixed with 30ml of distilled water and heated on a water bath for 5 minutes. To 5ml of the filtrate, 0.2ml of Fehling's solution A and B was added until it turns alkaline. The solutions were heated on a water bath for 2 minutes. A brick-red precipitate indicated the presence of glycoside.

Test for Terpenoids

Extract (0.lg) was dissolved in ethanol. Acetic anhydride (1ml) was added, followed by the addition of concentrated H2SO4. A change in color from pink to violet showed the presence of terpenoids.

Test for Flavonoids

A quantity (0.lg) of the extract was dissolved in water and filtered. O >ml of the filtrate. 3ml of lead ethanoate solution was added. Appearance of a buff-colored (pale yellow-brown) precipitate indicated the presence of flavonoids.

Test for Steroids

To 0.lg of extract, 2ml of acetic acid was added. The solution was cooled well in ice followed by the addition of concentrated tetraoxosulphate (VI) acid (H2SO4) carefully. Color development from violet to blue or bluish-green indicated the presence of a steroidal ring.

Test for Phenols

About 0.lg of extract was boiled with distilled water and then filtered. To 2ml of the filtrate, few drops of 10% ferric chloride solution were then added. A green-blue or violet coloration indicated the presence of a phenolic hydroxyl group.

Quantitative Phytochemical Analysis

Quantification of Flavonoid Content

This was determined according to the method of [7]. A quantity (0.lg) of the extracts was weighed out separately, macerated with 20ml of ethyl acetate, filtered through Whatman No. 1 filter paper and 5ml of the filtrate was pipetted out. Dilute ammonia (5ml) was added, shook and the upper layer of the mixture was collected. Absorbance at 490nm was measured

Quantification of Alkaloid Content

The determination of alkaloid was carried out accordingly to the method of [8]. A quantity (0.19) of the extracts was weighed out separately. Macerated with 20ml of 20% of H2SO4 in ethanol (1:1) and

was filtered. The filtrate (1ml) was pipetted out and 5ml of 60% H2S04 was added. After 5 minutes 5ml of 0.5% formaldehyde in 60% H2SO4 was also added. They were mixed together and are allowed to stand for 3 hours and absorbance at 550nm was measured.

Quantification of Steroid Content

The determination of alkaloid was carried out accordingly to the method of [9]. A quantity (0.lg) of the extracts was weighed out separately, macerated with 20ml of ethanol and filtered through Whatman filter paper. The filtrate (2ml) was pipetted out and 2ml of chromogen solution (4ml iron stock made up to 24ml with concentrated, H2S04 was added and the mixtures were left to stand for 30 minutes. Absorbance was measured at 550nm.

Quantification of Tannin Content

The determination of alkaloid was carried out according to the method of [10]. A known amount (O.lg) of the extracts was weighed; 50ml of distilled water was added and filtered. Then 5ml of the filtrate was pipetted out into a test tube and mixed with 0.1N and 0.008M potassium fcrricyanide. The absorbance was measured at 720nm.

Quantification of Saponin Content

Saponin content was determined by the method of [11]. This was done by macerating 0.lg of each extract with 20ml of petroleum ether. It decanted into a beaker and washed again with 10ml petroleum ether. The filtrate were combined and evaporated to dryness. The residue was dissolved with 6ml of ethanol. Then 2ml of the dissolved residue was put in a test tube and 2ml of chromogen solution (4ml iron stock made up to 24ml with concentrated H2SO4) added to it. It was allowed to stand for 30niinutes and the absorbance was read at 550nm against an ethanol blank.

Quantification of Terpenoid Content

The determination of terpenoid was carried out according to the method of [12]. A quantity (0.lg) of the extract was weighed out separately, macerated with 20ml of ethanol and filtered through Whatman No .1 filter paper. The filtrate (lml) was pipetted out and 1ml of 5% phosphomolybdic acid solution was added and shaken. Gradually lml of concentrated H_2SO_4 was added to each. The mixtures were left to stand for 30 minutes. Ethanol (2ml) was added and absorbance was measured at 700nm.

Quantification of Glycoside Content

The determination of glycoside was carried out accordingly to the method of [13]. The extract (0.lg) was weighed out separately, macerated with 20ml of distilled water and 2.5ml of 1 5% lead acetate was added and filtered. Chloroform (2.5ml) was added to the filtrates. Shakes vigorously and lower layer collected and evaporated to dryness. Glacial acetic acid (3ml) was also added together with 0.1ml of 5% ferric chloride and 0.25ml of concentrated H2SO4. The mixture was shaken and put in the dark for 2 hours. Absorbance was measured at 530 nm.

Quantification of Phenol Content

A quantity (0.lg) of the extracts was weighed out separately, macerated with 20ml of 80% ethanol and filtered through Whatman No.l filter paper. The filtrate (5ml) was pipetted out and ().5ml of folin-ciocalterus reagent was added. After 3 minutes, 2ml of 20% sodium carbonate (IV) was added and the mixtures were left to stand for 10minutes. Absorbance was measured at 650nm.

RESULTS

The yield obtained from the seed of *Arachis hypogeae* weighing 300g is 2.67% and this is shown in the table 1 below.

Table 1: Result of Extraction/Yield

Solvent	Weight of sample	Weight of extract	Yield (g)	% yield
Ethanol	300	8	0.0266	2.66

Result of Phytochemical Analysis

The Phytochemical analysis of the ethanolic extract *of Arachis hypogaea L.* revealed the presence ol phenol, flavonoid, alkaloid, saponin, steroid, tannin, glycoside, terpenoid.

Table 2: Result of Qualitative Phytochemical an Alysis
Preliminary Result of Phytochemical Analysis on *Araciі1si1ypogea*

PHYTOCHEMICAL	QUALITATIVE RESULT
Alkaloid	++
Flavoniod	+++
Saponin	++
Steroid	+
Tannin	+++
Glycoside	++
Terpenoids	+
Phenol	++

Key:
+ Slightly Present ++
Moderately Present +++
Highly Present

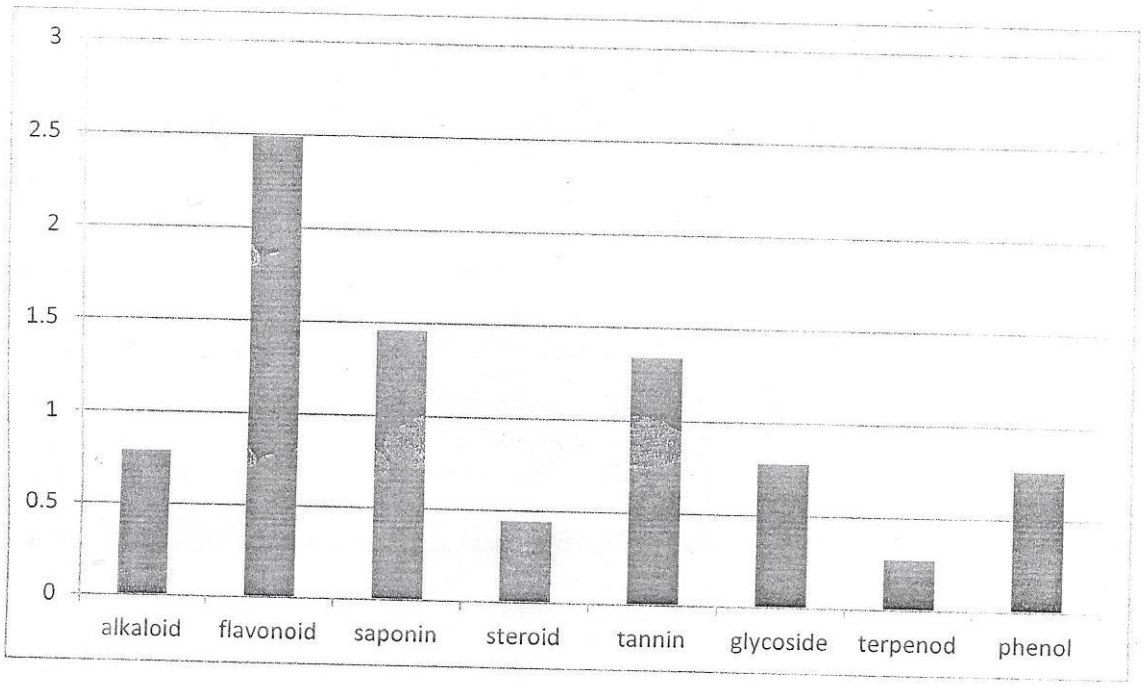
Results of the Quantitative Phytochemical Analysis of ***Arachis Hypogaea.***

Table 3: Quantitative Analysis of Phytochemical Con 1 Ent of Ethanolic Extract of *Arachis Hypogaea* Seed.

PHYTOCHEMICAL	CONCENTRATION
Alkaloid	0.79 ±0.10
Flavoniod	2.49 ±0.14
Saponin	1.46 ±0.24
Steroid	0.44 ±0.11
Tannin	1.34 ± 0.03
Glycoside	0.78 ± 0.06
Terpenoids	0.27 ± 0.07
Phenol	0.76 ±0.16

The result are presented as mean ± Standard Deviation, n=3

Figure 1. A Chart Showing the Quantitative Concentration of Ethanolic extract of ***Arachis Hypogaea.*** (Concentration Mg/G) (Mean ± Sem)



DISCUSSION

The percentage yield of ethanolic extract *of Arachis hypogaea* was found to be *4%* w/w. The seed extract *of A.hypogaea* analyzed is rich in Phytochemicals. The preliminary phytochemical screening of the legumes studied clearly showed that the legumes are nutritious and contained some Phytochemicals such as alkaloids, glycosides, tannins, flavonoids, sterols, saponins, terpenoids and

phenol. All these Phytochemicals present in these legumes compared favorably with those rcporled from some medicinal plants found in Nigeria [14]. Strongly presences of alkaloids found in various seed extracts *of A.hypogaea* are useful in the prolonging of the action of several hormones and acting as stimulants [15]. Flavonoids present in seeds enable food to be tasty which is in line with the work of dakora that flavonoids promote perculiar taste in prepared foods. Flavonoids are capable of treating certain physiological disorder and diseases. They are potent water soluble, super antioxidant and free radical scavengers which prevents oxidative cell damage and have strong anticancer activity which adds protection against all stages of carcinogenesis [16]. Presence of glycosides in agricultural seeds ol *A.hypogaea* as potential precursors of defensive metabolites could lead to a new appreciation of their roles in crop resistance to pests. Saponins are present the seeds of the *A.hypogaea* studied and are contained in appreciable quantities, which means it has cholesterol binding properties, and help in hemolytic activities. The high value of tannins in *A.hypogaea* seeds serves as astringent properties for healing of wounds and inflaming mucous membrane [17]. The phenols presence indicates that these legumes have ability to block specific enzymes that causes inflammation. They also modify the prostaglandin pathwavs thereby protecting platelets from clumping.

CONCLUSION

In conclusion the present screening of Phytochemicals in the seeds of groundnut plant possesses alkaloids, glycosides, flavonoids, terpenoids, tannins, steroids, phenols and saponins. This suggest that the seed part of the groundnut can be used as food supplements in everyday diet for normal metabolic activities of living organisms to reduce the anti nutritional effects and which are also useful in pharmaceutical industry

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