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Phytochemical and Anti-nutrient Compositions of *Juglas regia* Seeds

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ABSTRACT

This study was designed to determine the quantitative phytochemical and anti-nutrient compositions of *Juglas regia* seed. Spectrophotometric method was used for the study. The result of the phytochemical analysis revealed the presence of glycosides (21.3657±0.0321 mg/100g), flavonoids (739.4447±0.0451mg/100g), saponins (21.3657±0.0321mg/100g), tannins (3276.243±0.21049mg/100g), steroids (52.186±0.03mg/100g), terpenoids (517.6243±0.085 mg/100g), phenols (4362.186±0.0493mg/100g) and alkaloids (496.6267±0.0764mg/100g). The result equally showed that the seed contain the following anti-nutrients haemaglutinin (0.98±0.08 HU/mg), oxalates (1.30±0.51%), trypsin inhibitor (0.75±0.06 IU/mg), and phytates (3.31±0.06 mg/100g). This indicates that seed of *Juglas regia* contains appreciable amount of bioactive compounds which explains the use of this plant parts in ethno-medicine for the management of various diseases and the amount of anti-nutrients detected are below the permissible toxic level

Key Words: Phytochemical, Anti-nutrient, *Juglas regia,* Spectrophotometric and Seed

INTRODUCTION

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals [1]. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total [2]. Chemical compounds in plants mediate their effects on the human body through processes

identical to those already well understood for the chemical compounds in conventional drugs. Thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects [3].

Juglans regia L. (Walnut) is a monoecious, heterodichogamous, deciduous tree species valued for its high-quality timber as well as its nuts. It belongs to the family of *Juglandaceae* and the genus *Juglans.* The natural range of *J. regia* extends from China in the east to Turkey in the west and from Kazakhstan in the north to temperate regions of India and Nepal in the south. *J. regia* is grown commercially in China, USA (California), France, India, Italy and Spain for nut production (Manos and Stone, 2001). This specie has an aromatic phytochemical Juglone ($C_{10}H_6O_3$; 1, 4 – naptha quinone, 5 hydroxy –8Cl) found in all parts. This chemical is one of the oldest known allelopathic compounds in the history of cultivation and is also used as an active ingredient in herbal remedies and commercial dye. Juglone has been reported as an important therapeutic phytochemical and is under investigation for its reported anti-carcinogenic effects [4]. Walnut has been used globally in human nutrition since ancient times. The high protein and oil contents of the kernels of *Juglans regia* L. (*Juglandacea*) make this fruit indispensable for human nutrition. The seed part of the fruit (kernel) is consumed fresh, toasted, or mixed with other confectionaries. In the Middle East walnuts are added alone or along with almonds, date fruit, and raisin as a special pastry preparation called Ma'moul. Walnuts are nutrient-rich food due to high contents of fats, proteins, vitamins and minerals. They are also good source of flavonoids, sterols, pectic substances, phenolic acids and related polyphenols. The major components of walnut oil are triacylglycerols (980 g/kg oil), in which monounsaturated

fatty acids (FAs) (mainly oleic acid) and polyunsaturated FAs (PUFAs; linoleic and α-linolenic acids) are present in high amounts in all genotypes [5].

Walnuts have high amount of omega-6 and omega-3 PUFA, which are essential dietary fatty acids. Clinical studies suggest that omega-3 PUFA have significant role in prevention of coronary heart disease [6]. Oil rich in oleic acid displays greater oxidative stability therefore; it could be widely used as frying oil. According to an investigation conducted by several researchers, it was found that the average value for protein was 18.1% [7]. They are mainly composed of glutelins (about 70% of the total seed proteins) together with lesser amounts of globulins (18%), albumins (7%) and prolamins (5%) [8]. The amino acid (AA) composition of walnut flour is dominated by the acidic amino acid residues of aspartate and glutamate together with relatively high levels of arginine. Walnut proteins contain all essential AAs required for the needs of a human adult. The lysine/arginine ratio in walnut proteins is lower than those observed in other common vegetable proteins, and this fact has been identified as a positive feature in the reduction of atherosclerosis development [9].

Phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine. Epidemiological studies suggest that consumption of a diet high in fruits and vegetables are associated with a reduced risk of chronic disease. Unfortunately, there is not yet enough evidence to support the concept that phytochemicals are responsible for these effects [10]. Fruits and vegetables are important sources of a variety of beneficial agents including vitamins, minerals, fiber, and phytochemicals. More research is needed to fully explain the actions of phytochemical compounds in the human body. Hundreds of phytochemical compounds, with several different

biological functions, have been identified in plant-based foods. Therefore, consuming a variety of plant-based foods helps to ensure that individuals receive the optimum benefits from the fruits and vegetables consumed [11]. Anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrients. Nutrition studies focus on those anti-nutrients commonly found in food sources and beverages. Protease inhibitors are substances that inhibit the actions of trypsin, pepsin and other proteases in the gut, preventing the digestion and subsequent absorption of protein. Other anti-nutrients include oxalate, phytate and tannins which chelate metals such as iron and zinc and reduce the absorption of these nutrients, but they also inhibit digestive enzymes and may also precipitate proteins.

Drugs of Herbal origin are consumed because of their high nutrients and antioxidants potential. However, *Juglans regia* seed is one of the common seeds consume by Igbos in the South east of Nigeria more especially among Abakaliki indigenes of Ebonyi State, Nigeria. Therefore, this study was carried out to evaluate the phytochemical and anti-nutrient compositions of *Juglans regia* seed which is one of the major seed consume by Abakaliki people of Ebonyi State, Nigeria. The data gotten from this study would go a long way to stimulate interest in the utilization of these neglected seeds beyond their traditional use.

MATERIALS AND METHODS

Materials:

Equipment, instruments, chemicals and reagents used in this study were of analytical grade

Collection of Biological Materials: *Juglans regia* seed was obtained from Meat market at Abakaliki in Abakaliki Local Government area of Ebonyi State and allowed to dry between the periods of two weeks (4th of August to 18th of August, 2015) at room temperature. The plant sample was identified by a taxonomist, Prof. S.S. Onyekwelu of the Department of Applied Biology, Ebonyi State University,

Abakaliki, Nigeria. A voucher specimen was deposited at the herbarium in the Department of Applied Biology, Ebonyi State University, Abakaliki ,Nigeria, for reference purposes.

Figure 1: Fresh Seeds of *Juglas regia*

Figure 1: Dried Seeds of *Juglans regia*

Preparation of *Juglas regia* Seeds for Phytochemical and Anti-nutrient Analyses.

The seeds fruit of *Juglas regia* were dried at room temperature and ground to powdered form using electrical grinding machine. The paste sample was then stored in an airtight container and kept in the refrigerator until required.

Methods: Quantitative Phytochemicals were determined by the following methods:

Determination of flavonoids: This was determined by the method of Harborne (1973) [12].

Principle: Flavonoids reacts with dilute ammonia (NH3) to produce a coloured complex which can be measured spectrophotometrically at 470nm.

Procedure: Exactly 1g of the sample was weighed and macerated in 20mls of ethylacetate. It was filtered using Whatman filter pepper. Five mills of the filtrate was pipetted and added with 5mls of dilute ammonia. After that the upper layer was collected and absorbance was read at 490nm.

Determination of Tannins: This was determined by the method of Harborne (1973) [12].

Tannins reduce phosphotungstomolybdic acid in alkaline solution to produce highly coloured blue solution, the intensity of which is proportional to amount of tannins. The intensity is measured in spectrophotometer at 720nm.

Procedure: Exactly 1g of the sample was weighed and macerated in 20mls of distilled water and filtered. Exactly 5mls of the filtrate was pipetted and

0.3ml of 0.1n ferric chloride in 0.1NHCl was added. 0.3ml of 0.0008m potassium ferricyanide was equally added and absorbance at 720nm was measured against the blank.

Determination of Glycoside

This was determined by the method of Trease and Evans (1989) [13].

Procedure: Exactly 1g of the sample was weighed and macerated in 20mls of distilled water. After macerating, 2.5ml of 15% of lead acetate was added and filtered, then 2.5 mills of chloroform was added and stirred vigorously. The layer was collected and evaporated to dryness. The residue was dissolved with 3mls of glacial acetic acid. 0.1ml of 5% ferric chloride and 0.25mls of concentrated H_2S0_4 was then kept in the dark for 24hours. Absorbance was read at 530nm against the blank.

Determination of phenol: This was determined by the method of Harborne (1973) [12].

Principle: Phenols react with phosphomolybdic acid in folin-ciocalteau reagent in alkaline medium to produce a blue coloured complex (Molybdenum blue) which can be estimated spectrophotometrically at 650nm.

Procedure: Exactly 1g weighing was of the sample and macerated in 20mls of 80% ethanol and filtered with Whatman filter paper. 5mls of the filtrate was pipette d and 0.5mls of folin ciocalteau reagent was added. It was allowed to stand for 2 minutes and 2mls of 20% sodium carbonate was added. Then absorbance was read at 650nm.

Determination of Steroids: This was determined by the method of Harborne (1973) [12].

Procedure: Exactly 1g of the sample was weighed and macerated in 20mls of ethanol and filtered. Then 2mls of the filtrate was pipetted and 2mls of colour reagent was added and allowed to stand for 30mins. Then absorbance was taken at 550nm.

Determination of Terpenoids: This was determined by the method of Harborne (1973) [12].

Procedure: A known quantity of *Phoenix dactylifera* was weighed and macerated in 20mls of ethanol and was filtered properly with a whatman filter paper. 1ml of the filtrate was pippeted and 1ml of 5% phosphomomolybdic acid was added. Gradually, 1ml of sulphuric acid was equally added and allowed to stand for 30mins. Finally 2mls of ethanol was added and absorbance was read at 550nm against the blank.

Determination of Alkaloids: This was determined by the method of Harborne (1973) [12].

Principle: H_2S0_4 reacts with alkaloids in the presence of formaldehyde to form a coloured complex which is read spectophotometrically at 565nm.

Procedure: Exactly 1g of the sample was weighed and macerated in 20mls of ethanol in 20% sulphuric acid (1:1) and then filtered. 1ml of the filtrate was pipette and 5mls of 60% H_2S0_4 was added. After 5mins, 5ml of 0.5% formylladehyde in 60% H_2So_4 was also added, mixed and allowed to stand for 3hours. Absorbance was read at 565nm.

Determination of Saponin: This was determined by the method of Harborne (1973) [12].

Principle: Saponin reacts with anisaldehyde and ethylacetate to give a coloured complex which is read spectrophotometrically at 430nm.

Procedure: Exactly1g of the sample was weighed and macerated in 20mls of petroleum ether and decanted into a beaker. It was washed again with 10ml

of petroleum ether. The filtrate was combined and was allowed to evaporate to dryness. The residue was dissolved in 6mls of ethanol and 2mls of it was taken into a test tube. 2mls of chromogen solution was added and allowed to stand for 30mins. Absorbance was read at 550nm.

Determination of Cynogenic Glycosides: This was determined by the method of Trease and Evans (1989) [13].

Principle: Cyanogenic glycosides react to alkaline picrate under boiling temperature to produce a colour that is read spectrophotometerically at 490 nm.

Procedure: Exactly 1g of the sample was weighed and macerated in 50mls of distilled water. It was filtered properly with whatman filter paper. 1ml of the filtrate was pipette and 4mls of alkaline picrate solution was then added. It was then boiled for 5mins and allowed to cool. The absorbance was then read at 490nm.

Quantitative Analysis of Anti-nutrients: Anti-nutrient analysis of date fruits were carried out by the method of Association of Official Analytical Chemist (AOAC, 1997) [9] as follows:

Determination of Haemagglutinin

Procedure: A known weight of the sample was taken and dispersed in a 10mls normal saline solution and buffered at pH 6.4 with a 0.01m phosphate buffer solution and was allowed to stand at room temperature for 30mins and then centrifuge to obtain the extract. Exactly 0.1ml of the extract was diluted in a test tube and 1ml of tryspinezed rabbit blood was added. Then control was mounted on the test tube containing the blood cells and the both test tubes was allowed to stand for 4 hours at room temperature, 1ml of normal saline was added to all the test tubes and

allowed to stand for 10mins, after which the absorbance was read at 620nm specifically against the blank.

Determination of Trypsin Inhibitor

Procedure: Exactly1.0g of the sample was weighed and dispersed in 50mls of 0.5ml NaCl solution. The mixture was stirred for 30mins at room temperature and centrifuged. The supernatant was filtered with the Whatman filter paper and the filtrate is taken. 10mls of the filtrate was pipetted into a test tube and 20mls of the standard trypsin solution was added. A blank solution of 10mls of the same substrate was prepared in a test tube. The content of the test tubes was allowed to stand for at least 5mins and then measured spectrophometrically at 410nm.

Determination of Oxalate:

Procedure: Exactly 2.0g of the sample was weighed into a conical flask. 20mls of 30% HCl was added and was allowed to stand for 5mins. 4.0g of ammonium sulphate was equally added. It was stirred gently to dissolve and was allowed to settle. Supernatant was decanted into a 25mls volumetric flask and the volume was made up with 30% HCl. It was transferred into 50mls volumetric flask equal volume of diethyl ether. The pH was adjusted to 7.0 with either NH_4OH or CH_2COOH and was centrifuged at 3000rpm. It was equally decanted into a 250mls conical flask and titrated with 0.1M$KMnO_4$. The absorbance was read at 490nm.

Determination of Phytate:

Procedure: Exactly 0.5g of the sample was weighed into a 500 ml round bottom flask, and extracted with 100mls of 2.4% HCl for 1hr at room temperature and filtered. 5mls of the filtrate was pipetted and diluted to 25mls with water. From the diluted sample, 10mls was taken into a test tube through amber let resin grade 200-400 mesh to elude inorganic phosphates. 15mls of 0.7 M sodium chloride was added and the absorbance was read at 520nm.

RESULTS

Result of Phytochemical and Anti-nutrients Analyses of *Juglas regia* Seeds

The results of phytochemicals analysis revealed that phenol (4362.186±0.0493) had the highest value and glycoside (21.3657±0.0321) had the least value as shown in Figure 3 and 4. The results of anti-nutrient composition of *Juglas regia* seeds revealed that phytate (3.31±0.06) had the highest value, while trypsin inhibitor (0.75±0.06) had the least value as shown in Table 1.

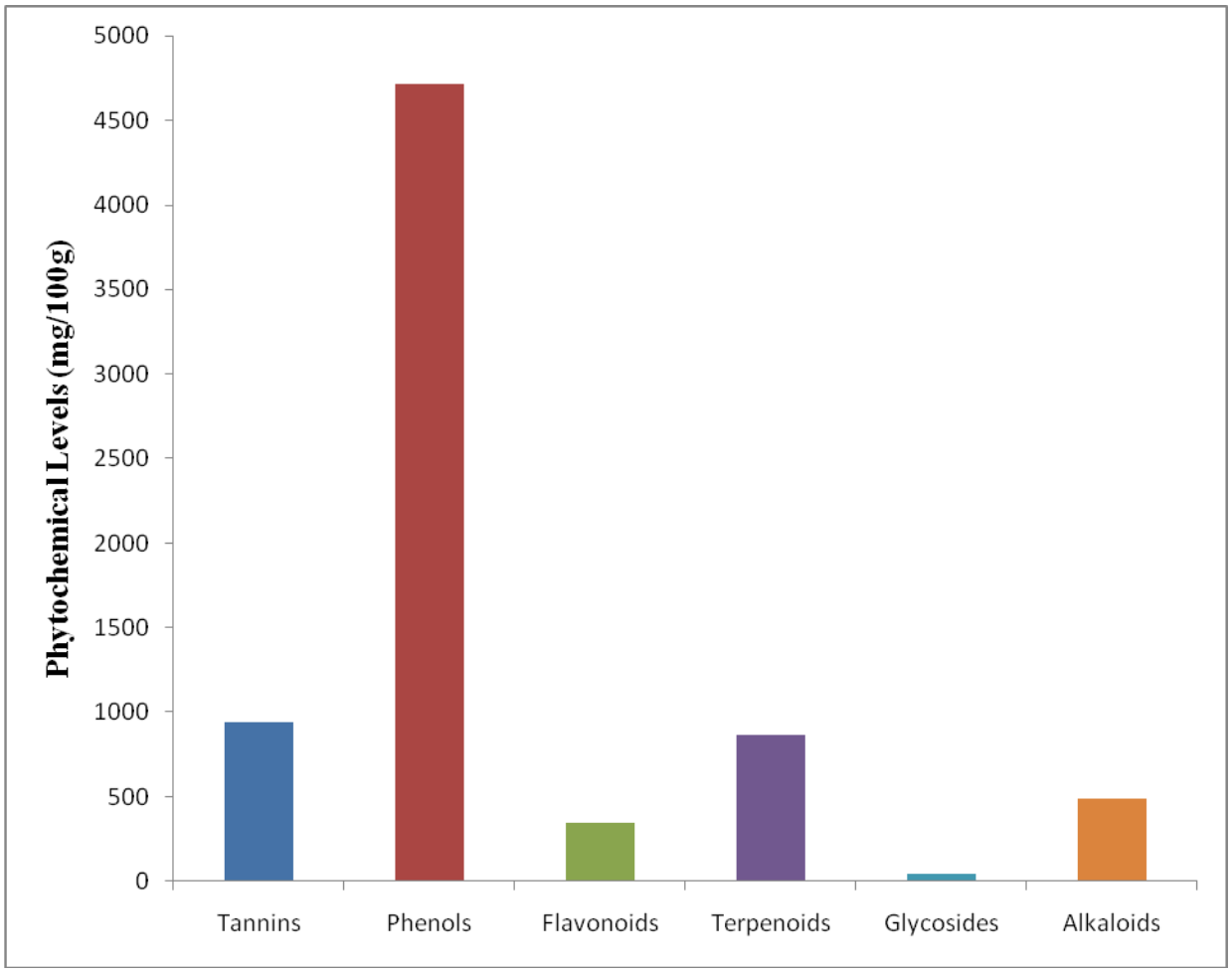

Figure 3: Phytochemical Compositions of *Juglas regia* seeds in mg/100g.

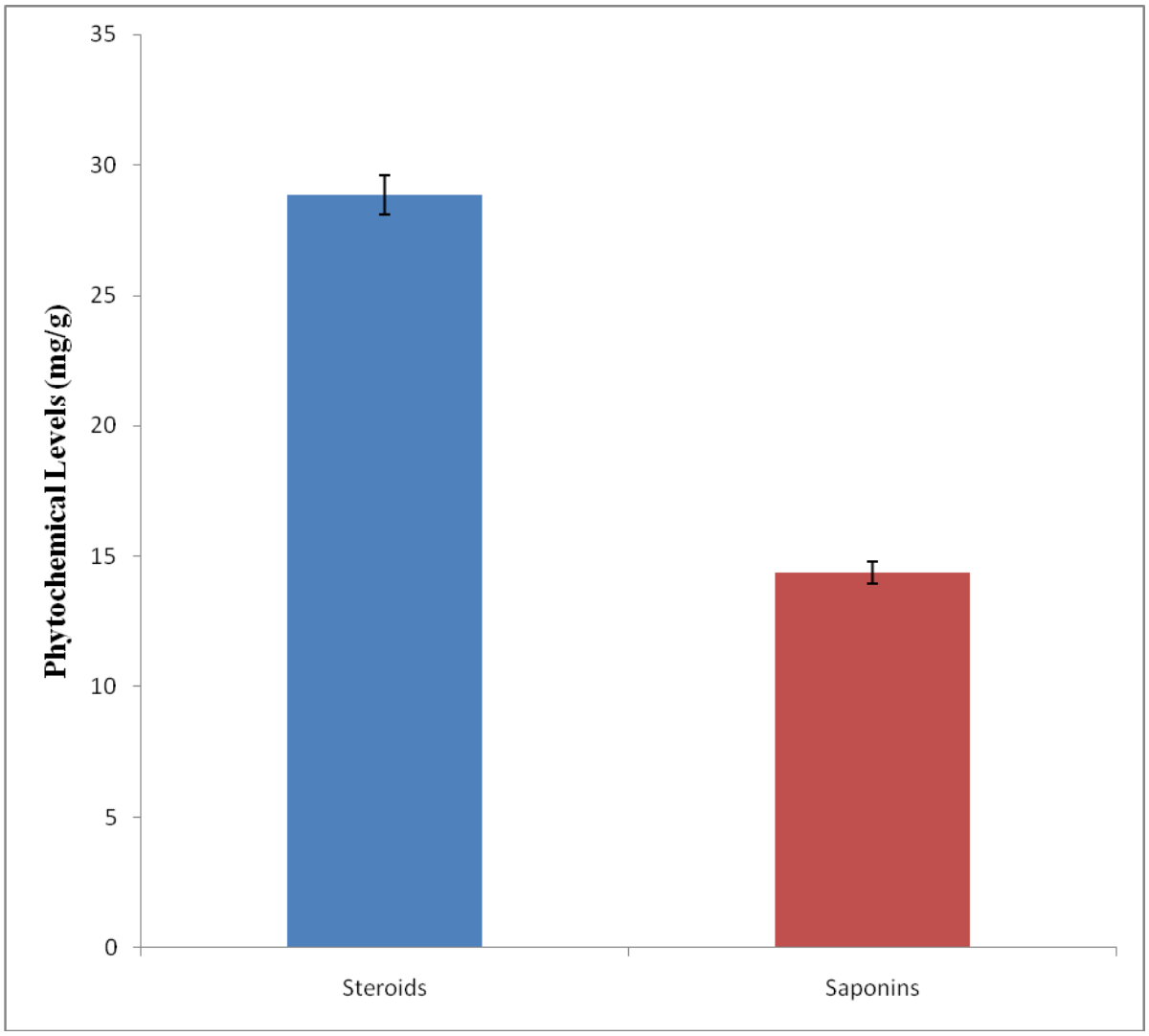

Figure 4: Phytochemical Compositions of *Juglas regia* seeds in mg/g

Table 1: Anti-nutrient Composition of *Juglas regia* Seeds

Anti-nutrients	Phytate (mg/100g)	Oxalate (%)	Haemaglutinin (HU/mg)	Trypsin Inhibitor (IUI/mg)
	3.31±0.06	1.30±0.51	0.98±0.08	0.75±0.06

DISCUSSION

Phytochemical analysis is very useful in the evaluation of active biological components of medicinal plants. The quantitative phytochemical analyses were carried out on the dried samples of *Juglas regia* seeds. Alkaloids, phenols, flavonoids, cyanogenic glycosides, tannins and saponin were revealed to be present as shown in Figures 3 and 4. The result of the study revealed that *Juglas regia* seed is rich in phytochemicals which correlate with the report of Aja *et al.*, (2010) [3] which reported that *Talinum triangulare* leaves are rich in phytochemicals. Phytochemical components are responsible for both pharmacological and toxic activities in plants. Some of these metabolites have been studied to be useful to both animal and plant itself. Though some of the constituents could be toxic to animals and man at higher concentration.

The result of the study also revealed high concentration of phenols in the seed of *Juglas regia.* This could be the reason behind their traditional uses as oral anesthetic/analgesic in products as reported by Pedro (2009) [14]. Phenols are strong antioxidants which prevent oxidative damage to biomolecules such as DNA, lipids and proteins which plays a role in chronic diseases such as cancer and cardiovascular diseases [15]. Phenols can be used in reduction of risk for infection in minor skin irritations and it also kills germs. Phenols can improve effectiveness at relieving itching and it can be added to lotion meant for the relief of insect bites and sunburn and other painful itching skin conditions [16].

The results obtained equally revealed that *Juglas regia* seed contained appreciable amount of tannins as shown in Figure 3. This indicates that *Juglas regia* seed have antimicrobial properties [17]. The result is in correlation with the report of Carson and Riley, (2003) [15], which reported that tannic acid, has anti-bacterial and astringent properties which have action upon mucous tissue such as tongue and inside the mouth. The indigestion of tannic acid cause constipation and can be used in the treatment of diarrhea. Tannins are polyphenols that are obtained from various parts of different plant belonging to multiple species. Tannins can also be effective in curbing hemorrhages and as well restrict bare swellings [18].

The obtained result revealed that flavonoids have an appreciable amount in *Juglas regia* seed as shown in Figure 3. It has been shown that flavonoids have wide range of biological and pharmacological activities such as: anti-allergic, anti-inflammatory, anti-oxidant and antimicrobial activities [19, 20]. Flavonoids have been implicated in the inhibition of pro-inflammatory activity of enzymes involved in free radical production, such as cyclooxygenase, lypoxygenase or inducible nitric oxide synthase and

modification of intracellular signaling pathway in cells [21, 22 and 23]. Flavonoids may help to provide protection against these diseases by contributing along with vitamins and enzymes to the total antioxidant defense system to human body [24].

The glycosides and terpenoids levels in *Juglas regia* seed as shown in Figure 3 revealed that the plant is not a good source of glycosides. This result supported the reports of Aja *et al.* (2015) [4] which reported high levels of phenols and other phyochemicals in *Dissotis rotundifolia* and *Cajanus cajan* leaves and seeds. Cyanogenic glycosides in plant-based food can improve glucose metabolism and can enhance the overall health of diabetic patients by improving the lipid metabolism, antioxidants status, also in improving capillary function and lowering of cholesterol level [25]. Glycosides contribute in the modification of tumourgensis and are also inhibiting carbohydrate mediated in tumor growth. Glycosides (Glycyrrhetinic acid) inhibit the enzyme 15-hydrox prostaglandin dehydrogenase which metabolizes the ether soluble prostaglandins (PGE_2) to active 15 keto –13 metabolite. This causes an increased level of prostaglandins in the digestive system. The prostaglandin inhibits gastric secretion and stimulates pancreatic and mucous secretion in the intestines and then may markedly increase the intestinal motility. Phosphate soluble prostaglandins (PGF alpha) stimulate the contraction of the uterus and 2 can cause abortion in a pregnant woman. Glycoside (Glacyrrhizin) is used in the treatment of chronic hepatitis and cirrhosis in Japan. It also inhibits the growth of several DNA and RNA viruses and inactivates herpes simplex virus particles [26].

Appreciable amount of alkaloids in *Juglas regia* seed obtained in the results showed that *Juglas regia* is a good source of alkaloids as shown in Figure 3. This result supported the reports of Aja *et al.* (2015) [5] which reported high levels of phenols and other phyochemicals in *Dissotis rotundifolia* and

Cajanus cajan leaves and seeds. Alkaloids have been implicated for inducing a stress response and apoptosis in human breast cancer cell [27]. Alkaloids which are nitrogen –containing naturally occurring compounds commonly found to have anti-microbial properties (Wasagu *et al.*, 2005). The alkaloids can be used as a central nervous system stimulant as well as powerful pain relievers [28].

Result of this study revealed high level of steroids in seed of *Juglas regia* as shown in Figure 2. High level of saponin was found in *Juglas regia* seed (Figure 3). This result supported the reports of Aja *et al.* (2015) [4] which reported high levels of phenols and other phyochemicals in *Dissotis rotundifolia* and *Cajanus cajan* leaves and seeds. Saponins bind to bile salt and cholesterol in the intestinal tract. Bile salts from small micelles with cholesterol facilitate its absorption. Saponin has been reported to cause reduction of blood cholesterol by preventing its reabsorption (Anwar *et al.*, 2007). Heffman (1997) reported that saponins inhibit sodium ion (Na^+) efflux by blockage of the entrance of Na^+ - Ca^{2+} anti-porter in cardiac muscle, which strengthens the contraction of heart muscle.

The result of anti-nutrients composition revealed low levels of anti-nutrients in seed of *Juglas regia* as shown in Table 1. This result supported the report of Aja *et al.* (2015) which reported low levels of these anti-nutrients in *Parkia biglobosa* fruits from Abakaliki, Ebonyi State, Nigeria. The anti-nutrients such as phytate, oxalate, trypsin inhibitor and haemagglutinin were observed to be very low in concentration in *Juglas regia* seed even below permissible toxicity levels [22]. This indicates probable lack of interference with the availability of mineral elements. This result is in agreement with the values obtained by Akubugwo *et al*, (2007) [5].

CONCLUSION

The present study confirmed that the *Juglas regia* seed contain appreciable amount of phytochemicals and low concentration of anti-nutrients even below the permissible toxic level.

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