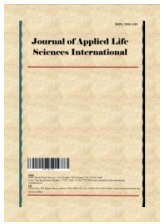

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Lipid Peroxidation Activity and Phytochemical Constituents of Extract of Groundnut Peels

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Authors' contributions

This work was carried out in collaboration among all authors. Author EFU designed the study, performed the statistical analysis and wrote the protocol. Author EOC wrote the first draft of the manuscript and managed the analyses of the study. Author OM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was designed to evaluate the phytochemical constituents and lipid peroxidation activities of extracts of two species of groundnut peels (Var-Hypogaea-Big and Var-vulgaris-Small).
Study Design: Twenty-four (24) male wistar albino rats where randomized into four (4) groups of six (6) rats each. Group A was the baseline, Groups B and C were given feed formulated with 1% groundnut peel extract of Var-hypogaea and Var-vulgaris respectively, group D is the control group fed with a standard feed.
Results: The phytochemical analysis was carried out using standard methods. Lipid peroxidation was determined by the thiobarbituric acid-reacting substances (TBARS) assay method. The phytochemical analysis revealed that the extract from the groundnut peels of Var-hypogaea-big contain alkaloids, tannins, saponnins, terpenoid, flavonoids phlobotannin, and glycosides. Also, the groundnut peel extracts of Var-vulgaris-small reveal the presence of alkaloids, tannins, saponnins, terpenoid, flavonoids phlobotannin and glycosides. There was a significant (p<0.05) increase in the malondialdehyde level of the groups that were fed with the feed compounded with both species of the groundnut compared with the baseline and normal control. However, the malondialdehyde level of the group fed with var-vulgaris was higher than that of Var-hypogaea.

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Conclusion: These results suggest that the groundnut peel extract of Var-hypogaea and Var-vulgaris is capable of inducing lipid peroxidation in living tissues. The groundnut peel does not only serve as a covering to the groundnut seed but may also protect the seeds from the effect of pests since it exhibits lipid peroxidative activity.

Keywords: Var-hypogaea-big; var-vulgaris-small; weight; lipid peroxidation; phytochemical.

1. INTRODUCTION

Currently, lipid peroxidation is often considered as the main molecular mechanisms involved in the oxidative damage to cell structures and in the toxicity process that lead to cell death. First, lipid peroxidation was studied for food scientists as a mechanism for the damage to alimentary oils and fats. Some researchers considered that lipid peroxidation was the consequence of toxic metabolites such as tetrachloromethane (CCl_4) that produced highly reactive species, disruption of the intracellular membranes and cellular damage [1]. Lipid peroxidation is a major cause of food deterioration and spoilage, affecting the colour, flavour, texture and nutritional value of food and food products [2].

The biological production of reactive oxygen species primarily superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) is capable of damaging molecules of biochemical classes including nucleic acids and amino acids. Exposure of reactive oxygen to proteins produces denaturation, loss of function, cross-linking, aggregation and fragmentation of connective tissues as collagen [3]. However, the most damaging effect is the induction of lipid peroxidation. The cell membrane which is composed of poly-unsaturated fatty acids is a primary target for reactive oxygen attack leading to cell membrane damage.

The lipid peroxidation of polyunsaturated fatty acids may be enzymatic and non-enzymatic. Enzymatic lipid peroxidation is catalyzed by the lipoxygenases family, a family of lipid peroxidation enzymes that oxygenates free and esterified PUFA generating as a consequence, peroxy radicals. Non enzymatic lipid peroxidation and formation of lipid peroxides are initiated by the presence of molecular oxygen and is facilitated by Fe^{2+} ions [4]. Oxidation processes are intrinsic to the management of energy in all living organisms and are, therefore, kept under strict control by various cellular mechanisms [5].

Oxidative breakdown of biological phospholipids occurs in most cellular membranes including mitochondria, microsomes, peroxisomes and plasma membrane. The toxicity of lipid peroxidation products in mammals generally involves neurotoxicity, hepatotoxicity and nephrotoxicity [6]. Oxidized lipids appear to have a signaling function in pathological situations and are proinflammatory agonists which contribute to neuronal death under conditions in which membrane lipid peroxidation occurs. For example, mitochondrial lipid cardiolipin makes up to 18% of the total phospholipids and 90% of the fatty acyl chains are unsaturated. Oxidation of cardiolipin may be one of the critical factors initiating apoptosis by liberating cytochrome c from the mitochondrial inner membrane and facilitating permeabilization of the outer membrane. The release of cytochrome c activates a proteolytic cascade that culminates in apoptotic cell death [7].

The importance of dietary antioxidant components for the prevention of chronic diseases and improvement of health has attracted huge research attention during the last few decades [8,9]. The aim of this work is to determine the phytoconstituents and the lipid peroxidation activity of the extracts of two species of groundnut peels (Var-hypogaea and var-vulgaris) in wistar albino rats.

2. MATERIALS AND METHODS

2.1 Sample Collection

The two varieties of Arachis hypogaea specie (Var-hypogaea and Var-vulgaris) of groundnut was purchased from Eke Awka Market.

2.2 Sample Extraction

The groundnut seeds were oven dried at 50°C after which the peels were separated from the seeds. The peels were grounded to powder. Exactly 150 g each of the two species of the groundnut were soaked in 1 litre of 70% absolute ethanol for 24 hrs for complete extraction to take place. It was then sieved and filtered using whatman no 1 filter paper. The filtrate was then

concentrated using water bath at 40°C. The extract was then stored separately in a universal bottle and kept in a refrigerator for use.

2.3 Preparation of Feeds

The feeds for the two test groups (Var-hypogaea and Var-vulgaris) were prepared using 1% of the extracts of groundnut peels of the Var-hypogaea and Var-Vulgaris respectively. Exactly 10 g of each of the extracts was weighed out, dissolved in distilled water and then mixed with 990 g of the Vital Feeds. It was allowed to dry under room temperature before using it to feed the animals.

2.4 Phytochemical Screening

Phytochemical tests were carried out on the ethanol extracts using standard phytochemical tests as described by Harbone [10], Sofowora [11], Trease and Evans [12]. The following phytochemicals were analysed: Terpenoids, Saponins, Tannins, Flavonoids, Phlobotannins, Alkaloids, Cardiac Glycosides and Phenolic Group.

2.4.1 Test for alkaloids

The powdered sample (5.0 g) was placed in the test tube and 20 ml of ethanol poured into the test tube. The mixture was allowed to boil for 2 mins in a water bath. It was cooled and filtered in 2 ml of the filtrate, 2 drops of Meyer's reagent was added (Solution of iodine and potassium iodine) which indicated redish brown colour. In another test tube, 2 ml of Hager reagent was added (which indicated yellow colour).

2.4.2 Test for terpenoids

The extract (5.0 ml) was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of inter face was formed to show positive result for the presence of terpenoids.

2.4.3 Test for cardiac glycosides

The extract (5.0 ml) was heated with 2 ml glacial acetic acid containing drop of ferric chloride solution with 1 ml of H_2SO_4. In Red groundnut brown ring on top indicate deoxysugar and a violet ring below the brown ring. In small java groundnut is the same with red.

2.4.4 Test for tannins

The dried powdered sample, 0.5 g was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for greenish black colour.

2.4.5 Test for saponins

Exactly 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. Frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion.

2.4.6 Test for flavonoids

Exactly 5 ml of dilute ammonia solution were added to a portion of aqueous filtrate of each plant extract followed by addition of concentration observed in each extract which indicate the presence of flavonoid, which later disappeared on standing.

2.4.7 Test for steroids

Exactly Two (2) ml of acetic anhydride was added with 2 ml of H_2SO_4 in the ethanol extract. The colour change from violet to blue or green indicates presence of steroids. There was no steroid in each sample.

2.4.8 Test for phlobotanins

Disposition of a red precipitate when an aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid which shows the presence of phlobotanins.

2.5 Lipid Peroxidation Determination

Lipid peroxidation was determined by the thiobarbituric acid-reacting substances (TBARS) Assay Method. Method by Buege and Aust [13].

Malondialdehyde (MDA) has been identified as the product of lipid peroxidation. Lipid peroxidation was therefore assayed by the measurement of MDA levels on the basis of MDA reacting with thiobarbituric acid at 532 nm. TBARS are products of the oxidative degradation of polyunsaturated fatty acids, particularly malondialdehyde. When membrane lipids are destroyed, MDA will be released. MDA is therefore determined in the serum.

2.5.1 Procedure

Exactly 0.4 ml of the serum was collected into the test tube, exactly 1.6 ml of 0.25 N HCl was added together with 0.5 ml of 15% trichloroacetic

acid and 0.5 ml of 0.375% thiobarbituric acid and then mixed thoroughly. The reaction mixture was then placed in 100 boiling water for 15 minutes, allowed to cool and centrifuged at 3000 revolution per minute for 10 minutes. The supernatant was collected and the optical density was recorded at 532 nm against reagent blank containing distilled water.

The lipid peroxide activity was calculated using the formular:

$$\text{Lipid peroxidation activity} = \frac{\text{Optical density}}{\text{Time}} \text{ x} \frac{\text{extinct coefficient}}{\text{amount of sample}}$$

Where the extinction coeff value is 1.56 x 10 raised to power of -5 (Unit) = m raised to -1/cm raised to -1. Optical Density = 532 nm. Time = 15 mins. Amount of sample = 15 mg.
The unit is expressed as umol/MDA/mg of serum.

2.6 Experimental Animals

Exactly 30 Wistar Albino Rats that are three months old weighing between 145 g and 150 g were purchased from the Animal House of Chris Farms in Awka, Anambra State and used for this work. They were acclimatized for two weeks prior to the commencement of experiment. They were kept at room temperature and maintained *ad libitum* on growers' mash. The animals were weighed and the initial weight taken before the commencement of the experiment.

2.7 Experimental Design

A total of 24 male wistar albino rats were randomized into four (4) groups of six (6) rats each.

Group A: This Group was used as the base line and did not receive any extract.
Group B: This group was given groundnut peel extract of Var-hypogaea for a period of two weeks.
Group C: This group was given groundnut peel extract of var- vulgaris for a period of two weeks.
Group D: This is the Control group. It was not feed with the extract.

2.8 Statistical Analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) software for windows version 21 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD. Statistical analysis of the results were performed by using ANOVA. The limit of significance was set at $p<0.05$. Data obtained were subjected to test of significance to determine if significance difference exists between the mean of the test and control.

3. RESULTS

3.1 Result of the Phytochemical Constituents of Groundnut Peels

Alkaloid, Tannin, Terpenoid and Phlobotannins were detected in the groundnut peels of Var-hypogaea in an abundant quantity while flavonoids and saponin were detected in trace amount. Also, the groundnut peels of Var-vulgaris contain Alkaloids, Phlobotannins, Cardiac glycosids in substantial quantities. Saponin was observed to be in a trace amount in the groundnut peels of var-vulgaris (Table 1).

Table 1. Results of the phytochemical analysis of the groundnut peels of Var-hypogaea and Var-vulgaris

Plant	Alkaloid	Tannin	Saponn	Steroid	Terpenoid	Flavonoid	Phlobatannin	Cardic glycoside
Red groundnut (Var-hypogaea)	+++	+++	+	-	+++	+	+++	++
Java groundnut (Var-vulgaris) Small type	+	++	+	-	++	++	+++	+++

Method of recording
Trace amount +; Abundant ++; Very abundant +++

3.2 Results of the Weight of the Animals for the Period of the Experiment

The mean ± SD for each of the groups were obtained in each group and represented in Table 2. Each of the groups showed increase in weight as the experiment progressed. However, the weight of the test groups significantly (p<0.05) increased more than the control group.

The group of rats that were fed with feed formulated with the groundnut peel of Var-hypogaea and Var-vulgaris ethanol extract showed 58.50% and 58.05% increase in weight respectively within the first week of the experiment which was higher than the percentage increase (51.58%) recorded in the control group within the same period (Table 3). In the second week of the experiment, a percentage increase in weight recorded for the group of rats fed with feed formulated with the groundnut peel of Var-hypogaea and Var-vulgaris ethanol extract are 67.14% and 69.43% respectively. These values were higher than the percentage increase (54.43%) recorded in the control group.

3.3 Results of the Lipid Peroxidation Activities of Groundnut Peels

The Lipid peroxidation of the test groups were observed to be higher than that of the control and baseline as can be clearly seen from Table 4. When the Lipid peroxidation activity of the two species of groundnut peels was compared it was found that Var-Vulgaris has more activity than var-hypogaea. Comparing the Test groups with the baseline and control showed a significant difference (p<0.05) in their lipid peroxidation activities.

4. DISCUSSION

The results of the phytochemical analysis of the groundnut peels of Var-hypogaea extract revealed the presence of alkaloids, tannins,

Table 2. The weight of the rats fed with var-hypogaea and var-vulgaris ethanol extract of groundnut peels for a period of two weeks

Groups	Initial weight (g)	Weight (g) after 1st Wk	Weight (g) after 2nd Wk
Group A (Baseline)	163.3 ±7.822		
Group B (Var-Hypogaea)	110.0 ± 2.733	128.7 ± 3.575*	147.7 ±3.801*
Group C (Var-Vulgaris)	99.17 ± 5.307	115.2 ±5.594*	137.7 ± 5.264*
Group D (Control)	117.3 ± 5.364	121.0 ± 5.556	127.7 ±5.702

** significant increase at p<0.05 compared to control group*

Table 3. The percentage change in weight of the rats fed with Var-hypogaea and Var-vulgaris ethanol extract of groundnut peels after first and second week

Groups	% change in weight after 1st week	% change in weight after 2nd week
Group A (Baseline)	-	-
Group B (Var-Hypogaea)	58.50	67.14*
Group C (Var-Vulgaris)	58.08	69.43*
Group D (Control)	51.58	54.43

** significant increase at p<0.05 compared to control group*

Table 4. Shows the lipid peroxidation activity of the groups

Groups	Lipid peroxidation x 10^{-10}
Group A (Baseline)	3.467 ± 1.640
Group B (Var-Hypogaea)	8.765 ± 1.085*
Group C (Var-Vulgaris)	10.505 ± 1.294*
Group D (Control)	3.238 ± 1.945

**significant (p<0.05) increase with respect to baseline and control group*

saponnins, terpenoid, flavonoids phlobotannin, and glycosides. Substantial quantities of alkaloids, tannins, terpenoids and phlobotannins were detected in the groundnut peels of Var-hypogaea extract. The groundnut peels extract of Var-vulgaris revealed the presence of alkaloids, tannins, terpenoid, phlobotannin and cardiac glycosides in abundance while saponins, and flavonoids were detected in moderate amount. It has been reported that the antioxidant properties of some plant products are effective mainly by scavenging of superoxide anion radicals [14]. The hydroxyl radical scavenging capacity of a drug is directly related to its antioxidant activity. The highly reactive hydroxyl radicals can lead to oxidative damage to DNA, lipids and proteins [15].

Alkaloids and terpenoids which were found in both extracts could be responsible for the peels antibacterial and antifungal properties which inhibit the invasion of micro-organisms in the groundnut seed. They could also be responsible for the reduced risk of fungal infection. Flavonoids was found in trace quantity in the groundnut peels of Var-vulgaris while it was found to be abundant in the groundnut peel extracts of Var-hypogaea. This flavonoid which has been known as an antioxidant protects the groundnut seed against the damaging effects of reactive oxygen species otherwise called free radicals such as singlet oxygen, superoxide, peroxyl radical and hydroxyl radicals and which results in oxidative stress leading to cellular damage.

The percentage rise in weight shows that groundnut peels could be used in the formulation of feeds for farm animals to boost growth and productivity. The induction of free radicals as evidenced by the higher levels of lipid peroxidation in the test groups over the control and baseline could mean that the groundnut peels contain pro-oxidants or principles that have high propensity to become oxidized therefore offering prorection on the lipids in the groundnut. This could be a protective mechanism of the peels on the groundnuts against attacks by microbes and pests. The free radicals being agents of oxidative stress and cellular damage (destruction of proteins, lipids and nucleic acids) might have made the groundnut peels unpalatable to microbes and pests thereby warding them off. The diseases associated with lipid peroxidation include Cancer, Diabetes and Hypertension [16].

Thus, when the peels or the extracts are consumed, they raise the levels of free radicals as shown by the higher level of lipid peroxidation in the tests over the control and baseline groups. However, in consideration of the fact that it can induce growth on animals, it is therefore recommended that it should be used in compounding of farm animal feed with oil seed cakes produced after mechanical extraction of oil. These types of cakes contain residual oils which contain antioxidants like vitamin E and beta-carotenes. These antioxidants will eliminate or lower the levels of free radicals in the farm animals bringing about healthier growth and development and also rendering the products from the farm animals like meat, milk among others safer for human consumption.

5. CONCLUSION

In conclusion, the groundnut peels of Var-hypogaea and Var-vulgaris have been found to contain important phytochemicals which could be responsible for the protection it confers to the groundnut seed. However, they may also contain pro-oxidants which become activated when removed from the seeds and can induce lipid peroxidation activity. The experiment also revealed that the extracts are capable of improving weight. The implication of this is that the groundnut peels need not be consumed. Also, lipid peroxidation is implicated in aging. People should ensure that the peels are properly removed before consuming the groundnut seeds.

ETHICAL APPROVAL

All authors on this note declare that “Principles of laboratory animal care” were followed. All experiments have been examined and approved by the ethics committee of Nnamdi Azikiwe University Awka, Nigeria in accordance with the requirements of the Animal Care and Use in Research, Education and Testing (ACURET).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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