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Investigation of some important phytochemicals, vitamins and mineral constituents of ethanol leaves extract of *Piper Nigrum*

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ABSTRACT

The pytochemicals, vitamins and mineral constituents of ethanol leaves extract of Piper nigrum was investigated in this study. The preliminary phytochemical screening showed that ethanol leaves extract of Piper nigrum contains alkaloids, flavonoids, steroids, tannins, saponins and terpenoids while cardiac glycosides were not dictated. The quantitative phytochemical analysis contains: alkaloids (17.27± 0.05 mg/g), steroids (6.06± 0.00 mg/g) , terpenoids (6.12 ± 0.03 mg/g), flavonoids (9.54± 0.05 mg/g), t annins (3.23 ± 0.04 mg/g), and saponins (3.68 ± 0.0 9 mg/g). The following amounts of vitamins and minerals found in the leaves extract; vitamin A (9.80 ± 0.02 mg/100g), vitamin C (12.42 ± 0.07 mg/100g), and vitamin E (7.25 ± 0.03 mg/100g); minerals: Iron (7.28 ± 0.025 mg/100g), ma gnesium (97.58± 0.03 mg/100g), and selenium (1.60 ± 0.025 m g/100). The results obtained revealed that the plant Piper nigrum is endowed with free radical scavenging molecules and it can be used as a potential source of natural antioxidants in oxidative stressed conditions.

Keywords: *Piper nigrum*, Phytochemicals, Vitamins, Minerals, Antioxidants.

INTRODUCTION

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Phytochemicals are naturally occurring and are believed to be effective in combacting or preventing disease due to their antioxidant effect [1][2]. The medicinal values of these plants lie in their component phytochemicals, which produce the definite physiological actions on human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds [3]. Some of these naturally occurring phytochemicals are anticarcinogenic and some others possess other beneficial properties, and are referred to as chemopreventers. One of the predominant mechanisms of their protective action is due to their antioxidant activity and the capacity to scavenge free radicals [4].

Black pepper belongs to the family *Piperaceae*. The members of the piperaceae family include the “pepper family” and they are the family of *Piperales* found in warm, tropical foliated and shady habitats.

Piper nigrum have recently attracted research interest because it possesses antioxidant properties against a variety of physiologically relevant free radicals [5]. Black pepper contains several powerful antioxidants and is thus one of the most important spices for preventing and curtailing oxidative stress. It exhibits immunomodulatory properties and is capable of boosting the number and efficiency of white cells, thereby assisting the body to mount a powerful defence against invading microbes and cancer cells [6]. The leaf of *Piper nigrum* has shown a potent stimulatory

effect on malanogenesis and this is due to the active constituent 3, 4-dimethyoxy-3, 4 desmethylene dioxycubebin and cubebin [7] These two constitutents have also been shown to have histamine release inhibitory activity [8].

In view of the reputed efficacies of this plant, the present study investigates qualitative and quantitative phytochemicals and antioxidant vitamin and mineral compositions of *Piper nigrum* leaves in order to ascertain its possible usefulness in oxidative stress conditions.

MATERIALS AND METHODS

Collection and identification of plant materials

The leaves of *Piper nigrum* were used for this study. The leaves of *Piper nigrum* were collected within University of Nigeria, Nsukka and were identified in the Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State. The fresh leaves were washed with clean water to remove dirt and sand, drained, and chopped. They were dried under shade for several days and then pulverized into fine powder.

Extraction of plant materials

A quantity, 500g of the powdered form of the leaves of *Piper nigrum* was macerated in 1.5 litres of ethanol for 48h. The solution was filtered with Whatman no. 4 filter paper and the filtrate was concentrated to a semi-solid residue in an oven at 60^{o}C.

Pytochemical screening

The preliminary phytochemical analysis of the leaves of *Piper nigrum* was carried out according to the method of [9] and [10] to identify its active constituents.

Quantitative phytochemical analysis

Alkaloid determination

The determination of alkaloid was as described by [9]. A portion (5 g) of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 2 h. This was filtered and the extract was concentrated on a water bath to one – quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract till a precipitate was formed. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavonoids determination

This was determined according to the method of [9] A quantity, 5 g of the sample was boiled in 50ml of 2M HCl solution for 30min under reflux. It was allowed to cool and then filtered through whatman No. 1 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with drop. The solution was filtered into a weighed crucible. The filtrate was heated to dryness in an oven at 60^{0} C. the dried crucible was weighed again and the difference in the weight gave the quantity of flavonoid present in the sample.

Steroids determination

This was determined by the method described by [4]. A known weight of each sample was dispersed in 100ml freshly distilled water and homogenized in a laboratory blender. The homogenate was filtered and the filtrate was eluted with normal ammonium hydroxide solution (pH 9). The eluate (2ml) was put in test tube and mixed with 2ml of chloroform. Ice-cold acetic anhydride (3ml) was added to the mixture in the flask and 2 drops of conc. H_2SO_4 were cautiously added. Standard sterol solution was prepared and treated as described above. The absorbances of standard and prepared sample were measured in a spectrophotometer at 420 nm.

Tannins Determination

The method of [11] was used for the determination of the tannin content. A quantity, (0.2g) of finely ground sample was weighed into a 50ml beaker. About 20ml of 50% ethanol was added and covered with paraffin and placed in a water bath at 77-80^{o}C for 1hr and stirred with a glass rod to prevent bumping. The extract was filtered using a double layer of Whatman No. 1 filter paper into a 50ml volumetric flask, and then 20ml distilled water, 2.5ml Folin-Denis reagent and 10ml of 17% Na_2CO_3 were added and mixed properly. The mixture was made up to mark with distilled water and allowed to stand for 20mins when a bluish-green colouration developed. Standard tannic acid solutions of range 0-1ppm were treated similarly as 1ml of sample above. The absorbances of the tannic acid standard solutions as well as samples were read, after colour development at 760nm.

Saponin Determination
The method used was that of [12]. The sample was ground and 20 g was put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at about 55^oC. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The concentrate was transferred into a 250 ml seperatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight; the saponin content was calculated.

Determination of vitamin contents
The vitamin assay was performed with the method of [13].

Vitamin A
A quantity, 1.0g of ground sample was macerated with 20ml of petroleum ether. This was decanted into a test tube and then evaporated to dryness. 0.2ml of chloroform-acetic anhydride (1:1, v/v) was added to the residue. Two (02) milliliters of TCA-chloroform in like (1:1 v/v) was added to the resulting solution and absorbance was measured at 620nm. Vitamin A standard was prepare in like manner and the absorbance taken at 620nm. The concentration of vitamin A in the sample was extrapolated from the standard curve.

Vitamin E
1g of the sample was macerated with 20ml of ethanol and then filtered. 0.2% ferric chloride in ethanol and 1ml of 0.5% α-α-dipyridine to 1ml of the filtrate. This was diluted to 5ml with distilled water. Absorbance was taken at 520nm. The standard solutions were prepared similarly and the concentration of vitamin E extrapolated from the standard curve.

Vitamin C
A quantity, 1g of sample was macerated with 20ml of 0.4% oxalic acid. This was filtered and to 1ml of filtrate was added 9ml of Indolephenol reagent. The standard solution of vitamin C was prepared similarly and the absorbances of the standard solution and the sample were read at 520nm. The concentration of vitamin C was extrapolated from the standard curve of vitamin C.

Determination of mineral contents
The method of [14] was used. Two grams of sample was weighed into a crucible and ashed into a furnance at 550^0C for 6 h. The ash is cooled, 6NHCl added and boiled for 10 mins, while covering the crucible with a watch glass. After boiling the sample, allow to cool and filter into 100ml volumetric flask. The crucible was washed with distilled water and the washings added to the ash filtrate. The ash filtrate was then made up to 100ml with distilled water. An aliquot of the filtrate was aspirated into the atomic absorption spectrophotometer and the absorbance values corresponding to different minerals recorded. The percentage of the elements in the samples was calculated from the absorbance values of the samples and standard solutions.

Determination of Magnesium
A precipitate formed in the previous test was removed it by filtration and made strongly alkaline with ammonia. A volume, $1cm^3$ of 10% sodium phosphate solution was added. The formation of a crystalline precipitate indicated the presence of magnesium.

Determination of Ferric Iron
Acidify several cubic centimeters of the solution with hydrochloric acid. Add about $1cm^3$ of 10% ammonium thiocyanate. The formation of a red colour indicated the presence of ferric iron. If negative, take a second portion and a few drops of hydrogen peroxide and worm. This will oxidize any ferrous iron (iron II) to ferric, which can be detected as above.

Statistical Analysis
Data were mean of 3 replicates ± SD. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 19. The data were expressed as mean ± standard deviation.

RESULTS

Qualitative phytochemical screening of ethanol extract of *Piper nigrum* leaves

Table 1 show that the ethanol extracts of *Piper nigrum* leaves contains alkaloids, flavonoids, saponins, steroids, terpenoids, and tannins. Glycosides were not detected in the extract.

Table 1: Qualitative phytochemical screening of ethanol leaves extract of *Piper nigrum*.

Phytochemicals	
Alkaloids	+++
Flavonoids	++
Steroids	++
Tannins	+
Saponins	+
Terpenoids	+
Cardiac Glycosides	-

Key: + slightly present, ++ moderately present , +++ Highly present , - absent

Qualitative phytochemical constituents of ethanol extract of *Piper nigrum* leaves.

Table 2: Shows the qualitative phytochemical contents of the extract. It shows that the ethanol extract of *Piper nigrum* leaves contains a high amount of alkaloids.

Table 2: Quantitative phytochemical constituents of ethanol extract of *Piper nigrum* leaves.

Pytochemical constituents	Mean ± SD
Alkaloid (mg/g)	17.27 ± 0.05
Steroids (mg/g)	6.06 ± 0.00
Terpenoids (mg/g)	6.12 ± 0.03
Flavonoids (mg/g)	9.54 ± 0.05
Tannins (mg/g)	3.23 ± 0.04
Saponins (mg/g)	3.68 ± 0.09

Vitamin contents of the ethanol extract the *Piper nigrum* leaves

Table 3: Shows the vitamin contents of the ethanol extract of the *Piper nigrum* leaves.

Table 3: Vitamin contents of the ethanol extract of *Piper nigrum* leaves.

Vitamin	Mean ± SD (mg/100g)
Vitamin A	9.80 ± 0.02
Vitamin C	12.42 ± 0.07
Vitamin E	7.25 ± 0.03

Mineral contents of the ethanol extract of *Piper nigrum* leaves.

Table 4 shows the mineral contents of the ethanol extract of *P. nigrum* leaves

Table 4: Mineral contents of the ethanol extract of *Piper nigrum* leaves.

Mineral	Mean ± SD (mg/100g)
Iron	7.28 ± 0.025
Magnesium	97.58 ± 0.03
Selenium	1.60 ± 0.025

DISCUSSION

The use of chemicals derived from plants to treat diseases has stood the test of time [15]. Plants with antioxidant activities have functioned as anti-radical chain breaker of free radical propagation. Among plants whose medicinal properties stood to test is the *Piper nigrum* plant.

The phytochemical constituents, vitamins and mineral contents of the leaves were investigated. The qualitative phytochemical analysis revealed the presence of terpenoids, alkaloids, flavonoids, tannins, steroids and saponins

which is in consonance with the reports of Shetty and Vijayalaxmi [16] while the absence of glycosides as reported is in contrast with the work of Arpita *et al*. [17] who reported the presence of glycosides in their work. Differences in these reports could be due to environmental factors, time of collection and handling.

The quantitative phytochemical analysis showed that the extract contains alkaloids (17.27 ± 0.05 mg/g), steroids (6.06 ± 0.00 mg/g), terpenoids (6.12 ± 0.03 mg/g), flavonoids (9.54± 0.05mg/g), and tannins (3.23± 0.09 mg/g). Some of these secondary metabolites have been known to possess antioxidant properties and hence can be useful for medicinal purposes. The quantitative phytochemical analysis in this study indicates higher concentrations of phytochemicals on the leaf extract compared to the stem as shown in tables 1 and 2 above. Phytochemicals are secondary plants metabolites responsible for many observed bioactivity of plant extracts. They are known to possess antioxidant, anti-inflammatory, antibacterial, immunomodulatory and antisickling antivities [18]. The presence of those metabolites no doubt is indication of the potential medicinal usefulness of the plant extracts. The result of this work showed that the *Piper nigrum* leaves extract possesses some biologically active compounds which could serve as potential sources of drugs. Saponins have been shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties [19]. Presence of tannins as shown in the result suggests the ability of this plant to play a major role as antidiarrhoec and antihaemorrhagic agent [20]. Flavonoids have been shown to have antibacterial, anti – inflammatory, anti allergic, antiviral antineoplastic activity [21]. Many of these alleged effects have been linked to their known functions as strong antioxidant, free radical scavenger and metal chellators [22]. Steroidal compounds are of importance in pharmaceuticals because of their relationship with compounds used as sex hormones [23]. The terpenoids have also been shown to decrease blood sugar level in animal studies [24].

The vitamin contents of the extract was found to be vitamin A (9.80 ± 0.02 mg/100g), vitamin C (12.42 ± 0.07 mg/100g), and vitamin E (7.25 ± 0.03 mg/100g). These show that the extract possess some antioxidant vitamins which can help in protecting the body against oxidative stress as reported by Nahak and Sahu [25]. As a result of the presence of ascorbic acid in the extract as shown in table 3, it can be used for the treatment of common cold and other diseases like prostate cancer [26]. Deficiency of ascorbic acid is associated with pains in the joint and defect in skeletal calcification, anaemia, manifestation of scurvy haemorrhage from mucous membrane of the mouth and gastrointestinal track [27]. The presence of vitamin E (table 3) may also be beneficial for coating our cell walls with a protective layer of lipids, diminishing the aging process in our tissues [28]. It may also help in reducing the negative effects of environmental pollutants and food-toxins in our bodies [29]. The quantity of vitamin A in the extract as shown in table 3 suggests it can affect some aspects of the adaptive immune response. Retinoic acid, a derivative of vitamin A, enhances cytotoxicity and T-cell proliferation [30]. Vitamin A deficiency correlates with decreased T_H2-cell responses [31].

The mineral contents of the leaves extract was found to be Iron (7.28 ± 0.025 mg/100g), Magnesium (97.58 ±0.03 mg/100g), and Selenium (1.60 ± 0.025 mg/100g). Magnesium is closely associated with calcium and phosphorus both in its distribution and its metabolism. Magnesium is an essential part of more than 300 enzymes in the body. These enzymes are body chemicals that help to regulate body functions, produce energy, make protein and contract muscles. Magnesium is found in all body tissues, but principally in the bones. Potassium and Magnesium are known to decrease blood pressure. Iron is an essential part of hemoglobin, which transports oxygen to the cells and makes use of the oxygen when it arrives. Iron is widely distributed in the body. It is found in the blood, liver, spleen and bone marrow. An iron deficiency can lead to anemia, along with fatigue, weakness, and increased risk for infections. Selenium works as an antioxidant with vitamin E to protect cells from damage that may lead to cancer, heart disease, and other health problems. Selenium appears to have a sparing action on vitamin E. The effects of a deficiency of selenium are not clear, but may involve the heart muscle or thyroid functioning [32].

CONCLUSION

The study reviews that the ethanol extract of *Piper nigrum* contains nutritive and medicinal values which may act as an adjunct in the management of oxidative stress conditions.

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