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Phytochemicals, Elemental Composition and Antioxidant Activity of *Pueraria phaseoloides*

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Abstract Phytochemicals, elemental composition and antioxidant activity of ethanolic extract of *Pueraria phaseoloides* leaves were investigated. The results showed the presence of six Phytochemicals: alkaloids, flavonoids, saponins, tannins, steroids and phenols. The micro and macro elements were determined using Flame Photometer and Atomic Absorption Spectrophotometer. The analyses revealed the presence of: calcium (2.44±0.087 mg/kg), Magnesium (0.58±0.035 mg/kg), Sodium (0.130±0.042 mg /kg), Potassium (1.60±0.02 mg/kg), Phosphorus (1.52±0.125 mg /kg) Manganese (0.032±0.010 mg/kg), Zinc (0.040±0.001mg/kg), Iron (0.071±0.039 mg/kg) and Copper (0.019±0.002 mg/kg) respectively. Antioxidant activity of the extract was determined using DPPH free radical scavenging method. The results showed a scavenging capacity comparable ($p > 0.05$) to that of ascorbic acid. Considering the presence of these important phytochemicals, essential elements and reasonable level of antioxidant activity, this plant has great potential for widespread use as cover crop for soil enrichment and as food/medicine for humans and livestock.

Keywords *Pueraria phaseoloides*, Phytochemicals, Elemental Composition, Antioxidant Activity

Introduction

Climate change and desertification in sub-Sahara Africa has contributed considerably to soil infertility leading to fodder/forage scarcity. This has had an adverse effect on food availability for livestock and humans by extension. In Nigeria, herders repeatedly encroach on farmlands in a bid to secure fodder for their starving livestock, resulting in the incessant farmers/herders clashes with substantial loss of lives and property. *Pueraria phaseoloides*, sometimes used in tropical and sub- tropical regions as cover crops and for soil enrichment has a great potential for use as fodder/forage source for livestock. *Pueraria spp.* is of the pea family and subfamily of papilonoideae. It has deep-rooted system, high accumulation of nitrogen and therefore high potential for soil fertility enrichment. Much of the highly priced inorganic phosphate fertilizers applied to soil is quickly immobilized and consequently unavailable to plants [1].

Hence rural farmers reportedly boost cropping systems with plant species that can mobilize P from Soil-P pools. This is not readily available to less P-efficient crops. Some leguminous cover crops are reported to possess a remarkable capacity to mobilize soil P through root exudates [2]. When these P-efficient plants mobilize P in excess of their own requirements, then the surplus may contribute to the nutrition of other less P-efficient crops, grown in rotation and/or intercropping. It is hypothesized in principle that *Pueraria phaseoloides*, a leguminous cover crop,

can mobilize more P from soil inorganic pools than natural re-growth of vegetation under a shortened fallow period, and this is then recycled through its litter and biomass to organic matter (OM). The OM, in turn, would be mineralized to release readily available forms of P to crops grown after the fallows. Hence, the spectroscopic determination of Phosphorus in the leaf extracts of the plant in question would lay credence to this hypothesis.

In addition to the potential applications of *Pueraria phaseoloides* listed, this plant is widely being used in ethno-medicinal purposes in Otuoke, a suburb in Bayelsa State and to large extent in the entire Southern region of Nigeria. The leaf extracts of *Pueraria phaseoloides* are used in the treatment of skin diseases and indigestion [3]. In north Africa, liquid extracts of *Pueraria phaseoloides* are used to effect blood clotting and to treat abdominal pains. Extracts of the leaves of this plant are also used as a disinfectant for old wounds and boils [5] and to treat patients with malaria and jaundice [4]. This investigation is thus aimed at assessing the phytochemicals, the micro and macro elements (nutritional potentials) as well as the antioxidant properties of *Pueraria phaseoloides* growing abundantly in the Otuoke and other parts of southern Nigeria, with a view to recommending its widespread use as a cover crop in fallow systems and as a viable source of forage/fodder in modern live-stock non open grazing techniques.

Materials and Methods

Collection and Preparation of Plant Materials

About 2 kg of fresh mature leaves of *Pueraria phaseoloides* were collected from an open field in Federal University Otuoke, Bayelsa State, Nigeria. The plant was identified by comparison with Herbarium Reference Material at Department of Botany, University of Benin, Edo State. The plant materials were thoroughly rinsed in distilled water, and then air dried for about two weeks. The dried leaves were ground into powder using an electric blender (Silver Crest SC 1589), and the resultant powdered sample was packed into sterile containers prior to extraction and analyses.

Figure 1: The Pueraria phaseoloides plant

Preparation of Plant Extract (Harborne, 1984)

250 g of air-dried leaves of *P. phaseoloides* were crushed and ethanolic extract prepared according to the method described by Harborne, [6]. About 30 g of the crushed sample was mixed with 120 cm^3 of the Analar grade ethanol in 250 cm^3 conical flasks. The flask containing the mixtures were carefully swirled for 30 mins after which the mixtures were filtered with Whatman filter paper No.1 (11cm) and concentrated to about 50% in a rotorvapor (Buchi 850V). The ethanolic extract were placed in air tight sample bottle and stored in the refrigerator.

Qualitative Determination of Phytochemicals

Test for Alkaloids

Wagner Test: Wagner's reagent was prepared by dissolving 1.27g of iodine and 20g of KI in 100ml of distilled water. Then, about 3 drops of Wagner's reagent was added to about 3ml of sample solution. The iodine reacts with iodide ion to give brownish solution, while the potassium ion K^+ bind covalently with nitrogen present in alkaloid

molecule producing reddish brown complex which is precipitate of potassium-alkaloid. The presence of reddish-brown precipitate confirms the presence of alkaloids in the sample.

Test for Flavonoids

2ml of extract in a test tube was mixed with few drops of ammonia solution. A yellow color appears and the solution becomes colorless when few drops of dilute H_2SO_4 was added. This indicates the presence of flavonoids.

Test for Steroids

2ml of the extract was added to 2ml of acetic anhydride and 1ml of dilute H_2SO_4. A change in the color of the solution from violet to green, confirms the presence of steroids.

Test for Saponins

2ml of water was added to 5ml of the sample filtrate. It was shaken vigorously and an observed persistent frothing indicates the presence of Saponins.

Test for Anthocyanins

2ml of the extract was measured and mixed with 2ml of dilute HCl. The solution changed to pale pink color which affirms the presence of anthocyanins.

Tests for Tannins

2ml of extract was measured and 2ml of ferric chloride was added. A resultant brownish green solution confirms the presence of tannins.

Test for Terpenoids (Salkowski Test).

About 2ml of chloroform and 3ml of conc. H_2SO_4 was added to 5ml of the extract. The formation of a reddish brown layer at the interface indicates the presence of terpenoids.

Test for Phenolic Compounds

2ml of the extracts was diluted in 5ml of distilled water. Thereafter two drops were added to the mixtures. When a dark green colored solution is obtained, it clearly indicates the presences of phenolic Compounds.

Elemental Analysis of the Sample

The levels of the following micro and micro elements: Calcium, Magnesium Zinc, Manganese, Iron, Copper and Phosphorus were determined using Buck Scientific 200 AAS [7] while Sodium and Potassium were determination by flame photometry.

Determination of the Extract's Antioxidant Activity via DPPH Radical Scavenging Method

Varying concentrations of ethanolic extract (0.1-0.5mg/ml) were mixed in a 1.5mM DPPH ethanolic solution. The solution was incubated at 37°C for 30mins and was measured calorimetrically at 518 nm. The positive control employed was Ascorbic acid under the same conditions. The lower absorbance of different concentrations of the extract of *Pueraria phaseoloides* at 518nm shows higher DPPH scavenging activities. The percentage DPPH radical scavenging activity of the extract was calculated from the decrease in absorbance at 518nm with reference to the negative control.

$$\% \text{ Inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100$$

A_0= Absorbance of negative control

A_1= Absorbance of samples.

Results and Discussion

Phytochemical Analysis

The result of the phytochemical screening of the leaves of *Pueraria phaseoloides* is as presented in Table 1 below:

Table 1: Result of Phytochemical Screening of *Pueraria phaseoloides*

Compounds	Ethanolic crude extract of *P. phaseoloides*
Alkaloids	+
Flavonoids	+
Steroids	+
Saponins	+
Anthocyanins	_
Tannins	+
Terpenoids	_
Phenolics	+

Key: Present = (+), Absent = (-)

Table 1 shows that flavonoids, alkaloids, steroids, saponins, tannins, and phenolic were present in ethanol extracts of the leaves of *Pueraria phaseoloides.* While anthocyanins and terpenoids were absent.

Flavonoids: protect the cells from oxidative damage that can lead to diseases conditions. It also helps regulate cellular activity and fight off free radicals that cause oxidative stress in the body. They are secondary metabolites and are responsible for photosynthesis [8]. Thus, the presence of flavonoids in ethanol extracts of the leaves of *Pueraria phaseoloides* is an indication of therapeutic importance of the plant.

Alkaloids: Alkaloids are generally bicyclic, tricyclic or tetracyclic derivatives of quinolizidine molecule. They are bitter and therefore considered as feed deterrent [9]. It protects the plants against insects, and also serve as regulatory growth factors. Therapeutically, they are known anesthetics and anti-inflammatory agents. Some examples are morphine, quinine, nicotine and ephedrine [10].

Saponins: decrease blood lipids, lower cancer risks and lower blood glucose response. Table 1. shows that saponins were present in ethanol extracts of the leaves of *Pueraria phaseoloides*. This result is similar to that obtained by Gafar and Itodo [1].

Steroids: decreases body fat percentage, increases muscle strength and power and enhanced recovery from workouts and injury. Table 1. shows that steroids were present in ethanolic extracts of the leaves of *Pueraria phaseoloides*.

Anthocyanins: Anthocyanins are water-soluble vacuolar pigments. They are pH dependent, thus, it may appear red, purple, blue, or even black depending on the pH. Fruits, vegetables, beans and grains that are generally rich in anthocyanins. Traditionally, anthocyanins-rich foods are used to prevent a number of disease conditions such as prostate enlargement, diabetic retinopathy, stroke, lower cholesterol and high blood pressure, Alzheimer's diseases etc [12]. There are claims that anthocyanins are rich in anti-oxidants and therefore boost the immune system and help to fight heart diseases, inflammation, viral infection and even cancer. Table 1 shows that the plant under investigation is rich in anthocyanins. Several vegetables consumed in Nigeria showed a marked anti-oxidant activity in linoleic acid model system ranging from 3.67% measured in A hybridus plant to 68.41% obtained in C. agentea Var Cristata [13].

In this study, anthocyanins were found to be absent. Similarly, Uzoekwe and Hamilton-Amachree [14] stated that leaf & bark of Njangsa plant showed presence of alkaloids, flavonoids, steroids, tannins but reported an absence of anthocyanins.

Tannins (Tannic acid): Tannins are complex polyphenolic compounds found mostly at the bark of some plants. It removes harmful microbes from the body, and fight against harmful bacteria, viruses and fungi by speeding up blood clotting. Tannins also have a healing effect on cut and wounds. Table 1. shows that tannins were present in ethanol extracts of the leaves of *Pueraria phaseoloides*. This result is similar to that obtained by Gafar and Itodo, [11].

Terpenoids: Have been found to be useful in the prevention and treatment of several diseases including cancer. Table 1. shows that terpenoids were present in ethanol extracts of the leaves of *Pueraria phaseoloides*.

Phenolics: Some pharmacological and biochemical properties namely: anti-inflammatory, antioxidant, antiviral, anticancer activities have long been associated with these compounds.

Table 1. shows that phenolics were present in ethanol extracts of the leaves of *Pueraria phaseoloides*. Similarly, Aune, [15], who investigated plants foods, reported presence of phenolic compounds in number of plants. The extracts of the leaves of *Pueraria phaseoloides* which are used for various traditional medicinal purposes (as a disinfectant, in treating wounds and boils, patients with malaria and cancer, skin diseases and diabetes) was found to contain the secondary metabolites listed: alkaloids, flavonoids, steroids, saponins, tannins and phenolics in this study. This justifies its successful application over the years in traditional medicine. These findings are in line with an earlier study by Aune [15], which reported that *Pueraria phaseoloides* has no known toxicity, rather its phytochemical constituents contribute immensely to its widespread medicinal.

Elemental Analysis

The results of elemental composition of the leaf extract of *Pueraria phaseoloides* is presented in table 2 below.

Table 2: Elemental Composition (mg/kg) of ethanolic leaf extract of *P. phaseoloides*

Parameters	Mean± SD
Macro Element	
Calcium	2.44±0.087
Magnesium	0.58±0.035
Sodium	0.13±0.042
Potassium	1.60±0.0.02
Phosphorus	1.52±0.125
Micro Element	
Manganese	0.032±0.010
Zinc	0.040±0.001
Iron	0.071±0.039
Copper	0.019±0.002

Key: SD (Standard Deviation)

Table 2 shows that *P. phaseoloides* leaves were found to have relatively higher quantities of calcium, potassium and phosphorous. Other elements such as copper, iron, zinc, manganese and sodium were present but relatively found in lower concentrations.

Macro Elements

Vegetables and spices are rich sources of minerals [16]. Macro element are the mineral elements of which the body needs a substantial amount and are more important than other minerals [17]. Macro elements present in *Pueraria phaseoloides* are:

Calcium: is needed to build and maintain strong bones, and 99% of the body's calcium is in the bones and teeth. The macro element Calcium has also been reported to contribute to the maintenance of healthy communication between the brain and other body parts. Table 2 revealed that value of calcium obtained in this study (2.44±087mg/kg) is comparable to that of leafy vegetables consumed in Nigeria which was reported by Otitoju et al; [18]. In that study the amount of calcium ranged between 1.76mg to 8.76mg.

Magnesium: helps to maintain normal nerve and muscle function, supports a healthy immune system, keeps the heart beat steady and helps bones to remain strong. Magnesium helps in the production of protein and energy. The value obtained for Magnesium is 0.58±0.087mg/kg a relatively close value (0.61 mg/kg) for green leafy vegetables consumed by the Edo people of southern Nigeria [19].

Sodium: the body uses sodium to maintain fluid level. The well-being of the kidney, liver and heart are largely dependent on a balance of fluid and sodium. It also regulates blood pressure (Brazier, 2017). The concentration sodium obtained in this study is 0.13±0.042mg/kg, which is much lower than 2.41mg/kg reported by Haliwell, [20].

Potassium: This is a very important mineral in the body. Fluid balance and muscle contractions are largely regulated by it. Moreover, a potassium-rich diet is also known to aid in the reduction of blood pressure, water retention and protection against stroke. In this study, the concentration of potassium was found to be 1.60±0.02mg/kg, which is comparable to 1.63mg/kg reported by Mensa, [19] for vegetables consumed in Edo State.

Phosphorus: The body needs Phosphorus to make protein which is essential for proper growth, maintenance of cells and tissues. Table 2 above showed the value for phosphorus to be 1.52±0.125mg/kg. Similarly, Uzoekwe and Hamilton-Amachree [14] also reported a comparative value for the methanol leaf extract of Njasan plant. Additionally, the phosphorus content reported in this study also justifies the use of the ''*Pueraria* fallow system'' in an earlier study and the positive effect recorded on soil phosphorus fractions and crop Phosphorus uptake [21].

Micro Elements

Micro Element are elements which are required by plants in only very small amount. Micro elements obtained from the extraction *of Pueraria phaseoloides* are;

Manganese: Contributes to bone formation, blood clotting, and reduction of inflammation. The value for manganese in this study is 0.032±0.010 and it is in line with to the values reported by Tubek, [22] (manganese: 0.05mg/kg).

Zinc: The presence of this micro element in the body helps the immune system and metabolism function. Zinc is also aids the wound healing process. The value measured for zinc is 0.040±0.001 and this is in line with values reported by Aune, [15] in an earlier study (zinc:0 .06mg/kg).

Iron: the body uses iron to make hemoglobin, a protein in the red blood cells that carries oxygen from the lungs to all parts of the body. The value obtained in this work is 0.071±0.0101mg/kg.

Copper: is an essential nutrient for the body. It helps to maintain healthy bones, blood vessels, nerves and immune function. The value found in this study (0.019±0.002mg/kg), is close to the result (copper- 0.03mg/kg) obtained by Tubek [22]. They are important in the formation of bones and teeth as a cofactor or enzymes. The minerals present in low concentrations are iron, zinc, copper and sodium. They perform various important functions in living things like the formation of hemoglobin, growth and sexual maturation [19].

Antioxidant Activity

The results of anti-oxidant activity of *Pueraria phaseoloides* using ascorbic acid as the control is presented in Table 3 below. For various concentrations (0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml) of the plant extract, the absorbance measured at 518nm were 0.374, 0.438, 0.491, 0.442 and 0.420 respectively. The percentage inhibition for the above stated sample concentrations were 75.41±0.129, 69.88±0.089, 67.71±0.111, 70.94±0.113 and 72.38±0.103 respectively. In humans many diseases are associated with the presence of free radicals. Antioxidants such as ascorbic acid can scavenge free radicals and thus minimize the harm in the body. A free radical is an atom or molecule with unpaired electron. This makes the molecule to be reactive and aims to achieve stability through electron pairing with biological molecules such as proteins, lipids and DNA in a normal cell and thus causing cell damage [23]. In this study, using DPPH method, the scavenging activity of *Pueraria phaseoloides* with inhibition ranging from 67.71%-75.41%, is comparable ($p>0.05$) to 76.85%-84.8. % inhibition recorded for ascorbic acid. There are evidences that some dangerous pathophysiological processes such as diabetes, cardiovascular diseases and even cancer are associated with accumulation of free radicals [23]. Therefore, ethanolic extract of *Pueraria phaseoloides* leaf is a potential source of natural anti-oxidants and can be used as source of pharmaceutical products. The result of anti-oxidant activity of the leaves of *P. Phaseoloides* are shown Table 3 below.

Table 3: Anti-Oxidant Activity of Ethanolic Extract of *Pueraria Phaseoloides*

Sample	Conc. (mg/ml)	ABS @ 518nm	% inhibition
Ethanolic	0.1	0.374	75.41±0.129
Crude	0.2	0.438	69.88±0.089
Extract of	0.3	0.491	67.71±0.111
P.P.	0.4	0.442	70.94±0.113
	0.5	0.420	72.38±0.103
Ascorbic	0.1	0.290	80.93±0.036
Acid	0.2	0.318	79.09±0.016
	0.3	0.352	76.85±0.040
	0.4	0.289	76.85±0.116
	0.5	0.231	84.81±0.020

% Inhibition = $\frac{(A_0 - A_1)}{A_0} \times 100$

A_0= Absorbance of negative control

A_1= Absorbance of samples

Ascorbic acid strengthens the guard against viruses and germs as, it is a multi-purpose nutrient than the Ascorbic acid extracted from the leaves of *Pueraria phaseoloides*. And the results obtained from data in Table 3. Also shows that the leaves absorbance of different concentrations of the extract of the *P. Phaseoloides* shows higher DPPH scavenging activities. The results obtained in Table 3 is similar to the results obtained by Sofowora [24].

Conclusion

This study reports the presence of important bioactive molecules such as alkaloids, flavonoids, saponins, tannins, steroids and phenols in the ethanolic extracts of *Pueraria phaseoloides.* The presence of these secondary metabolites is an indication of therapeutic importance of the plant in the traditional medicine. The extract was found to contain relatively high values of micro and macro elements with a potential to contributing positively to the nutritional requirements of humans and livestock. Furthermore, the anti-oxidant analysis showed a relatively high scavenging capacity comparable to that of ascorbic acid.

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