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PHYTOCHEMICAL SCREENING AND PHARMACOGNOSTIC PROPERTIES OF *PEURARIA PHASEOLOIDES* LEAVES (ROXB) BENTH. (FABACEAE)

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ABSTRACT: *Pueraria phaseoloides is an herbaceous twining vine and much branched. It belongs to the family fabaceae. Evaluations of the leaves were carried out to determine the phytochemical, macroscopic, microscopic, chemomicroscopic, and physicochemical analysis using standard methods. The phytochemicals detected in the ethanol leaf extract were alkaloids, saponins, tannins, flavonoids, triterpenoids, cardiac glycoside, carbohydrate and reducing sugar. The macroscopic examination revealed fresh leaves are dark green, glabrous, cuneate, alternate and pubescent. Microscopic examination indicated the presence of calcium oxalate crystals, starch grains, xylem, phloem, trichomes, epidermal cells, collenchyma cells, amphistomatic stomata and reticulate vessels. Chemomicroscopic characters present are starch grains, lignified tissues, calcium oxalate crystals, cystolith, tannin, and cellulose. The physicochemical evaluation indicated 5.8% moisture content, 1.3% total ash value, 0.5% acid insoluble ash value, 1.0% water soluble ash value, 8.0% water soluble extractive value and 6.0% alcohol soluble extractive value. This study is useful in pharmacognostic standardization of this plant.*
KEYWORDS: Pueraria phaseoloides, phytochemical, pharmacognostic, microscopic, chemomicroscopic, physicochemical.

INTRODUCTION

Most plants are often made up of some important phytochemicals as well as various chemical constituents but these plants can never be recognised if they are not evaluated and tested in the laboratory using suitable chemical tests and physiochemical parameters. *Pueraria phaseoloides* commonly known as tropical kudzu is an herbaceous twining vine and much branched, attaining 15 meters in length (Acevedo-Rodríguez Pedro, 2005). *Pueraria phaseoloides* belongs to the family Fabaceae. Fabaceae is one of the largest families of flowering plants. The family includes about 745 genera and 19500 species that can be found throughout the world, growing in many different environments and climates. Tropical kudzu is especially important as a component of grazed and ungrazed cover crop mixtures in rubber, oil-palm and coconut plantations in South-East Asia, Africa and tropical America. In East Africa, it is grown as a cover crop in plantations of sisal (*Agave sisalana* Perrine). In South-East Asia, tropical America and Australia it is also used as a pasture legume. Its ability to smother weeds makes it a useful pioneer legume often grown in combination with other more permanent species (Soria *et al*., 2002). Pharmacognosy is concerned with description and

identification of plants including their history, commerce, collection, preparation and storage and is of fundamental importance for pharmacopoeial identification and quality control purpose. Pharmacognostic study of plant drugs involves the sources of the drug, morphological character, histological character, chemical constituents and their qualitative test, various physic). Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. Hence, Standardization is an integral part of establishing the correct identity of the crude drug before any drug can be included in the pharmacopoeia (Kunle *et al*., 2012). Finally, revealing of the presence of phytochemicals will also help to open up other areas of use of this plant as well as research.

LITERATURE/ THEORETICAL UNDERPINNING

Pueraria phaseoloides is an herbaceous twining vine, much branched, attaining 15 m in length. Stems are cylindrical; leaves alternate, trifoliolate; leaflets 3-12(14) × 2.9-8.7(13) cm, chartaceous, ovate or rhombic, the lateral ones asymmetrical, the apex acute, the base cuneate on the central leaflet, rounded-obtuse on the lateral ones, the margins entire; upper surface dark green, dull, pubescent, especially on the veins; lower surface pale green, strigose, with prominent venation**.**

Figure 1: Pictorial diagram of *Pueraria phaseoloides*

Scientific Classification
Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Fabales

Family: Fabaceae
Subfamily: Faboideae
Genus: Pueraria
Species: *Pueraria phaseoloides*

Common Names

Tropical kudzu (*Pueraria phaseoloides*) is known by different names in different locations across the globe (Maesen, 1994):

- Tropical kudzu, puero (Australia) (En). Kudzu tropical, puero (Fr)
- Indonesia: kacangruji, krandang (Javanese), fuobanga (Ternate)
- Malaysia: kacanghijauhutan, tampongurat
- Philippines: singkamasaso (Tagalog), bahay (Bikol), vaay (Ivatan).
- Burma (Myanmar): peying pin
- Laos: pièd, s'üakpièd
- Thailand: thua-sianpa (central)
- Vietnam: dâu ma, dâudai, dâurùng.

In comparison with other legume species tropical kudzu has been ranked highly as a shade-tolerant plant. Under a regime of more than 50% shade, tropical kudzu is still comparatively productive, but in mixtures it gives way to other species. Seedling growth of tropical kudzu is only moderately vigorous during the first 3-4 months. Seedling vigour is superior to other cover crops such as centro (*Centrosema pubescens* Benth.) and calopo (*Calopogonium mucunoides* Desv.).Tropical kudzu is especially important as a component of grazed and ungrazed cover crop mixtures in rubber, oil-palm and coconut plantations in South-East Asia, Africa and tropical America. Its ability to smother weeds makes it a useful pioneer legume often grown in combination with other more permanent species. As widely used material in oriental herbal medicines, Pueraria plants have been extensively studied for their chemical content. It appears that the plants are rich sources of polyphenols and polyphenolic glycosides (Kinjo *et al.,* 1987). Isoflavones and their glycosides are principal bioactive constituents. The wide scope of the nature of secondary metabolites in Pueraria plants is reflected by the presence of complex triterpene saponins as well as of various volatile flavour components. Therefore, a review on the isolation, identification, and analysis of pertinent constituents should be of interest to researchers studying the properties and bioactivities of Pueraria plants (Kinjo *et al.*, 1987).

Pharmacognostic standardization of crude drugs is a series of laboratory experiment which reveals and assembles a set of inherent peculiar characteristics such as constant parameter, definite, qualitative and quantitative values or specific and unique features on the basis of which similar herbal medicine claim to be the same, can be compared for the purpose of authenticity, efficacy, genuiness, purity, reproducibility and overall quality assurance. The broad use of herbal drugs in conventional medicines, standardization becomes an important measure for ensuring quality, purity and authenticity of the crude drugs. First step in this context is authentification of plant species which can be done by morphological and anatomical analysis or pharmacognostic analysis. It is one of the simplest and cheapest methods for establishing the correct identification of the source materials (Nirmal *et al.,* 2012; Kumar *et al*., 2012a).

The present study reports the detailed phytochemical screening and pharmacognostic parameters of *Peuraria phaseoloides* leaves (roxb) benth. (fabaceae). These parameters will be

useful in complete authentification and standardization of the crude extract, which can guarantee the quality and purity of the drug and maintain its therapeutic efficacy.

MATERIALS AND METHODS

Plant materials

Pueraria phaseoloides leaves were collected in July 2019 from Nando in Oyi Local Government Area of Anambra State, Eastern Nigeria. The plant was identified and authenticated by a taxonomist in the Pharmacognosy and Traditional Medicine Department of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Nigeria. Herbarium specimen deposited with herbarium number PCG 474/A/030.

Equipments

Microscope (Finlab, Nigeria), Hot air oven (Genlab, UK), Electronic weighing balance (Ohaus Corp, USA), water bath (Serological, England), beakers (Pyrex;10, 50, 100 and 1000 ml), measuring
cylinders, hand grinding machine (Ohaus Corp, USA), syringes and needles (1, 2 and 10 ml capacity), refrigerator (Thermocool, England), cotton wool (Pyrex. Nig).

Reagents and chemicals

Concentrated sulphuric acid (Versha Chemicals, Belgium), naphthol solution in ethanol (Molisch reagents) (Nalco Chemicals, USA), Ammonium solution (Shakti Chemicals, India), Aluminum
chloride (Neel Chemicals, India), Fehling solution A and B (Alpha Chemika, India), Hager's reagent (saturated solution of picric acid) (Alpha Chemika, India), Wagner's reagent (iodine and potassium iodide) (Alpha Chemika, India).

Preparation of plant material

The leaves were dipped in water to remove dust and unwanted particle. They were air dried at room temperature for two weeks. The dried leaves were pulverized with an analytical milling machine and sieved to control the particle size. Then it was stored in an airtight container for further analysis (Bruce *et al*., 2016).

Extraction

A quantity (600 g) of the powdered leaves was extracted using ethanol (2500 ml) with occasional stirring for 72 h by cold maceration. The mixture was sieved using porcelain cloth and filtered with a filter paper. The filtrate was dried *in vacuo* at 40°C. The extract was stored in a refrigerator for use (Onyegbule *et al*., 2019).

Phytochemical analysis

Qualitative phytochemical analysis

The plant crude extracts were tested for the presence Reducing sugar, Hydrogen cyanide, Soluble carbohydrate, Tannins, Alkaloids, Steroids, Terpenoids, Phenol, Flavonoids, Saponins and Glycosides using standard methods (Evans, 2002).

Quantitative phytochemical analysis

The coarse powder of the plant material were tested to determine the quantity of Reducing sugar, Hydrogen cyanide, Soluble carbohydrate, Tannins, Alkaloids, Steroids, Terpenoids, Phenol, Flavonoids, Saponins and Glycosides present (Edeoga and Gomina, 2000).

Pharmacognostic studies

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Macroscopic examination

Macroscopic studies were carried out by using organoleptic evaluation method. The shape, size, colour, odour, taste, base, texture, margin, apex of the leaf of plant were observed (Evans, 2002).

Microscopic examination

Microscopic studies were carried out by preparing thin sections of leaf. The thin sections were further washed with water, staining was done by clearing in chloral hydrate solution then heat fixed and allowed to cool, then mounted using glycerine. The specimen was gently covered with a cover slip and placed on the stage of the microscope for observation (10x, 40x) (Khandelwal, 2008).

Quantitative leaf microscopic investigation

Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, and vein – islet number and vein let termination number were carried out on epidermal strips. Foliar epidermis of the adaxial (upper surface) and abaxial (lower surface) surfaces of the leaves were prepared by clearing method. The leaf samples were cleared by soakingin commercial bleach "Hypo" containing 3.5% sodium hypochlorite for 18 hrs. Then, epidermal strips of the leaf samples were scrapped gently with the aid of a pair of forceps and placed on a clean slide, and then stained with Safranin solution and covered with a cover slip (Nwafor *et al.,* 2019). The slides were viewed under a light phase contrast microscope.

Chemomicroscopic examination

Examination of the powder for lignin, starch, mucilage, calcium oxalate crystals, cellulose, fatty oil and protein were carried out using standard techniques. Chemomicroscopy was conducted on the powders to determine the presence of starch, calcium oxalate crystals, Gums and Mucilage, Cellulose and lignified vessels. A judicious quantity of the sample was dropped on a glass slide. One drop of chloral hydrate was dropped and passed over a bunsen burner repeatedly until bubbles formed (Evans, 2002). This signified the successful clearing of the tissues.

Physicochemical analysis

The parameters which were studied are moisture content, ash values and extractive values (Eleazu and Eleazu, 2012; AOAC, 2005; Tatiya *et al*., 2012).

RESULTS

Table 1: Qualitative Phytochemical Analysis of *P. phaseoloides* leaf extract

Phytochemical	**Crude extract**
Alkaloids	+
Saponins	++
Tannins	++
Flavonoids	++
Steroids	-
Terpenoids	+
Cardiac Glycosides	++
Carbohydrates	+
Proteins	-
Reducing sugars	++

+ =Moderately present, ++=Abundantly present, - =Absent

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Table 2: Quantitative Phytochemicals Analysis of *P. phaseoloides* leaf extract

Phytoconstituents	**Crude extract (%)**
Alkaloids	5.1
Saponins	8.7
Tannins	9.0
Flavonoids	8.8
Steroids	0
Terpenoids	5.4
Cardiac Glycosides	8.6
Carbohydrates	4
Proteins	0
Reducing sugars	8.2

Table 3: Macroscopic Features of *Pueraria phaseoloides*

FEATURES	**OBSERVATION**
Colour	Dark green
Taste	Bitter
Margin	Entire
Apex	Acute
Venation	Pinnate
Texture	Hairy
Surface	Glabrous
Base	Cuneate
Leaf Arrangement	Alternate
Size	3-12 x 2.9-8.7cm
Petiole	Pubescent

Result for Fresh Leaf Microscopy

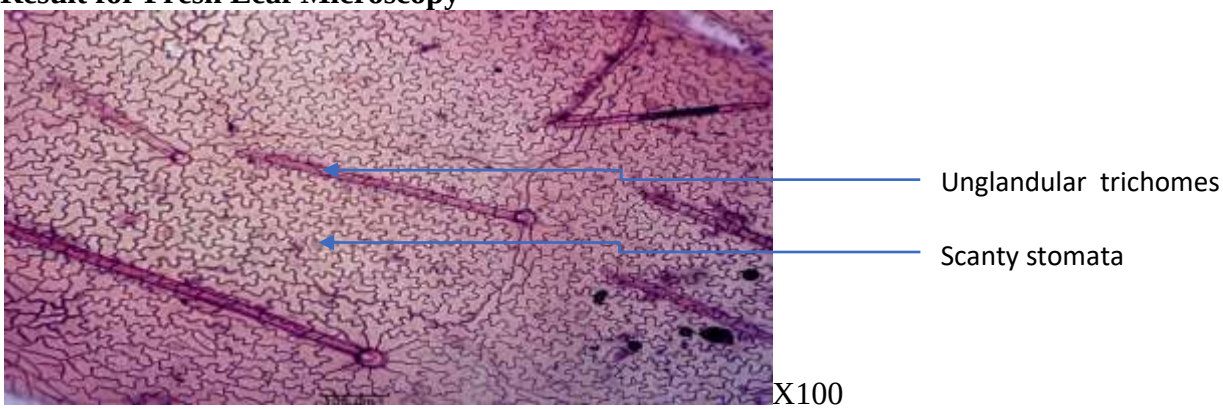

Figure 2: Adaxial surface of *Pueraria phaseoloides* showing unglandular trichomes and scanty stomata

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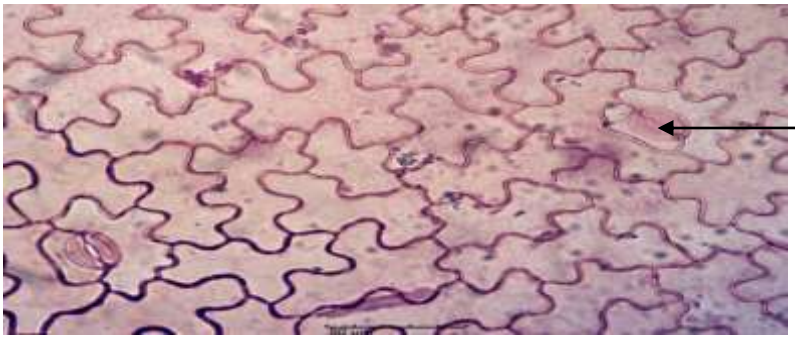

Figure 3: Adaxial surface of *Pueraria phaseoloides* showing amphiparacytic stomata

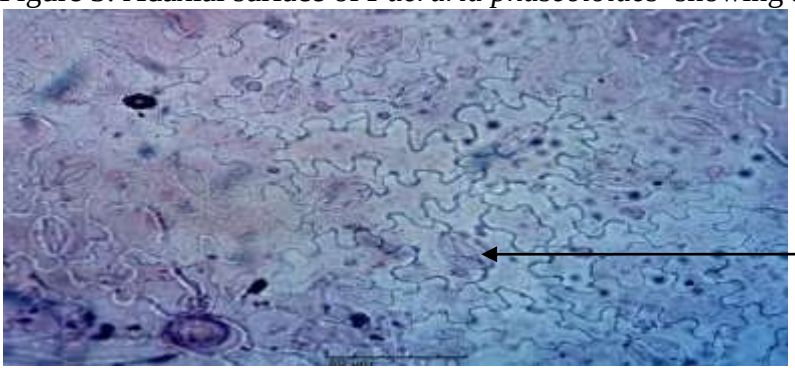

Figure 4: Abaxial surface of *Pueraria phaseoloides* showing anomocytic stomata

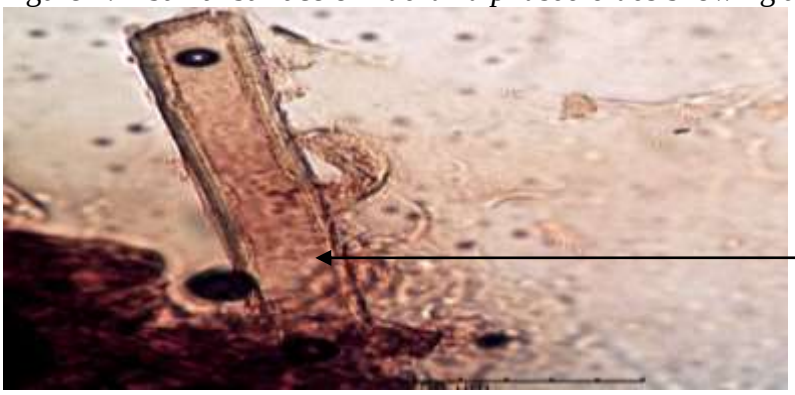

Figure 5: Chemomicrograph of *Pueraria phaseoloides* Leaf Powder, Showing Fragmented Trichome

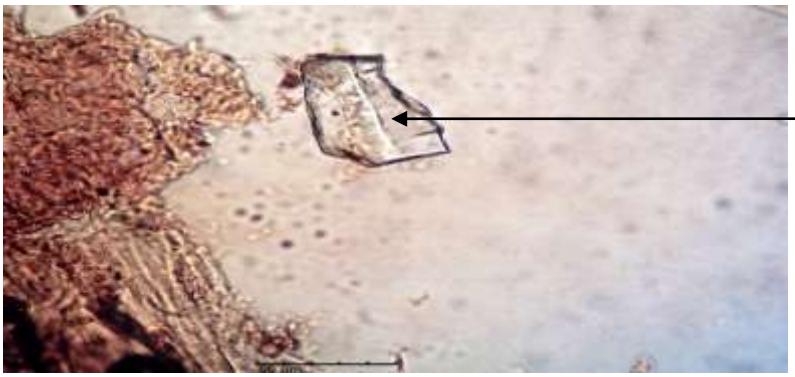

Figure 6: Chemomicrograph of *Pueraria phaseoloides* Leaf Powder, Showing Crystal of Calcium Oxalate

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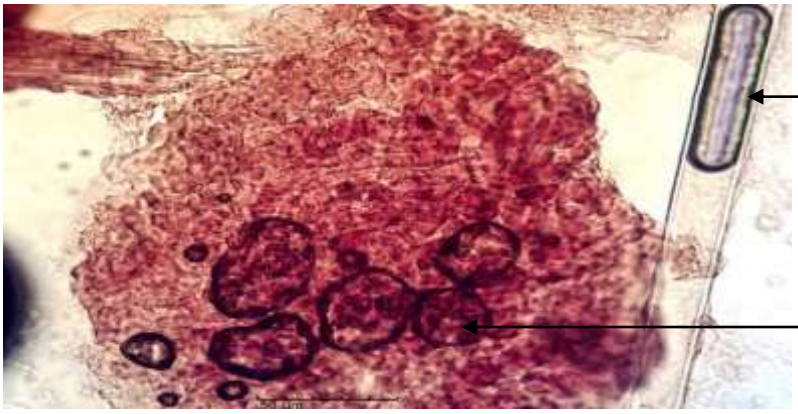

Figure 7: Chemomicrograph of *Pueraria phaseoloides* Leaf Powder, Showing Fragmented Trichome with Cystoliths and Crystals of Calcium Oxalate

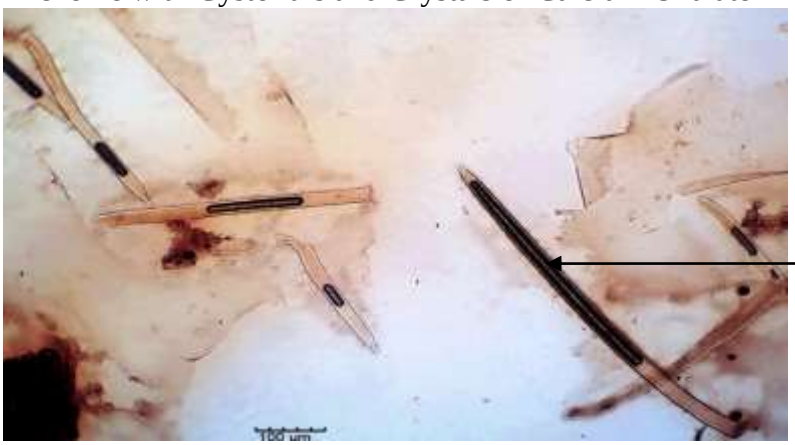

Figure 8: Chemomicrograph of *Pueraria phaseoloides* Leaf Powder, Showing Fragments of Unicellular Non-glandular Trichomes

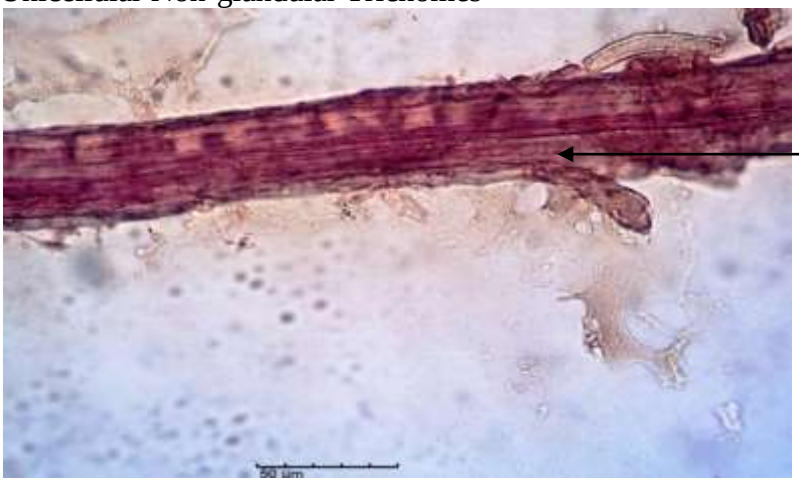

Figure 9: Chemomicrograph of *Pueraria phaseoloides* Powder, Showing Lignified Vessel Elements and Fibres

Table 4: Quantitative leaf microscopy of *Pueraria phaseoloides*

Parameter	**Range (mm^{-2})**
Palisade ratio	6.25 ± 0.05
Stomata number	25.48 ± 0.22

Stomach index	11.76 ± 0.15
Vein islet number	7.84 ± 0.05
Veinlet termination number	6.63 ± 0.25

Table 5: Chemomicroscopy of *Pueraria phaseoloides* Leaf

Test reagent	Observation	Inference
Sample + Phloroglucinol + Conc. HCl	Red colour observed	Lignin present
Sample + Iodine	Blue colour observed	Starch present
Sample + Ruthenium red	Red colour not observed	Mucilage absent
Sample + Hydrochloric acid	Bright crystals dissolved	Calcium oxalates Crystal present
Sample + Chlor-Zinc Iodine or N/50 iodine + 66% H2SO4	Blue colour observed	Cellulose present
Sample + Sudan IV reagent	Pink colour not observed	Fatty oils absent
Sample + 1% Picric acid and Million's reagent	Red colour not observed	Protein absent

Table 6: Physicochemical Analysis of *P. phaseoloides* Leaves

Parameter	Composition (% w/w)
Moisture content	5.8
Total Ash Value	1.3
Acid Insoluble Ash Value	0.5
Water Soluble Ash Value	1.0
Alcohol Soluble Extractive Value	8.0
Water Soluble Extractive Value	6.0

DISCUSSION

Several plant-based foods and herbs contain powerful phytochemical substances that can improve the quality of our health. Phytochemicals protect us against many diet related diseases. Results of the phytochemical screening of *Pueraria phaseoloides* ethanol leaf extract showed the absence of steroids and proteins while alkaloids, saponins, tannins, flavonoids, terpenoids, cardiac glycosides, carbohydrates, and reducing sugars were present. These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals. Plants used in the treatment of diseases are said to contain bioactive principles with biological activity some of which are responsible for the characteristic odour, pungencies and color of plant, while others give the particular plant its culinary, medicinal or poisonous virtue (Usunobun *et al*., 2015). The qualitative phytochemical screening of *Pueraria phaseoloides* was similar to what was obtained in Bruce *et al*., 2019, Onyegbule *et al*., 2019, Falodun *et al*., 2011, and Ihekwereme *et al*., 2020.

It has been reported that flavonoids and phenolics are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activity (Pourmorad *et al*., 2006; Ugwu *et*

al., 2013) and they might induce mechanism that affect cancer cells and inhibit tumor invasion (Rafat *et al*., 2008). These activities could be attributed to their ability to neutralize and quench free radicals (Ugwu *et al*., 2013; Omale and Okafor, 2008). It can also be due to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Rice-Evans *et al*., 1995). Herbs that have tannins as their component are astringent in nature and are used for the treatment of intestinal disorders such as diarrhoea and dysentery (Bajai, 2001), thus supporting the reasons why *Pueraria phaseoloides* has position among medicinal plants used for the treatment of microbial infection. Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues (Okwu and Emineke, 2006; Li *et al*., 2003; Adegboye *et al*., 2008). Alkaloids are beneficial chemicals to plants serving as repellent to predators and parasites. This probably endows these group of agents its antimicrobial activity. Several alkaloid-containing medicinal plants are reported to have been used by the early man as pain relievers, as recreational stimulants or in religious ceremonies to enter a psychological state to achieve communication with ancestors or God (Heinrich *et al*., 2005; Gurib-Fakin, 2005). Saponins are believed to react with the cholesterol-rich membranes of cancer cells, thereby limiting their growth and viability (Roa *et al*., 1995). Saponins have the property of precipitating and coagulating red blood cells (Yadav and Agarwala, 2011). Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al*., 2000; Okwu, 2004). Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation (Just *et al*., 1998; Okwu and Emineke, 2006, Liu and Henkel, 2002). Cardiac glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure (Yukari *et al*., 1995).

Macroscopic characteristics reveal that the leaves are dark green in colour, simple leaf in composition, alternate in arrangement, margin is entire, apex is acute, base is cuneate, surface is glabrous, venation is pinnate, texture is hairy, colour is dark green, petiole is pubescent and size are between 3-12cm (length) by 2.9-8.7cm (breadth). Fresh leaves have a characteristic odour and bitter taste. These parameters could be useful in the preparation of the herbal section of proposed Nigerian Pharmacopoeia and will aid in the physical or phenotypic identification of the plant. Any crude drug which is claimed to be *Pueraria phaseoloides* but whose characters significantly deviate from the accepted standard above would then be rejected as contaminated, adulterated or fake.

Microscopical features revealed that the powder contains numerous prisms and amorphous shaped calcium oxalate crystals, starch grains, lignified tissues, cystoliths, tannins and cellulose. The quantitative microscopic evaluation of fresh leaves indicate the presence of stomata on both surfaces of leaves which are anomocytic and paracytic in nature. The micro and macro morphological feature of the leaf described distinguishes it from other members of the genera. Studies of macroscopic and microscopic study can be valuable source of information which is usually and helpful in evaluation of purity and quality of a crude drugs. Numerical data and quantitative leaf microscopy are parameters that are unique to the plant and are required in its standardization.

Pharmacognostic and physicochemical studies of whole plant act as a reliable tool for plant identification and detecting adulteration (Desai and Chanda, 2014; Onyegbule *et al*., 2020; Bruce *et al*., 2016).

The various physicochemical parameters of leaves and powder revealed moisture content (5.8%), total ash value (1.3%), acid insoluble ash (0.5%), water soluble ash (1.0%), alcohol soluble extractive value (8.0%) and water soluble extractive value (6.0%). The physicochemical analysis (% w/w) revealed that the moisture content (5.8) of the plant fall within the acceptable limit since the general moisture content requirement of crude drugs is expected not to exceed 14% (British Pharmacopoeia, 2011). Therefore the moisture content of the plant is not too high (falls within the limit of the general requirement of 8-14%), indicating less probability of microbial degradation. Excess moisture in crude drug may lead to the breakdown of important constituent and the growth of microorganisms especially during storage of drug (Adesina *et al*., 2008 and Bruce *et al*., 2019). Total ash value is (1.3%), which can also be used to detect foreign organic matter and adulteration of sand or earth (Kunle *et al*., 2002). Acid insoluble ash value is (0.5%) as compared to that of *Atropa belladonna* L. leaves which is not more than 4% (British Pharmacopoeia, 2011), water soluble ash value is (1.0 %). The water soluble ash value is used to estimate the amount of inorganic compound present in drugs (Tatiya *et al*., 2012). The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent (Ozarkar, 2005). Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not (Tatiya *et al*., 2012). Water soluble extractive value of *Pueraria phaseoloides* leaves is 6.0%, as compared to that of *Azadirachta indica* A. Juss. leaves which is less than 20% (British Pharmacopoeia, 2011). The alcohol soluble extractive value of *Pueraria phaseoloides* leaves is 8.0%, compared with Bruce *et al*. (2019), which reported the alcohol soluble extractive value of 9.0%. This suggests that the use of alcohol as an extractive solvent is a better choice for the polar metabolites present in the plant. The results of this investigation could serve as a basis for proper identification, collection and investigation of the plant.

IMPLICATION TO RESEARCH AND PRACTICE

The wide scope of the nature of secondary metabolites in *Pueraria phaseoloides* plant is reflected by the presence of complex triterpene, saponins as well as of various volatile flavour components. Therefore, a proper review on the isolation, identification, and analysis of pertinent constituents should be of interest to researchers studying the properties and bioactivities of *Pueraria phaseoloides* plants.

CONCLUSION

The current investigation reveals the phytochemical screening and pharmacognostic properties of the leaves of *Pueraria phaseoloides*. The high content of poly-phenolic secondary

metabolites (alkaloids and flavonoids) in *Pueraria phaseoloides* and it's used in complementary medicine are indications that the plant is of great potential for wide range of applications in ethnomedicine.

FUTURE RESEARCH

Standardization is an integral part of establishing the correct identity of the crude drug before any drug can be included in the pharmacopoeia and the presence of these phytochemicals would help to open up other areas of use of this plant.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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