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Research Article

Pharmacognostic Study of *Parquetina nigrescens* (Afzel.) Bullock (Periplocaceae)

Sopeyin A O, Ajayi G O*

Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria.

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ABSTRACT

Parquetina nigrescens (Afzel) Bullock of the family Periplocaceae leaves are widely used in African traditional medicine for treatment of helminthiasis, insanity, gonorrhea, menstrual disorders, as an aphrodisiac and as a cardiac-tonic however, there are no information on the standardization of this plant. The aim of this study is to provide values that can be used for identification and determination of its quality; these include the macroscopic, microscopic, phytochemical screening, fluorescence and physicochemical analyses of the leaf and stem of *P. nigrescens*. Phytochemical screening of the ethanolic extract of the leaf and stem showed the presence of reducing sugars, tannins, terpenoids, saponins, flavonoids, alkaloids and cardiac glycosides. Quantitative determination of saponin and flavonoid content of the leaf were 2.5% and 10.9% respectively. The determined mean values for total ash, acid insoluble ash and water soluble ash were 16±0.36, 7.9±1.21, 11.1±0.60 (% w/w) respectively. Microscopic examination of the leaf revealed *P. nigrescens* is hypostomatic with paracytic stomata, presence of non-glandular trichomes (multicellular and possibly stellate) in the abaxial epidermal layer, prismatic calcium oxalate crystal, and polygonal epidermal cells with straight anticlinal walls. The characteristic microscopic features of the petiole include collenchyma, sclerenchyma, parenchyma and xylem vessels. Other microscopic characters in the transverse section of the stem include epidermal cells, sclereids, vascular bundles, collenchyma, and sclerenchyma amongst others. The average length and width for stoma and trichomes are 72±1.9, 17±1.5; 82.5±1.2, 77.5±1.0 (µm) respectively. Presence of hairs was observed on the anticlinal wall in the adaxial surface. The macroscopic examination of the leaf showed leaf arrangement - opposite, both surfaces – glabrous, leaf shape – cordate, leaf veination – pinately veined and a distinction in colour of both surfaces with adaxial surface a darker shade of green in comparison to the abaxial surface.
The qualitative, quantitative and physiochemical parameters established in this study will provide standards that can be used in the identification, authentication and quality control of *P. nigrescens*.

Keywords: *Parquetina nigrescens*, pharmacognostic study, qualitative, quantitative and physiochemical parameters

INTRODUCTION

Since the evolution of humans, plants have been used in a multitude of ways amongst which is as medicinex[1]. The use of plants as medicine has become more popular due to the claims of adverse effects caused by orthodox drugs and disease causing organisms becoming resistant to available drugs amongst others.

Ensuring the quality of medicinal plant products by making use of current control techniques and using suitable standards has been emphasized by the World Health Assembly[2]. Determination of certain pharmacognostic characters is very useful in the standardization of crude drugs to be used as medicine.

Also, the identity and purity of medicinal plants and crude drugs can be ascertained by studies such as macroscopic, microscopic, phytochemical screening, physicochemical screening and fluorescence analysis is[3].

Parquetina is a monotypic genus with *Parquetina nigrescens* being the only species in it. It is commonly found in secondary forests and around villages in Senegal and Nigeria. It is a perennial plant with twining stems; often herbaceous but becoming woody with age. It has relatively large and coriaceous leaves 10-15cm long, 6-8cm broad, and fleshy coriaceous corolla with inside pink, maroon or deep crimson to black-violet, and pubescent or hirsute stamens with pollen in tetrads[4].

It is mostly used by traditional healers in the treatment of anaemia in humans[5,6], helminthiasis, gonorrhea, menstrual disorders, and as an aphrodisiac; Also, as a cardiac-tonic and in the treatment of wounds[7].

This study is aimed at producing data on the different pharmacognostic parameters of *P. nigrescens* that will be used in its identification, authentication and quality control.

MATERIALS AND METHODS

The specimen of *Parquetina nigrescens* containing leaves and stems was identified and authenticated by Mr T.K. Odewo of the University of Lagos Herbarium, Lagos state (LUH), Nigeria where vouchers was also deposited as *P. nigrescens*- LUH 2757.

Macroscopy

*Author for Correspondence

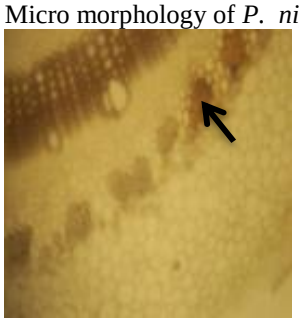
Macro morphology of *P. nigrescens* leaves

Figure 1: *Parquetina nigrescens* leaves

Lower surface (Abaxial)

Upper surface (Adaxial)

Micro morphology of *P. nigrescens* stem, petiole and leaf



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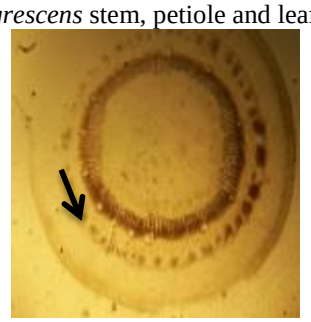

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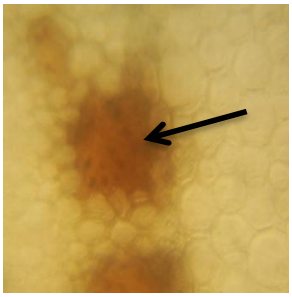

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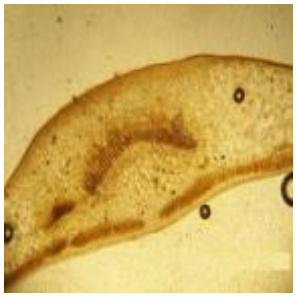

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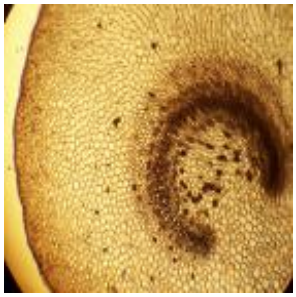

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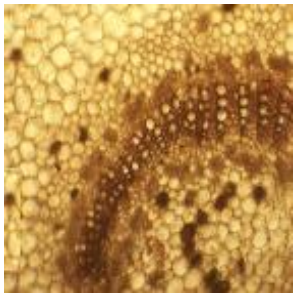

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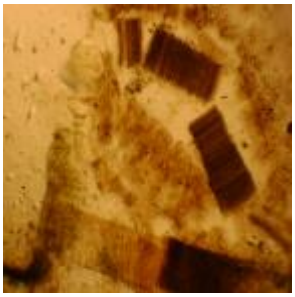

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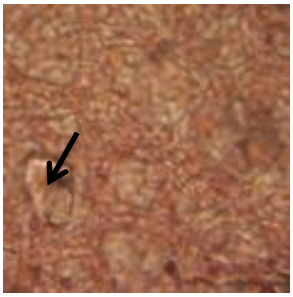

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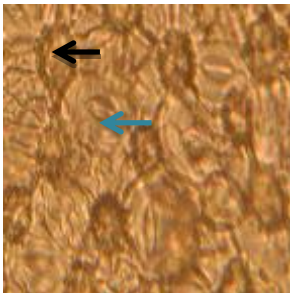

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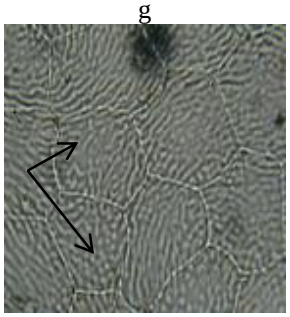

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Figure 2: (a), (b) Transverse section of *P. nigrescens* stem showing vascular bundle arrangement and sclereids (arrow) x200. (c), Sclereid in transverse section of *P. nigrescens* stem x400. (d) Transverse section of *P. nigrescens* leaf midrib x400. (e) x200, (f) x400, Transverse section of petiole of *P. nigrescens*. (g) Vascular bundles in transverse section of petiole of *P. nigrescens* x400. (h), Abaxial surface of *P. nigrescens* leaf showing spongy mesophyll and calcium oxalate crystal (arrow) x400. (i), Abaxial surface of *P. nigrescens* leaf showing stomata (blue arrow) and trichomes (black arrow) x400. (j), Adaxial surface of *P. nigrescens* leaf showing hairs on anticlinal wall and epidermal cells and (k), showing striations x400.

The various morphological characteristics of the leaf was studied and recorded appropriately.

Microscopy

Using free hand sectioning, very thin sections of the transverse section of the leaf across the mid rib, the stem and the petiole were obtained. The sections were rinsed in

Table 1: Macroscopic Characteristics of *P. nigrescens* leaf

Character	Observation
Abaxial leaf colour:	Light green
Adaxial leaf colour:	Dark green
Odour:	Odourless
Texture:	Glossy
Leaf type:	Simple
Phyllotaxy:	Opposite
Leaf apex:	Acuminate
Leaf shape:	Cordate
Leaf base:	Cordate
Leaf veination:	Pinnately veined
Adaxial leaf surface:	Glabrous
Abaxial leaf surface:	Glabrous
Leaf length:	6.5 cm – 18.3 cm
Leaf width:	5.4 cm – 16.8 cm

Table 2: Qualitative characters of *P. nigrescens* leaf

Leaf surface	Adaxial	Abaxial
Cell shape	Polygonal	Circular
Cuticular ornamentation	Thick	Thick
Anticlinal wall pattern	Straight/Wavy	Straight/ Wavy
Stomata type	Absent	Paracytic
Presence of trichome	Present	Present
Striation	Present	Present
Trichome type	Absent	Non glandular, stellate
Crystals	Absent	Prismatic calcium oxalate
Oil globules	Absent	Absent

Table 3: Quantitative parameters of *P. nigrescens* leaf adaxial epidermis

Parameters	Adaxial epidermis
Length of stomata	72±1.9 µm
Width of stomata	17±1.5 µm
Length of trichome	82.5±1.2 µm
Width of trichome	77.5±1.0 µm
No of Epidermal cells	37±2.1
No of Stoma	16±1.5
Stomata index	26.1 to 28.6 to 33.3

petri dishes containing water. Drops of different grades of ethanol: 50–100% was in turn added to dehydrate the cells. The sections were later stained with phloroglucinol and 25% hydrochloric acid before mounting in glycerine on the glass slide and covered with cover slips. The prepared slides were examined under light microscope at magnification x100 and x400[8,9].

Also, the abaxial and adaxial surface of different sections of the fresh leaf of *P. nigrescens* was obtained. The portions were placed in beakers containing chloral hydrate solution for about four (4) hours to completely clear the epidermal layers. The cleared epidermal layers were then rinsed in a Petri dish containing distilled water. Drops of different grades of ethanol: 50–100% was added in turn to dehydrate the cells. The preparations were later stained with Safranin O in 50% alcohol for about five minutes before mounting in glycerine on the glass slide. The epidermis was mounted on glass slide with the uppermost surfaces facing up and covered with cover-slips. Slides were examined under light microscope at x100 and x400 also; photomicrographs were taken using a digital camera (Kodak 8.1 mega pixel) fitted on an eye piece of the microscope used for examination. Quantitative and qualitative characters of the leaf epidermis were assessed[8,9].

Slides were prepared for each epidermal surface. Stomata number, stomata index palisade ratio, and the length and width of stoma and trichomes were recorded according to Gray, 1964; Evans, 2002[8,9]. Stomata index was calculated using the formula:

$$\text{Stomata index} = \frac{\text{Stomata number}}{\text{Number of epidermal cells + stomata number.}} \times 100$$

Fluorescence analysis

The fluorescence properties of plants samples were studied under ultra violet (UV) light adopting previously described methods[10,11]. The behaviors of the samples with different chemical reagents were studied and fluorescence characters were observed in daylight and under Ultra Violet lamp at 254 and 365 after 1-2 min.

Phytochemical and Physicochemical Screening

Following the methods of Evans and Sofowora, 1993, 2002[9,12] various phytochemical tests were carried out to determine the presence or absence of various phytochemicals and results reported accordingly.

Percentage flavonoid and saponin contents were determined using the methods of Boham and Kocipai, 1994[13]. Also total ash value, acid insoluble ash value, water soluble ash value, alcohol soluble extractive value, water soluble extractive value and the moisture content of *P. nigrescens* was carried out according to (Ajazuddin and Shailendra, 2010)[14].

RESULTS, DISCUSSION AND CONCLUSION

It has been widely observed and accepted that the medicinal value of plants lies in the chemical constituents present in the plant[15]. Phytochemical screening of the ethanolic extract of *Parquetina nigrescens* leaf and stem revealed the presence of flavonoids, saponins, terpenoids, and cardiac glycosides also, the presence of tannins and alkaloids in the stem extract. Quantitative determination of the leaves of *Parquetina nigrescens* contained 2.5% saponins and 10% flavonoids. Flavonoids have been shown to protect body cells from damage and maintain capillary integrity by functioning as anti-oxidants[16]. Flavonoids are also used as effective constituents of several pharmaceuticals in the treatment of capillary fragility and phlebosclerosis[17]. Orally administered flavonoids have also been observed to inhibit vascular permeability and prevent pulmonary haemorrhage [18]. The presence of flavonoids could be the reason for its ethno botanical use as an anti-anaemic agent.

Table 4a: Fluorescence Characters of the powdered samples of *P. nigrescens leaf*

Particulars of treatment	Visible light	UV light	
Leaf powder +		254nm	365nm
1N HCl	Brown green	Bright bottle green	Mud brown
Conc. HCl	Brown green	Bright bottle green	Mud brown
Leaf Powder only	Green	Bright bottle green	Grey/ash
Conc. HNO_3	Yellow	Bright bottle green	Orange to brown
50% HNO_3	Light brown	Bright bottle green	Light brown
Conc H_2SO_4	Dark green	Dark green	Mud brown
50% H_2SO_4	Dark green	Dark green	Mud brown
Glacial acetic acid	Brown	Bright bottle green	Brown
10% $FeCl_3$	Brown	Brownish green	Brown
10% NaOH	Green	Green	Green
Methanol	Green	Green	Brown
Water	Dark green	Pale green	Brown
50% KOH	Dark green	Dark green	Black
Picric acid	Yellow green	Bright bottle green	Brown
Dil. NH_3	Dark green	Dark green	Dark green
Dil. NH_3+conc HNO_3	Brown	Bright bottle green	Brown
Acetone+Methanol	Green	Green	Green
10% Iodine	Brown	Green	Brown

Table 4b: Fluorescence Characters of the powdered samples of *P. nigrescens* stem

Particulars of treatment	Under visible light	Under UV light	
Stem powder +		254nm	365nm
1NHCl	Brown	Yellow green	Light brown
Conc HCl	Reddish brown	Bright bottle green	Mud brown
Stem Powder only	Brown	Green	Brown
Conc HNO_3	Orange	Bright bottle green	Orange
50% HNO_3	Orange	Bright bottle green	Orange
Conc H_2SO_4	Black	Black	Black
50% H_2SO_4	Black	Black	Black
Glacial acetic acid	Brown	Bright bottle green	Brown
10% $FeCl_3$	Brown	Dark green	Brown
10% NaOH	Brown	Black	Black
Methanol	Brown	Bright bottle green	Brown
Water	Brown	Green	Brown
50% KOH	Brick red	Green	Brown
Picric acid	Yellow	Bright bottle green	Yellow
Dil NH_3	Brown	Bright bottle green	Brown
Dil NH_3+conc HNO_3	Orange	Green	Brown
Acetone+Methanol	Brown	Green	Brown
10% Iodine	Brown	Bright bottle green	Brown

Table 5: Phytochemical screening of ethanol extract of *P. nigrescens* leaf & Stem

Chemical constituent	Inference	
	Leaf	Stem
Reducing sugar	+	++
Tannins	-	+
Phlobatannins	-	-
Terpenoids	+	++
Saponins	+	+
Flavonoids	++	+
Alkaloids	-	+
Cardiac glycosides	+	+
Anthraquinones	-	-

The average total ash value of the leaves of the plant as a result of the quantitative analysis was 16 % w/w. The acid insoluble ash was 7.9%w/w while that for water soluble ash was 11.1%w/w. The water soluble extractive and the ethanol soluble extractives were 19.3%w/w and 12%w/w respectively.

The macroscopical examination of the leaf revealed its various morphological characters. Phyllotaxy which is the position of the leaves on the stem is opposite. It was observed that both the abaxial (lower) and the adaxial (upper) surfaces of the leaves were smooth with no hairs i.e. glabrous. There was a distinction in the colour of both surfaces with the adaxial surface being a darker shade of green in comparism to the abaxial surface.

From the microscopic examination of the leaves, *P. nigrescens* was hypostomatic i.e. stomata was found only

Table 6: Physiochemical analysis of *P. nigrescens* leaf

Parameters	Percentage (% w/w)
Saponin content	2.5
Flavonoid content	10.9
Total ash	16±0.36
Acid insoluble ash	7.9±1.21
Water soluble ash	11.1±0.60
Water soluble extractive	19.3±0.63
Acid soluble extractive	12±0.49
Moisture content	34.4±0.36

in the abaxial surface of the leaf (fig 2i); the stomata type is paracytic. Trichomes are unicellular or multicellular outgrowths that originate from the aerial epidermis and which vary in morphological features, location and mode of secretion[19]. A long history of published literature indicates that the type and density of trichomes differ among species and may vary in organs of the same plant[20]. The trichomes present in the abaxial epidermal layer (fig 2i) is non glandular, multicellular and possibly stellate. The measurement of the average length and width of the stomata and trichomes were 72 µm and 17 µm for stomata and 82.5 µm and 77.5 µm for trichomes. In medicinal plants, trichome characters have been reported to act as biomarkers to identify the plant even in the raw material or powder form. The presence of glandular trichomes in many of the medicinal plants is considered indicative on the concentration of secondary metabolites with pesticidal, pharmacological, and fragrant properties[21]. The adaxial surface had no stomata in it; presence of hairs (fig 2j) was observed on the anticlinal wall.

Collenchyma cells were mostly found below the epidermis in stem and petiole anatomy. Functions of collenchyma cells include; support and transport of nutrients[22]. Parenchyma cells were present in both the petiole and stem anatomy (fig 2 a & c). The parenchyma cells vary in size, they were not spherical having intercellular spaces. Functions of parenchyma cells vary depending on their location; they aid in wound healing, regeneration[23]. Sclerenchyma majorly provides mechanical support. They are of two types (sclereids and fibres) and contain lignin. Sclereid type (fig 2c) in *P. nigrescens* is brachysclereid (stone cells) they were iso-diametric with thick cell walls. The sclereids in the stem anatomy were arranged as a continuous layer in the periphery of the vascular bundles.

Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Much phytochemical fluorescence is seen when suitably illuminated. The fluorescence colour is specific for each compound. A non-fluorescent compound may fluoresce if mixed with impurities that are fluorescent. Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which is not visible in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives after reacting with different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation[24].

The results from this study have provided information on the phytochemical and physicochemical parameters of *P. nigrescens* also, the morphological and anatomical features of the leaves, stem and petiole. The various fluorescence characters have been revealed, all of which give the important diagnostic characters of *Parquetina nigrescens*. These parameters can be used for proper identification and quality control of the plant.

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