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Pharmacognostic Evaluation and Physicochemical Analysis of *Paullinia pinnata* L. (Sapindaceae)

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The current resurgence of public interest in herbal medicines calls for heightened studies on their quality and safety. The major setback in promoting the use of these herbal medicines is the lack of standardization and the confusion in the identification and authentication of these plants and their substitutes or adulterants. In this study, the pharmacognostic and physicochemical features of the leaves, stem bark and roots of *Paullinia pinnata,* an African shrub used traditionally for wound healing, treatment of dysentery and also as an aphrodisiac, was evaluated. The study included macroscopy, microscopy, physicochemical and phytochemical screening. Physicochemical parameters evaluated varied widely among the various parts studied which could be used in their identification. The study also revealed the presence of acicular calcium oxalate crystals, anomocytic stomata, wavy anticlinal walled epidermal cells and unicellular and multicelluar uniserate clothing trichomes in the leaves. Morphological, anatomical and physicochemical studies of *P. pinnata* provides simple and reliable standards which could be useful for the proper identification of *P. pinnata*.

Keyword: Extractive Values, Macroscopy, Microscopy, *Paulinia pinnata*, TLC

1. Introduction

The use of herbs and herbal products, in both developing and developed countries, for the treatment of various diseases has increased dramatically in recent years. However the major drawback in promoting the use of medicinal plants is the lack of standardization as well as the confusion in the identification of the plant and their substitutes or adulterants. To ensure reproducible quality of herbal plants, authentication is invaluable. The pharmacognostical studies not only give the authentication but also quality and purity standards of the plant. According to the WHO, the macroscopical and microscopical description of a medicinal plant is the first step towards establishing the identity and degree of their purity.

Paullinia pinnata L. (Sapindaceae) commonly known as Sweet gum, 'Toa-ntini' (Akan in Ghana) is a woody creeper or climber with rigid stems. The leaves are pinnate with five leaflets, the terminal leaflet being the largest. In traditional medicine, various parts of *P. pinnata* are used for treating various diseases. ln South West Nigeria, the leaf juice of *P. pinnata* is used for the treatment of sore throat [8], an infusion is used for fever while the roots are used for the treatment of leprosy, jaundice, snake bites [9], nausea and vomiting. [6] The whole plant is used in Ghana to treat dysentery. The roots, mashed

with seeds of *Piper guineense*, are applied as a styptic to cut veins and to treat leprosy[7]. The roots are also chewed for coughs and pulmonary diseases, gonorrhoea, fractures or abscesses or used on open sores. It is also used as aphrodisiac[1]. Previous phytochemical investigations of *P. pinnata* have shown the presence of triterpene saponins and cardiotonic catechol tannins. [4] Two flavone glycosides, diosmetin-7-O-(2''-O-β-D-apiofurasosyl-6''-acetyl-β-D-glucopyranoside) and tricetin-4'-O-methyl-7-O-(2''-O-β-D-apiofurasosyl-6''-acetyl-β-D-glucopyranoside), have also been isolated from the leaves[2]. Miemanang *et al.*[12], also reported the isolation of paullinoside A, paullinomide A, *β*-sitosterol and *β*-amyrin from the leaves, while Annan and Houghton,[3] isolated paullilupeone and paullilupeol from the roots. In the present studies, various pharmacognostic and physicochemical features of the leaves, stem bark and roots of *P. pinnata* were evaluated in an attempt to create a pharmacopoeial standard for the plant.

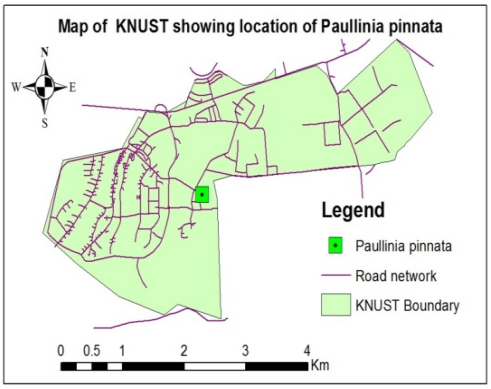

Fig 1: Map of KNUST showing the location of *P. pinnata*

2. Material and Methods

2.1 Collection and Authentication of Plant Material

Fresh leaves, roots and stems of *Paullinia pinnata*, located on latitude 6º 40'.88 N and longitude 1º 33'58.11 W (using Garmin 12 GPS, Figure 1), were collected in September, 2011 from the Physique Garden at the Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi and authenticated at the Department of Herbal Medicine where a herbarium specimen has been deposited. Photographs of the aerial parts of plant were taken (Figure 2A).

2.2 Preparation for Examination

Collected plant parts including; leaves, stems, and roots were cleaned of foreign matter and other contaminants. These were chopped into pieces, shade dried for two weeks and powdered. Microscopic evaluation was done according to standard methods[5,14,15].

2.3 Macroscopic Examination

The fresh leaves of *P. pinnata* were examined and characterized according to the shape, colour, margin, texture, apex, presence or absence of petiole and base symmetry.

2.4 Microscopic Examination

Free hand transverse sections of the midrib of a fresh leaf and tendrils of the stem were cut with a razor blade and mounted in chloral hydrate solution. Micrographs of the mounts were taken with the aid of a light microscope camera (Olympus software). Sections of the fresh leaf were also cleared for about 1 hour in chloral hydrate solution (80%^{w}/v) and used for surface data determination. The presence or absence of the following was observed: epidermal cells, stomata (type and distribution), epidermal hairs (type and distribution of trichomes).

2.5 Physicochemical Parameters

Powdered samples were subjected to physicochemical analysis including water and alcohol soluble extractives, total ash, acid-insoluble ash, water soluble ash, mineral content and moisture content determination according to the methods outlined by Khandelwal[10].

2.6 Thin Layer Chromatography Analysis

The chemical fingerprint was determined using thin-layer chromatography (TLC). Chloroform extracts of the leaves and roots (the part commonly used) were applied to aluminium precoated silica gel plates 60 $_{F254}$ (0.25 mm thick). The plates were developed in a chamber

containing pet-ether and chloroform (2:8) as the mobile phase. The TLC plate was air-dried and visualized under ultraviolet light (365 nm). The dried TLC was then sprayed with an anisaldehyde – sulphuric acid, vanillin – sulphuric acid and 5% concentrated sulphuric acid reagent respectively and heated at 105° C for 5 min[12].

3. Results/Discussion

3.1 Macromorphology

The results of the organoleptic and macroscopic examination established that the plant is a woody climber with a ridged stem, forked tendrils at the nodes, a conspicuously winged rachis and petiole, compound imparipinnate leaves which are papery in texture, olive-green in colour, oblong to obovate in shape, bland in taste with a characteristic odour. The apex of the leaf is acuminate with an irregularly crenate margin, a symmetrical base. The leaflets are about 11.4-13.8 cm in length and 5.6-6.6 cm in width with 5.6-6.3 cm long petioles. The odd leaflets of the imparipinnate leaf appear to be larger than the other four in each case (Figure 2A). These are unique of this species and can be essential in preliminary identification of the plant

3.2 Micromorphology

Microscopic examination of sections of the leaf revealed the presence of acicular calcium oxalate crystals, anomocytic stomata (more on the lower than the upper surface), wavy anticlinal walled epidermal cells (Figure 2B) and unicellular and multicelluar uniserate clothing trichomes (Figure 2D). Powdered samples of the leaf revealed the presence of starch grains, lignified fibres with no oil glands. A transverse section of the midrib and tendril shows circularly arranged vascular bundles (Figure 2E). The midrib and tendrils have a distinct appearance. The vascular bundle are uniquely marked with secondary cambium separating the phloem (outer) and xylem (inner) vessels. The palisade tissue is interrupted in the midrib with collenchyma occurring above and below the vascular bundles (Figure 2E). In the quantitative microscopy, a palisade ratio of 5.0-6.8, a stomatal index of 2.2-4.9 and 13.0-18.5 for upper and lower leaf surfaces respectively, vein-islet and veinlet termination numbers of 8-10 in each case were obtained (Table 1). The length of the covering trichomes was 120-250µm (Table 1). The powdered root showed the presence of numerous prismatic calcium oxalate crystals, lignified pitted vessels, lignified pitted fibres, numerous starch grains and abundant stone cells (Figure 3).

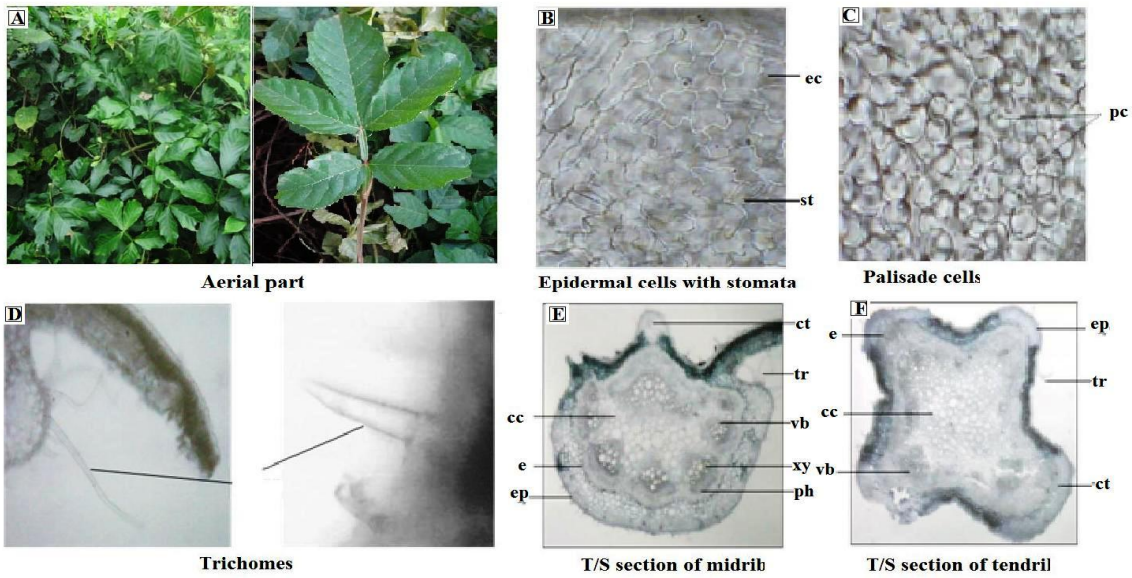

Fig 2: Aerial part of *P. pinnata* (A) and micrographs of sections of the leaves (B-F). **cc**-collenchyma cells, **e**-endodermis, **ep**-epidermal cell, **ct**-cortex, **tr**-trichomes, **vb**-vascular bundle, **xy**-xylem, **ph**-phloem

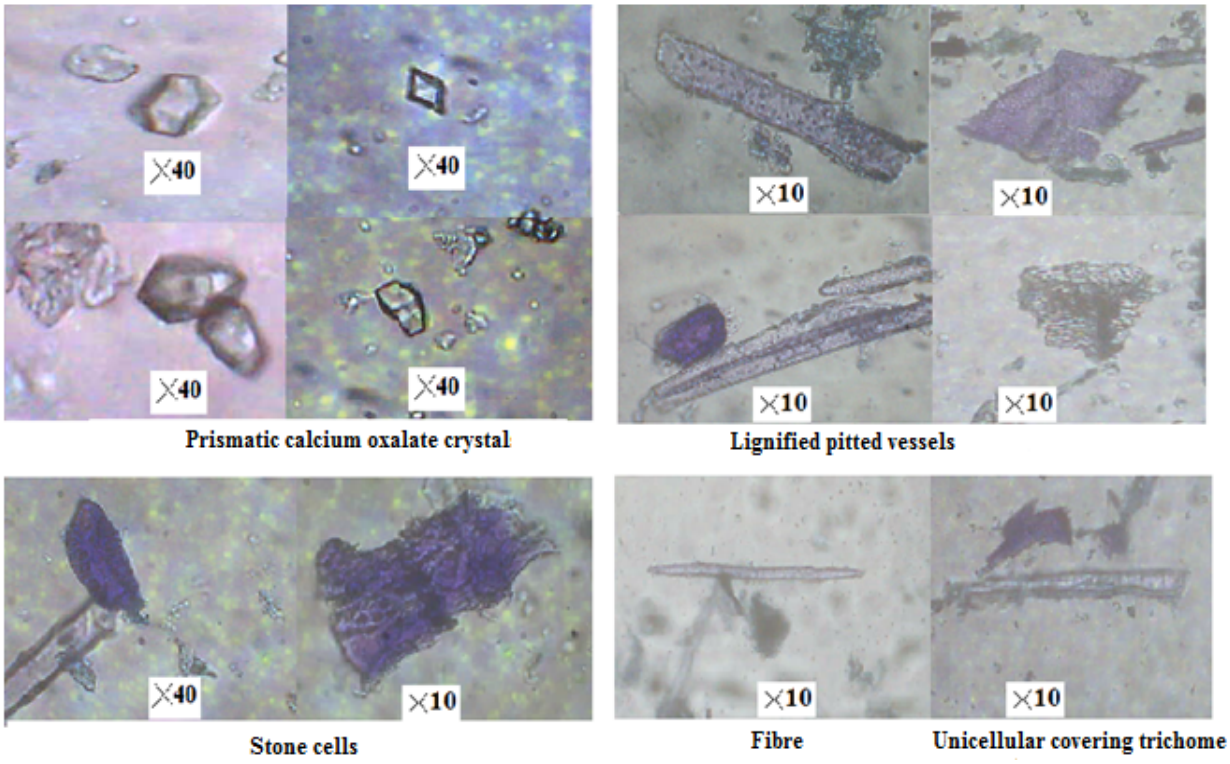

Fig 3: Micrographs of cellular components of the powdered root of *P. pinnata*

3.3 Physicochemical Parameters

Physicochemical data obtained varied widely among the different plant parts used. The total ash was highest for the leaves sample (12.33%) followed by the roots (3.86%) and the stems powder (2.04%). This trend persisted for the acid-insoluble ash (Table 2). The stems however had a higher water soluble ash than the roots but not the leaves. For the extractive values, the leaves had the highest values for all the solvents used indicating that the plant probably stores a higher amount of its secondary metabolites in the leaves. Per the four solvents used in this study, methanol extracted more constituents followed by distilled water and the least was chloroform. This variation can influence the choice of solvents during extraction of *P. pinnata* constituents. It also indicates the presence of relatively polar constituents and non polar constituents.

The moisture content determined for the fresh samples is a vital parameter in circumstances where the plant samples are collected and processed or sold afresh. Moisture content of the air dried samples is useful in determining the efficiency of the drying process and the probability of deterioration of the product due to higher moisture content[13]. This will serve as a guide to processing, preservation and storage of the crude drug. The moisture content was highest in the roots (Table 2). Some minerals such as sodium, zinc, calcium, magnesium and aluminium form an essential component of the human system and their presence in crude drugs is vital. Others like mercury, lead, arsenic, cadmium, manganese and copper in minute quantities may be detrimental to the health of people who consume products containing them. The study revealed that the essential minerals are all within the daily recommended quantities with calcium being the most abundant. The heavy metals however, occurred in trace amounts which are negligible in that the quantities present are below the permissible limits[15] and may not be detrimental to human health (Table 3).

Table 1: Surface data parameters of the leaves

Parameter	Range	Average
	U/S L/S	L/U Ratio
Stomatal index	2.2-4.9 13.0-18.5	3.3-6.0
Stomatal number	1 7	
Vein islet number	8-10	9
Veinlet termination number	8-10	9
Palisade ratio	5.0-6.8	6.0
Covering trichomes	120-250µm	180µm

U/S = upper surface, L/S = lower surface

Table 2: Physicochemical parameters of the leaves, roots and stem bark of *P. pinnata*

Parameter	Results %$^{w}/_{w}$		
	Leaves	Roots	Stems
Total ash	12.33	5.44	6.65
Water soluble ash	3.56	1.26	3.73
Acid insoluble ash	2.04	1.10	0.89
Water soluble extractive	11.92	7.70	5.89
Ethanol soluble extractive	8.79	5.24	2.80
Chloroform soluble extractive	5.54	3.40	1.50
Methanol soluble extractive	12.69	8.62	11.67
Moisture content	7.98	9.80	8.70

Table 3: Mineral content of ethanolic root extract

Mineral content	Quantity (ppm)
Magnesium (Mg)	76.50
Calcium (Ca)	59.00
Sodium (Na)	0.07
Manganese (Mn)	20.00
Zinc (Zn)	<0.01
Cadmium (Cd)	866.00
Cpper (Cu)	1851.50
Iron (Fe)	492.00

3.4 Chromatographic Fingerprint

Analytical TLC of the leaves and roots on silica gel revealed the presence of certain prominent spots/ compounds which could be used in the identification of *P. pinnata*. The TLC chromatogram of the roots (Figure 4A) showed three characteristic spots with R_f values of 0.22, 0.39 and 0.78. The compound, with R_f = 0.22, showed characteristic light green fluorescence at 365 nm. Similarly, that of the leaves showed three characteristic spots with R_f values of 0.24, 0.4 and 0.56. However, the spots from the leaves did not show any marked fluorescence at 365 nm. Thus the TLC fingerprint showed characteristic profiles that could be used as markers for quality evaluation and standardization of *P. pinnata*.

4. Conclusion

The pharmacognostic standards for *P. pinnata* are being reported for the first time in this study. The standards established in this study will help minimize the adulteration of samples of *Paullinia pinnata* and will also be of great use for researchers, manufacturers and individuals in selecting the authentic plant material for research, drug production or as a home remedy. In addition, the results of this investigation may be useful in the preparation of a medical monograph for this plant.

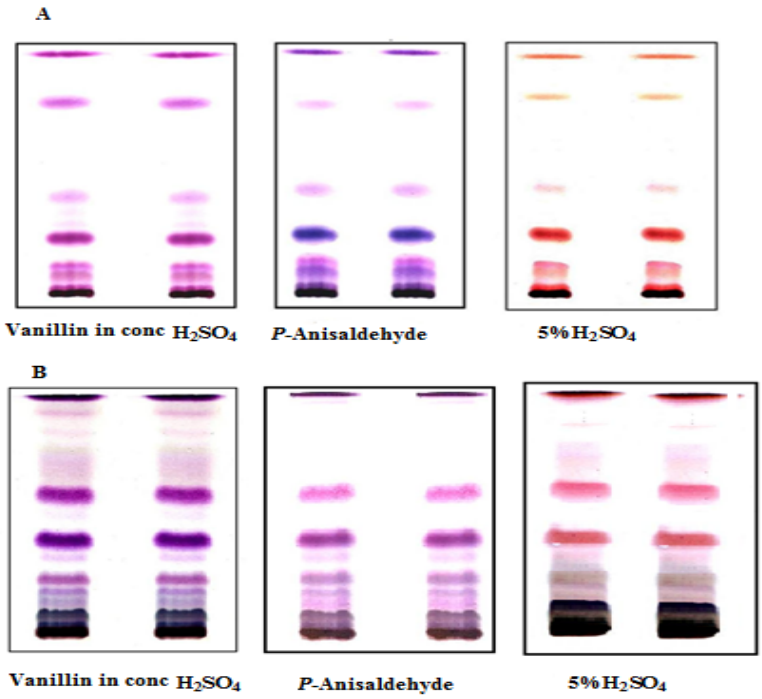

Fig 4: TLC chromatogram of the roots (**A**) and leaves (**B**) of *P. pinnata*

5. Acknowledgement

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