

Ethnopharmacological characteristics of *Pycnanthus angolensis* (Welw.) Warb. (Myristicaceae), a plant with antiparasitic and antiviral effects

ABSTRACT

Background: *Pycnanthus angolensis* (Welw.) Warb. (Myristicaceae) is a medicinal plant used in traditional Ivorian medicine. A recent ethnobotanical survey revealed this plant in the traditional treatment of viral and parasitic pathologies.

Aim: The present study aims to highlight the distinctive ethnopharmacological characteristics of *Pycnanthus angolensis*.

Methods: The aim was to identify some groups of chemical compounds by thin layer chromatography, to assay some minerals and finally to characterise the specific anatomical and micrographic features of the plant.

Results: Terpenes and sterols, saponosides, flavonoids and tannins are the main phytocompounds revealed. Magnesium with 621.3 mg/100 g dry matter is the most abundant mineral. Anatomical sections and plant powder revealed starch grains, calcium oxalate crystals, secretory pockets and tector hairs that are responsible for the formation of various biological substances in the plant.

Conclusion: These results add to the data on *Pycnanthus angolensis*, a taxon much used in traditional Ivorian medicine for the treatment of antiparasitic and antiviral diseases.

Keywords: *Pycnanthus angolensis*, ethnopharmacological characteristics, antiparasitic, antiviral, Côte d'Ivoire

1. INTRODUCTION

Pycnanthus angolensis (Welw.) Warb. or *Pycnanthus kombo* (Baill.) Warb., commonly known as African nutmeg, is a tree species found in West and Central Africa. Different parts of the tree are traditionally used for a wide range of medicinal purposes, including the treatment of pain and fungal infections [1]. Various preparations of the bark, and other parts as well but to a lesser extent, are used in traditional medicines [2]. Topical application of the bark powder and decoction is used for skin cancer in Ghana, while decoction of its stem bark and leaves are also used for wound healing [3]. According to N'Guessan et al. [4], stem bark is used to facilitate delivery. Its chemical analysis also revealed the presence of isoflavone, terpenoid-type quinone, and cyclolignene derivatives [5] justifying various biological activities. Elsewhere, the plant is used as an analgesic, carminative, anthelmintic, anti-inflammatory, haemostatic and antimicrobial [6]. Akinyeye and Olatunya [7] reveal that *P. angolensis* is also used in the treatment of female infertility, rheumatism, sore throat and bronchopneumonia. In vitro studies by Kamanzi [8] (2002) revealed that *Pycnanthus angolensis* (stem bark) is not cytotoxic.

Recent studies have revealed its use in Côte d'Ivoire in the traditional treatment of parasitic and viral pathologies. Several studies have been carried out on *Pycnanthus angolensis*. It is important to compare all these data obtained from the plant with that found in Côte d'Ivoire.

The present study aims to highlight the distinctive ethnopharmacological characteristics of *Pycnanthus angolensis*.

2. MATERIAL AND METHODS

2.1 Material

The parts of *Pycnanthus angolensis* used were the stem bark and the young stem. The stem bark was used for phytochemistry, mineralogy and micrography. The young stem was used for anatomical-histological cross-sections. These organs were harvested on the Yakasse-Attobrou (Adzope, Côte d'Ivoire). The geographic coordinates are 6°10'51" N and 3°39'26" W.

2.2 Methods

Phytochemistry

This phase started with the extraction of the different phytochemicals. The plant powder was introduced into a 50 mL Falcon tube and cold macerated for 24 hours in the extraction solvent (96% ethanol). The filtrate obtained was used for phytochemical screening.

Phytochemical screening of the extracts was performed on HPTLC plates (20 cm × 10 cm) silica gel [60 F] _254 (Merck, Darmstadt, Germany).

10 µL of extract were deposited in a 1 cm strip with a semi-automatic sample dispenser (CAMAG, Linomat 5, Switzerland) along the baseline 8 mm from the bottom edge of the plate. The distance between the spots is 3.4 mm. The distance between the first spot and the left edge of the plate and between the last spot and the right edge of the plate is 20 mm. A constant application rate of 100 nL/s was used. Linear upward development with 10 mL mobile phase was performed in a CAMAG double-trough glass chamber lined with filter paper and previously saturated with mobile phase vapour for 20 minutes. The development distance was approximately 70 mm. The plates were dried after development using a hair dryer. In the double trough chamber, the mobile phases were:

- Terpenoids, eluted with hexane/ethyl acetate system 20:4, v/v and revealed by Liebermann Burchard reagent;
- Saponosides, eluted with ethyl acetate/petroleum ether 2:1, v/v revealed with sulphuric anisaldehyde;
- Flavonoids and tannins: ethyl acetate/formic acid/acetic acid/water 100:11:11:26, v/v/v. Flavonoids were revealed by Neu's reagent, tannins by FeCl₃ (2 %).

Mineralogy

For the determination of mineral elements, the Analyst Pinnacle 900T air-acetylene flame atomic adsorption spectrometer (Perkin Elmer) was used. The wavelengths of the elements to be analysed were first defined on the instrument (324.75 nm for copper, 213.86 nm for zinc, 248.33 nm for iron, 285.2 nm for magnesium, 766.49 nm for potassium, 279.48 nm for manganese and 589.0 nm for sodium). Then, the different readings of the calibration ranges were used to establish the calibration curve translating absorbance as a function of concentration. Finally, the samples to be analysed were presented to the apparatus in order to determine their absorbances. A blank is necessarily passed between the passage of two different solutions.

Anatomo-histology

It consisted of making thin cross-sections of a portion of *Pycnanthus angolensis* stem using a new razor blade. The stem was inserted into sorghum or polystyrene pith. The whole set of sections was soaked in diluted bleach for 20 min followed by rinsing with plain water. The sections were then soaked in acetic water for 15 minutes to neutralise the excess bleach, which is chemically basic and makes the cell walls receptive to the dye. After this step, the sections obtained were stained with carmino-green (a mixture of carmine alumina and iodine green) and then rinsed one last time with water. Thus, the cell walls were stained according to their chemical nature (cellulosic walls in pink and lignified walls in green). The stained sections were then mounted between slides and coverslips in a drop of glycerine water for observation under a photonic microscope connected to a computer. After observation, the different sections were photographed.

Micrography

On an object slide, a drop of 10% potash was placed. This preparation was sprinkled with a pinch of fine plant drug powder obtained using a 500 µm mesh sieve. This was covered with a slide without leaving any air bubbles. The powder thus treated was observed with an Optika Microscopes Italy photonic microscope, connected to a tablet. Observations were made at x40 and x100 magnification in order to look for characteristic elements or organ fragments. These observed elements were then photographed.

3. RESULTS AND DISCUSSION

Systematic position

Kingdom: Plantae

Clade: Angiosperms

Clade: Dicotyledons

Order: Magnoliales

Family: Myristicaceae

Genus: *Pycnanthus*

Species: *Pycnanthus angolensis* (Welw.) Warb.

Synonyms

- *Myristica angolensis* Welw.
- *Pycnanthus kombo* (Baill.) Warb
- *Pycnanthus microcephalus* (Benth.) Warb.

Common names: Iromba, Tallow Tree, White Cedar, Wild Muscat

Local names : Etrain (Baoulé), Dign (Yacouba), Iromba (Guéré), Guilo (Attié), Tiè (Brong)

Herbarium specimen number

CSRS : N°10-CSRS004841-A4-C88-R1-E4-F4-P1

Botanical description

Pycnanthus angolensis is an evergreen, monoecious or dioecious, medium to large tree reaching 25-35(-40) m tall; bole generally straight and cylindrical, branchless to 15(-25) m tall, up to 120(-150) cm in diameter, generally lacking buttresses; outer bark greyish brown, with orange-brown exudate; crown small, with branches perpendicular to bole. The leaves are alternate distichous, simple and entire (Figure 1A); the blade is oblong to oblong-lanceolate with a cordate base; the apex is acuminate, dark green above, glaucous below. The young leaves are velvety with reddish brown hairs, but glabrescent, pinnately veined with 20-40 pairs of lateral veins. The inflorescence is an axillary panicle, often on leafless twigs, red-hairy, with flowers in numerous padded clusters. Flowers unisexual, regular, very small, sessile, with a 3-lobed perianth covered with dark brown hairs; male flowers with 2-4 stamens, threads fused into a column; female flowers with a superect ovary, sessile, 1-locular, stigmas 2, sessile. The fruit is an ellipsoid to oblong or globose drupe, clustered, yellowish orange at maturity, fruit wall rather hard and leathery, splitting longitudinally by 2 valves, containing 1 seed (Figure 1B). The seed is ellipsoid, aromatic, dark brown, with a pink to red aril, lacinated almost to the base [9,10].



Figure 1: A. *Pycnanthus angolensis*; B. Fruits

Habitat and geographical distribution

Pycnanthus angolensis (Welw.) Warb. (Myristicaceae) is widely distributed in the tropical Africa (Figure 2). Its phytogeographical distribution is Guinean-Congolese [11]. *P. angolensis* is a widespread species in secondary forests. It immediately repopulates old abandoned clearings.



Source: <https://africanplantdatabase.ch/en/nomen/177516>

Figure 2: Geographical distribution of *Pycnanthus angolensis* in Africa

Traditional uses in Côte d'Ivoire in the treatment of parasitic and viral diseases

The exudate of the trunk applied as an eye instillation treats Onchocerciasis. Against Schistosomiasis, the decoction of the stem bark is taken as a drink. The same beverage is prescribed as a drink against HIV-AIDS and other viral pathologies. Several phytomolecules extracted from *Pycnanthus angolensis* have antiviral and antiparasitic activities [12].

Other therapeutic uses

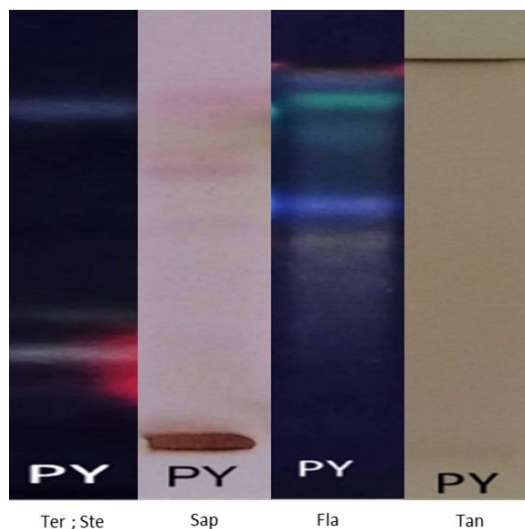
The stem bark of *Pycnanthus angolensis* is recommended for the treatment of infectious diseases. The exudate is indicated against gingivitis and the stem is used to clean the teeth.

Krief [13] confirmed the latter use. The decoction of the stem bark is prescribed against viral diseases. The antimicrobial effects of *Pycnanthus angolensis* [14]. En outre, some research shows a wide range of biological activities, including antioxidant, antiviral, anti-inflammatory, antibacterial, antiallergic, hepatoprotective, cytotoxic, estrogenic, antiestrogenic and pro-apoptotic [1, 15, 16, 17, 18, 19, 20].

The exudate is used cutaneously against skin diseases (scabies, chickenpox, leprosy) [21].

Phytochemistry

The chromatogram (Figure 3) shows the phytochemical composition of the stem bark of *Pycnanthus angolensis*. The presence of terpenes and sterols, saponosides, flavonoids and tannins is observed. These same phytochemicals have been highlighted by some authors [14, 22].



Ter ; Ste : Terpenes and sterols; Sap : Saponosides ; Fla : Flavonoids; Tan : Tannins

Figure 3: Chromatogram of the ethanolic extract of *Pycnanthus angolensis* stem bark

Mineralogy

Table 1 shows the mineralogical composition of the stem bark of *Pycnanthus angolensis*. The amount of magnesium is 621.3 mg/100 g dry matter. It is the most abundant mineral. Ngbolua et al. [23] determined Mg (0.04 ± 0.002 %MS) and Fe (0.007 ± 0.002 %MS).

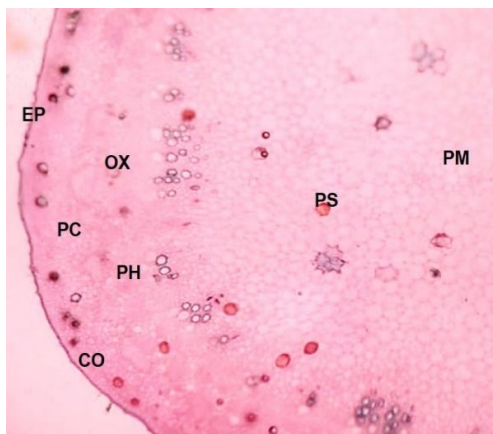
Table 1: Composition and mineral content of *Pycnanthus angolensis* stem bark

Samples	Mg	Cu	Fe	Mn	Zn
mg per 100 g dry matter	621.3	0.27	23.3	10.16	20.3

Mg: Magnesium; Cu: Copper; Fe: Iron; Mn: Manganese; Zn: Zinc

Anatomy

Figure 4 shows a cross-sectional portion of a *Pycnanthus angolensis* stem. From the periphery to the centre of the organ, epidermis, collenchyma, cortical parenchyma, phloem, xylem and medullary parenchyma can be seen. Jackson and Snowdon [24] observed the same tissues in other Myristicaceae. Schizogenic secretory pockets and calcium oxalate crystals are also seen. Indeed, a calcium deposit is observed in a container containing latex taken from the trunk of *P. angolensis*. Secretory pockets are cavities in the parenchyma of the *P. angolensis* stem. These cavities are lined with cells that excrete products that they have synthesised.

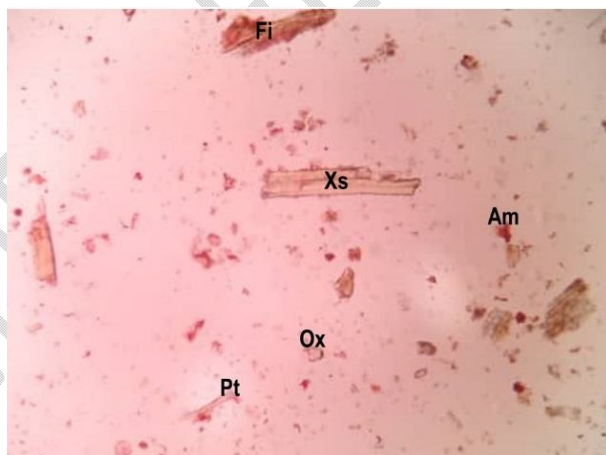


EP: epidermis; CO: collenchyma; PC: cortical parenchyma; OX: oxalate crystals; PH: phloem; XY: xylem; PS: secretory pocket; PM: medullary parenchyma

Figure 4: *Pycnanthus angolensis* young stem cross-section (G: x100)

Micrography

Microscopic observation of *Pycnanthus angolensis* powder identified starch grains, calcium oxalate crystals and tector hairs (Figure 5). Oxalate (1.46g/100g) and carbohydrates (0.92 mg/g) including starch grains were measured in some parts of *Pycnanthus angolensis*. Udeozo et al. [22]. Furthermore, studies have shown that the Myristicaceae family, particularly in the species *Myristica fragrans*, contains abundant starch grains and crystals [24].



Am: starch grain; Ox: calcium oxalate crystals; Fi: fibers; Pt: tector hairs; Xs: spiral xylem

Figure 5: Microscopic elements observed in *Pycnanthus angolensis* stem bark powder (G : x100)

4. CONCLUSION

The study identified the distinctive ethnopharmacological features of *Pycnanthus angolensis* (Fabaceae). Distinctive features such as starch grains and calcium oxalate crystals be observed. In addition, the phytochemical composition justifies the traditional use of *Pycnanthus angolensis* in the traditional treatment of lymphatic filariasis.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

REFERENCES

1. Gustafson K, Wu Q-L, Asante-Dartey J, Simon JE. *Pycnanthus angolensis*: Bioactive Compounds and Medicinal Applications. ACS Symposium Series. 2013. 1127(5):63-78.
2. K. Fern, A. Fern, R. Morris, Useful Tropical Plants Database, Recuperadode <http://tropicaltheferns.info>, 2014.
3. Agyare C, Asase A, Lechtenberg M, Niehues M, Deters A, Hensel A. Anethnopharmacological survey and in vitro confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. J. Ethnopharmacol. 2009;125(3):393-403.
4. N'Guessan K, Konan EK, Tiébré MS. Plants used in the treatment of gyneco-obstetric disorders by the Abbey and Krobou peoples of Agboville (Ivory Coast). Phytotherapy. 2009;7:262-274.
5. Fort DM, Ubillas RP, Mendez CD, Jolad SD, Inman WD, Carney JR, Chen JL, Ianiro TT, Hasbun C, Bruening RC. Novel antihyperglycemic terpenoid-quinones from *Pycnanthus angolensis*. J. Org. Chem. 2000;65(20):6534-6539.
6. Burkill HM. The useful plants of West Tropical Africa. Royal Botanical Gardens. 2000;2:144-150.
7. Akinoye RO, Olatunya AM. Phytochemical screening and mineral composition of the bark of some medicinal trees in Ondo State, Nigeria. Medicinal and Aromatic Plant Research Journal. 2014;2(3):44-49.
8. Kamanzi AK. Medicinal plants of Côte d'Ivoire: Phytochemical investigations guided by biological tests. State Doctorate of Science, Plant Biology, University of Cocody, Abidjan (Ivory Coast). 2002;176p.
9. Adjanohoun EJ, Aboubakar N, Dramane K, Ebot ME, Ekpere JA, Enow-Orock EG, Focho D, Gbilé ZO, Kamanyi A, Kamsu KJ, Keita A, Mbenkum T, Mbi CN, Mbiele AL, Mbome IL, Mubiru NK, Nancy WL, Nkongmeneck B, Satabié B, Sofowora A, Tamze V & Wirmum CK. Contribution to ethnobotanical and floristic studies in Cameroon. CSTR/OUA, Cameroon. 1996;641p.
10. Zirihi GN. Botanical, pharmacological and phytochemical studies of some antimalarial and/or immunogenic medicinal plants used among the Bétés of the Department of Issia, in the West of the Ivory Coast. State Doctorate Thesis, University of Cocody-Abidjan, Ivory Coast. 2006;181p.
11. Lusuna, KM. Contribution to the inventory of charcoal and canoe plants on Mbie Kisangani Island (DRC). Unpublished TFC, FaS /Unikis, 2000;pp10-22.
12. Andres A, Donovan SM, Kuhlenschmidt MS. Soy isoflavones and virus infections. J Nutr Biochem. 2009;20:563-569.
13. Krief S. Plant secondary metabolites and animal behavior: Health monitoring and feeding observations of chimpanzees (*Pan troglodytes schweinfurthii*) in Uganda.

Biological activities and chemical study of consumed plants. Life Sciences. National Museum of Natural History, MNHN Paris, France. 2003;346p.

14. Gnahoue G, Bene K & Coulibaly K. Botanical study, phytochemical screening and in vitro anticandidal activity of *Pycnanthus angolensis* (welw.) warb. (Myristicaceae). European Scientific Journal. 2015;11(36):241-252.

15. Li L, Wang HK, Chang JJ, McPhail AT, McPhail DR, Terada H, Konoshima T, Kokumai M, Kozuka M, Estes JR, Lee K-H. Antitumor agents, 138. Rotenoids and isoflavones as cytotoxic constituents from *Amorpha fruticosa*. J Nat Prod. 1993;56:690-698.

16. Nkengfack AE, Azebaze AG, Waffo AK, Fomum ZT, Meyer M, van Heerden FR. Cytotoxic isoflavones from *Erythrina indica*. Phytochemistry. 2001;58:1113-1120.

17. Hodek P, Trefil P, Stiborová M.. Flavonoids – potent and versatile biologically active compounds interacting with cytochromes P450. Chem Biol Interact. 2002;139:1-21.

18. Li F, Awale S, Tezuka Y, Kadota S. 2008. Cytotoxic constituents from Brazilian red propolis and their structure – activity relationship. Bioorg Med Chem Lett 16: 5434-5440.

19. Martín MA, Serrano AB, Ramos S, Pulido MI, Bravo L, Goya L. Cocoa flavonoids up-regulate antioxidant enzyme activity via the ERK1/2 pathway to protect against oxidative stress-induced apoptosis in HepG2 cells. J Nutr Biochem. 2010;21:196-205.

20. Orhan DD, Ozcelik B, Ozgen S, Ergun F. Antibacterial, antifungal, and antiviral activities of some flavonoids. Microbiol. Res. 2010;165:496-504.

21. Elouma Ndinga AM. 2015. Inventory and chemical analysis of plant exudates commonly used in Congo-Brazzaville. Paris-Sud University, France. 211p.

22. Udeozo IP, Ejikeme CM, Eboatu AN, Arinze RU. The Efficacy of *Pycnanthus angolensis* Timber: An Assay of its Properties, Chemical Constituents and Functional Group Analysis. Global J. Biotech. & Biochem. 2015;10(3): 121-125.

23. Ngbolua K-t-N, Justin A. Asimonyio, Ndrodza N, Mambo B, Bugenth P, Isangi Y, Mukirania JK, Ratsina L, Ngombe NK, Mpiana PT. 2016. Nutritional value and cyanhydric acid content of eight plant species consumed by *Okapia johnstoni* (Mammalia: Giraffidae) in Democratic Republic of the Congo. International Journal of Innovation and Scientific Research. 2016;23(2):419-427.

24. Jackson BP, Snowden DW 1990. Atlas of microscopy of medicinal culinary herbs and spices. Belhaven Press, 25 Floral Street, London WC2E 9DS. 1990;257p.