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PHARMACOGNOSTIC STUDIES AND ANTIMICROBIAL ACTIVITY OF *CRESCENTIA CUJETE* LINNAEUS STEM BARK (BIGNONIACEAE)

Ugwoke Christopher Emeka Chukwunonye*, Diovu Edith Obioma, Anze Sunday Patrick Gaza and Chukwube Vincent Obisike

Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka, Nigeria.

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Abstract:

Crescentia cujete tree is widely and various used for wood, household materials, culinary and therapeutic purposes. Virtually all parts of this tree are useful across tropical and subtropical continents. ***Aim****: this study covers the pharmacognostic standardization of the stem bark of C. cujete to ensure identity, quality control and purity of the plant drug material. The antimicrobial potential was also assayed to form a scientific basis for such claims by traditional therapists.* ***Materials and Methods****: This involved macroscopical studies via botanical examination techniques, anatomical sections of fresh samples of the stem bark, microscopic studies of powdered stem bark, with photomicrograps, and physicochemical (analytical) evaluation. Methanolic crude extract and various fractions were made and tested on clinical isolates of Bacillus subtitlis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Candida albicans and Aspergillus niger, using agar well (8 mm) diffusion.* ***Results:*** *the tree has pale to brown multiple trunks, smooth surface and brown nodules. Cut stem bark is cylindrical. Microscopy revealed calcium oxalate embedded in the cork, bundles of elongated fibres, sclerenchymatous and phloem parenchymatous cells. Alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, resins, carbohydrates, proteins, and fatty oils were detected. Antimicrobial inhibition was observed in the reconstituted extract and serial dilution for MIC. This was concentration dependent and increased in the order: EF>MF>HF>MCE.* ***Conclusion:*** *The Pharmacognostic features revealed are essential in creating a monograph of the plant. More so, the observed antimicrobial activity gave credence to claims of its use in ethnopharmacology.*

Keywords: *Crescentia cujete, macroscopical, physicochemical, clinical isolates, parenchyma*

Corresponding Author:

Ugwoke Christopher Emeka Chukwunonye,

Department of Pharmacognosy and Environmental Medicine,
University of Nigeria Nsukka,
Nigeria.

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INTRODUCTION:

Crescentia cujete L. also called calabash tree (English) or *Obele ukpachi* (Igbo) is an evergreen tropical tree of the family *Bignoniaceae* [1]. It is native to Central and South America, and naturalized in India. In the wild it is commonly found on hillside pastures especially in dries areas and can be propagated by seed or stem cuttings but grows slowly [2]. *C. cujete* produces a poisonous fruit pulp but the seeds are could be cooked and eaten. Other parts are claimed to have varied therapeutic uses viz. treatment of respiratory illnesses such as bronchitis, influenza or asthma by reducing the characteristic coughing and spasms caused by the ailments, gastrointestinal disturbances and intestinal disturbances by reducing pain and diarrhoea [3]. It also helps to stimulate or increase insulin production, lower blood pressure and to treat infections caused by *Staphylococcus aureus*. Preparations containing this plant is reported to induce labour went taken by pregnant women. An infusion of the flowers is used to remedy earache and whooping cough, and the seed as a contraceptive remedy [1, 4].

This study evaluated the pharmacognostic features of *Crescentia cujete* in order to highlight these essential inherent peculiar analytical characteristics to foster authentication and quality assurance.

Fig. 1 – *Crescentia cujete* tree

MATERIALS AND METHODS:

Collection and identification of plant materials

Fresh stem of the bark of *Crescentia cujete* were collected in November 2013 in Enugu-Ezike, Enugu State Nigeria. Fresh twigs were included and authenticated by Mr. A.O. Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD) 110 Aku Road Nsukka, Enugu State, Nigeria. A voucher specimen was deposited and designated INTERCEDD1113/Big.

Preparation of plant material

The stem bark was washed, cut into pieces and air-dried for 21 days. The dried samples were triturated using an electrical grinder. The fine powder was stored in amber coloured glass containers until required for use.

Macroscopical Examinations

The macroscopical features of the leaf and stem bark were described according to standard methods [5, 6]; organoleptic properties – colour, odour and taste [6] were noted.

Anatomical sectioning

Using a sledge microtome, a thin Transverse Section (TS) of the stem bark was made according to reported method [7]. The distribution of tissues and structural details of individual cells were observed and photomicrographs were taken with the aid of Motic image plus 2.0 digital Camera.

Physicochemical evaluation of the crude drug

Standard and reported procedures [8, 6, 7, 9, 10, 11] were used to evaluate the coarse powder of the stem bark of *C. cujete* for following analytical parameters: moisture content (LOD method), total ash, water soluble ash value, acid insoluble ash value, sulphated ash value, alcohol extractive value and water extractive value.

Analytical thin-layer chromatography (TLC)

TLC plates were prepared by coating the plates (10 x 20 cm) with silica gel G60 F254, with the help of a binder using a suitable spreader. The TLC plates (0.25 mm layer) were reactivated at 100o C for 5 min in an oven before use. A slight line (1.5 cm) from the base of each plate was carefully marked. A drop of the samples was placed along the line centered as much as possible. These applications were duplicated and allowed to dry up. These plates were placed in a 1 cm layer of solvent system at the bottom of the developing chamber and the lid was placed over. Inside the developing chamber (or chromatographic tank), the chromatogram was developed. After 1 hr, the plates were removed from the tank and the solvents were allowed to evaporate. The major spots were located using iodine chamber at 100o C as the detecting reagent. From the chromatograms developed, the retardation factors (Rf-values) of the major spots were calculated using the formula:

$$\mathbf{Rf} = \frac{\textbf{Distance moved from origin by a solute}}{\textbf{Distance moved from origin by solvent front}}$$

Crude extraction

About 369 g of the powdered stem bark was macerated with 80 % methanol. A mechanical shaker was used to shake the mixture for 6 hours and allowed to stand at room temperature for 66 hours and filtered. The residue was subjected to alternative soaking in fresh solvent for 24 hours and filtration to attain some level of exhaustive extraction, judged by

the loss of colour of the filtrate [12]. The collective filtrate was concentrated using a rotary evaporator at 40 – 50o C to obtain. The Methanol crude extract (MCE) was transferred to a sterile sample container and preserved in a refrigerator at 4o C until required.

Fractionation

The Methanol crude extract was adsorbed on silica gel G60 F254 and eluted in succession with n-hexane, ethyl acetate and methanol to yield the respective fractions.

Phytochemical analysis

Various qualitative phytochemical tests were carried out on both the MCE and fractions based on standard [13, 6] to screen for the presence of alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, proteins, fats and fatty oils, resins, carbohydrates and reducing sugars.

Antimicrobial assay

This was by means agar well (8 mm) diffusion method [14] with Nutrient Agar (NA) and Saboraud's Dextrose Agar, SDA, (Oxoid) plates for bacteria and fungi respectively. The extracts and fractions were respectively reconstituted to 300 mg/ml in Dimethylsulfoxide (DMSO) as solubilizing medium whereas 50 mg/ml Ciprofloxacin was used as reference drug. These were tested on clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhi* and *Candida albicans*. Incubation was at 37o C for 24 hours for bacteria and *Candida albicans*. The Inhibition Zone Diameter (IZD) was measured to the nearest mm. The Minimum Inhibitory Concentration (MIC) was determined using the serial dilution method [15].

RESULTS:

Macroscopical features of the stem bark are presented in Table 1.

The transverse section (TS) of the stem bark of *C. cujete* (Fig.2) showed upper epidermis with characteristic cell inclusions. The cortex is made up of isodiametrically shaped outer collenchymas and inner parenchyma, phloem cells, pitch parenchyma with thick walls and sclerenchymatous cells. The powder microscopy (Fig. 3 – 6) showed bundles of fibres with embedded rosettes of calcium oxalate, bundles of sclerenchyma cells and fragments of phloem parenchymatous cells with uniseriate medullary rays.

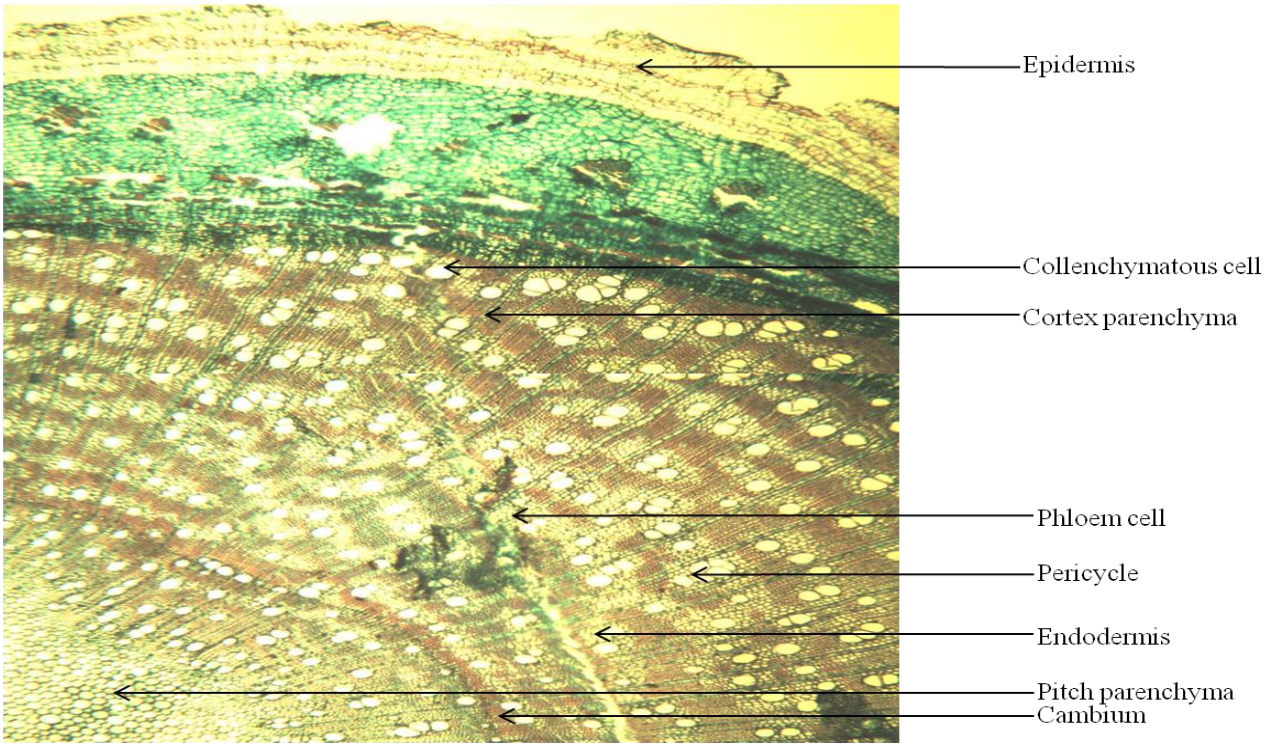

Fig. 2 – Transverse Section of the stem bark of *Crescentia cujete*

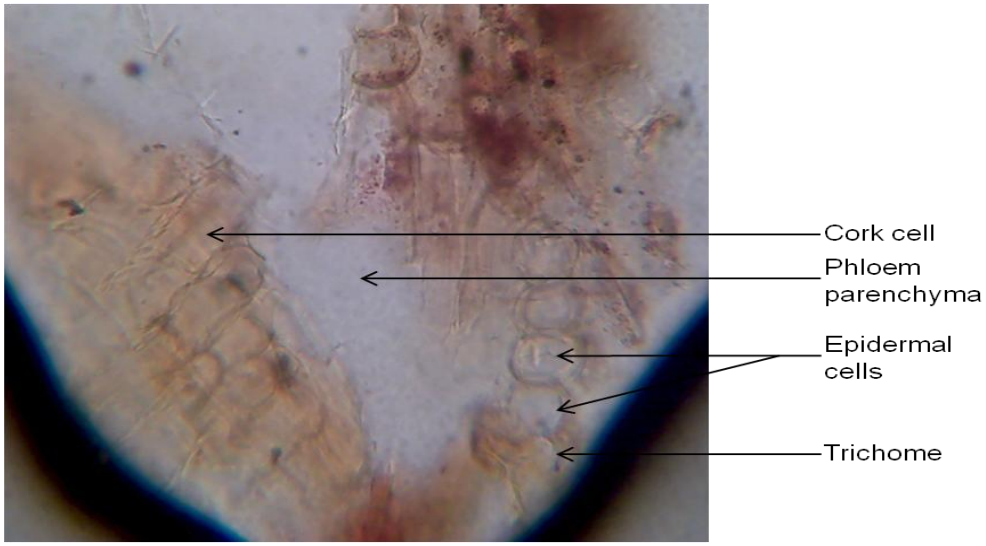

Fig. 3 – Cork and fragment of phloem parenchyma cells with uniseriate medullary rays

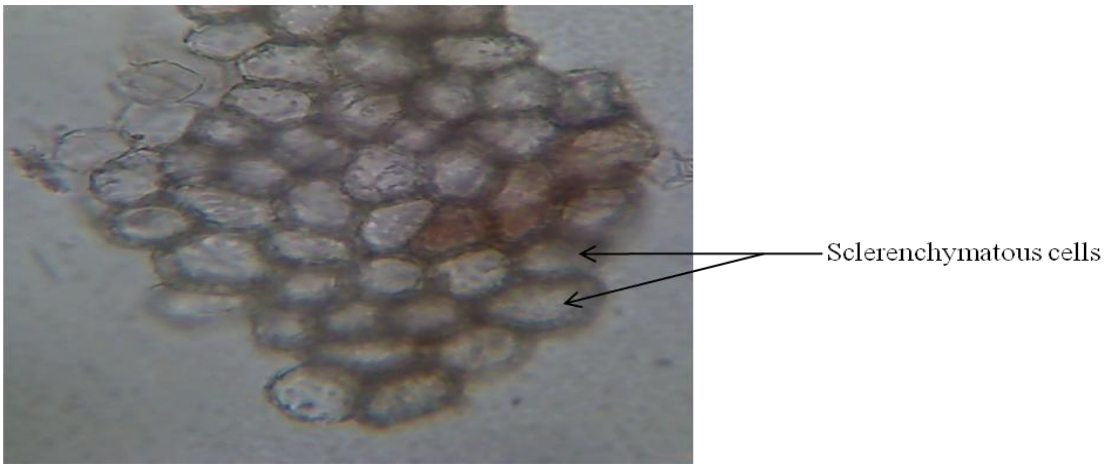

Fig. 4 – Bundle of sclerenchyma cells

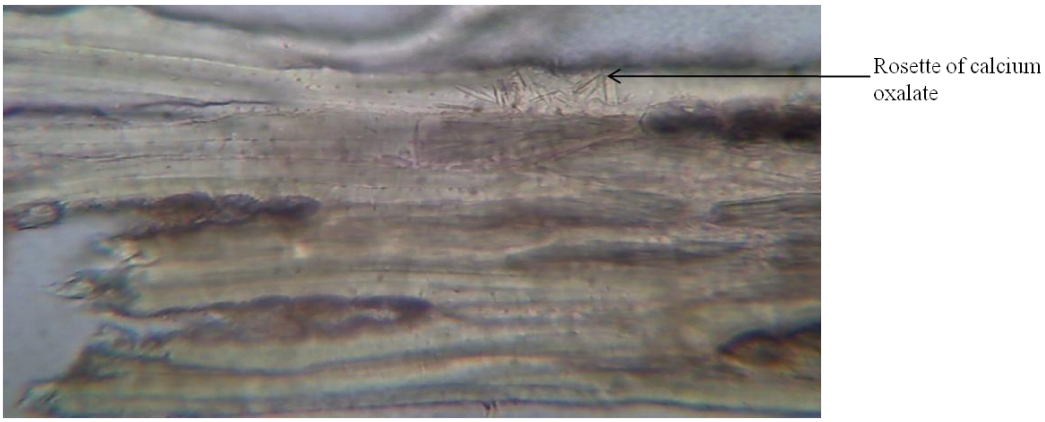

Fig. 5 – Bundles of fibres with embedded calcium oxalate

Fig. 6 – Phloem parenchyma cells

Table 1 – macroscopical features of *Crescentia cujete* stem bark

S/N	Character	Observation
1	Colour	Pale brown
2	Odour	Characteristic
3	Surface	Smooth with spot of brown nodules
4	Trunk	Multiple trunks without thorns
5	Fracture	Evident; trunk hard and brittle when dry
6	Shape	Cylindrical
7	Internodes	Short

Table 2 – physicochemical (analytical) features of *Crescentia cujete* stem bark

S/N	Parameters	% composition
1	Moisture content (LOD) (% w/w)	12.54 ±0.01
2	Total ash (% w/w)	06.60 ±0.06
3	Acid-insoluble ash (% w/w)	03.58 ±0.00
4	Water soluble ash (% w/w)	07.50 ±0.06
5	Sulphated ash (% w/w)	08.00 ±0.00
5	Water soluble extractive value (% w/w)	01.20 ±0.02
6	Alcohol soluble extractive value (% w/w)	01.10 ±0.02

Analytical TLC for the methanol crude extract (MCE) of *C. cujete* showed the following R_f – values in different solvent systems (Table 3) shows four spots for water extract and six spots for methanol extract while the two solvent mixtures of chloroform and methanol (1:1), chloroform and ethanol (1:1) revealed two spots for water extract and five spots for ethanol extract.

Table 3 – Analytical TLC of *Crescentia cujete* stem bark MCE

Extract	R_f – values in Chloroform: Methanol: Ammonium hydroxide (5:4:1)	R_f – values in Chloroform: Methanol (1:1)	R_f – values in Chloroform: Ethanol (1:1)
Water extract	0.42, 0.48, 0.55, 0.64	0.55, 0.64	0.50, 0.70
Methanol Crude Extract	0.37, 0.55, 0.60, 0.65, 0.70, 0.79	0.50, 0.65, 0.75, 0.77, 0.80	0.20, 0.30, 0.45, 0.50, 0.60

The relative yield on extraction and fractionation are presented in Table 4.

Table 4 – yield on extraction and fractionation of *Crescentia cujete* stem bark

Component	Yield (g)	Percentage yield (w/w)
Methanol Crude Extract	32.50	08.21
N-hexane fraction	09.50	02.39
Ethyl acetate fraction	12.50	03.16
Methanol fraction	10.50	02.65

Qualitative phytochemical screening revealed the presence of various plant secondary metabolites (Table 5)

Table 5 – phytochemicals of *Crescentia cujete* stem bark

S/N	Phytochemicals	Relative Abundance			
		MCE	HF	EAF	MF
1	Alkaloids	+++	+	++	+++
2	Saponins	++	–	–	++
3	Tannins	++	–	+	+++
4	Flavonoids	++	+	++	+
5	Steroids	++	+	++	++
6	Terpenoids	+++	++	+++	++
7	Proteins	+++	+	++	++
8	Fats and fatty oils	+++	+++	++	++
9	Resins	+++	+	++	+
10	Carbohydrates	++	–	+	++
11	Reducing sugars	++	–	+	++

Key: – absent, + present, ++ more present, +++ abundant

The result for antimicrobial sensitivity test indicated that the *C. cujete* possessed broad spectrum of activity (Table 6). The crude extract showed highest zone of growth inhibition with mean IZD of 12 mm and MIC of 15.625 mg/ml against *B. subtilis* and least inhibition with mean IZD of 6 mm and MIC of 125 mg/ml against *P. aeruginosa*. Hexane fraction showed highest zone of growth inhibition with mean IZD of 12 mm and MIC of 15.625 mg/ml against both *S. aureus and B. subtilis*, and least inhibition with mean IZD of 4 mm and MIC of150 mg/ml against *P. aeruginosa*. Ethyl acetate fraction showed highest zone of growth inhibition with mean IZD of 14 mm and MIC of 7.813 mg/ml against *S. aureus* and *B. subtilis*, and least inhibition with mean IZD of 4 mm and MIC of 125 mg/ml against *P. aeruginosa*. Methanol fraction showed highest zone of growth inhibition with mean IZD of 14 mm and MIC of 7.813 mg/ml against *S. aureus* and least inhibition with mean IZD of 4 mm and MIC of 150 mg/ml against *P. aeruginosa.* The Ciprofloxacin showed highest zone of growth inhibition with mean IZD of10 mm and MIC of 3.125 mg/ml against *B. subtilis* and MIC of 0.25 mg/ml against *C. albicans*.

Table 6 - Antimicrobial Inhibition (mm) of Methanol Crude Extract and Fractions of *C. cujete* stem bark

Microorganisms	300 mg/ml				5 mg/ml
	MCE	HF	EAF	MF	Ciprofloxacin
S. aureus	10.00	12.00	14.00	14.00	08.00
E. coli	08.00	10.00	08.00	04.00	06.00
P. aeruginosa	06.00	04.00	06.00	04.00	03.00
B. subtilis	12.00	12.00	14.00	10.00	10.00
S. typhi	10.00	08.00	12.00	06.00	
C. albicans	08.00	06.00	10.00	08.00	08.00

DISCUSSION:

This study has revealed specific inherent qualitative and quantitative analytical features of *C. cujete* stem bark on the bases of which its authenticity and purity can be verified. The macroscopic properties (Table 1), which also covers Organoleptic features via botanical description has provided the simplest and quickest means for identification and authentication of the plant. It is also reputed in quality assurance of the herbal material. The microscopic anatomical structures (Fig. 2 – 6) provide closer cellular discrimination in their intact natural arrangement. This is essential for authentication as no two plant species will possess exactly the same cellular patterns qualitatively and quantitatively in all respects. The physicochemical properties (Table 2) are useful in detection of adulteration of the original plant material, thereby useful in quality assurance. Total ash value is useful for the exclusion of drugs which have been coated with lime, chalk or calcium sulphate to improve their appearance. The solvent soluble extractive yields (Table 2) are useful to indicate chemicals that are extractable or exhausted by particular solvents [8] and thus useful in quality assurance. The moisture content gives a guide to the extent of care during storage of the herbal material to avoid microbial deterioration and consequently loss of active chemical constituents. The analytical TLC (Table 3) revealed that increased number of solvent mixtures increases the number of spots. The presence of the various phytochemicals has been previously reported in the leaves of *C. cujete* [3]. The crude extract and fractions of *C. cujete*, exhibited significant antimicrobial activity against the study microorganisms (Table) and can this can be linked to the presence of various phytochemicals such as tannins, flavonoids, alkaloids, saponins and steroidal components [17] in varying concentrations. The crude extract and fractions were more sensitive on Gram-positive than the Gram-negative bacteria, and activity was concentration-dependent when compared with the standard drugs.

CONCLUSION:

This study has specified salient pharmacognostic parameters that are essential for correct identification and quality assurance. As well it substantiated the use of this plant in ethno medicine for treatment of various ailments caused by the microbial pathogens that were sensitive to *C. cujete* crude extract and fractions in this study. The findings of this research would therefore be instrumental in creating a monograph for *Crescentia cujete*.

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