Investigations on the Morphology, Anatomy, Phyto-chemistry and Anti-microbial activities of the stem and leaves of *Euphorbia kamerunica* Pax

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Abstract

Euphorbia kamerunica is commonly used by traditional healers to prepare medicine for many ailments in Nigeria. The present study revealed the morphological features, anatomical properties (leaf epidermis), phyto-chemical constituents (qualitative) and anti-microbial activities of Euphorbia kamerunica. It was observed that both morphological features and anatomical properties have taxonomic significance. Also, both antimicrobial activities and phyto-chemical studies support Euphorbia kamerunica to be a potential source of useful drugs. Further studies are required in order to isolate the active components from the crude plant extracts for proper drug development. This is necessary in drug research so as to prevent adulteration of the medicinal plant.

Keywords; *Euphorbia kamerunica, Medicinal plants, Pharmacognosy, Phyto-chemicals, stomata.*

Introduction

In developing countries, plants are the main sources of drugs administered by man to cater for his health challenges and general body maintenance. In order to ensure that the qualities of these plant products are reproducible, correct identification of the starting materials is paramount. Proper and correct identification (that is based on the external morphology) is only possible when a complete plant specimen is available. In order to be certain that the correct species has been acquired; the material should be authenticated using macroscopic and microscopic botanical examinations (Breeman *et al.* 2007). This is because adulteration of the real plant is inevitable.

In Nigeria, a lot of plants are useful in herbal medicine. *Euphorbia* plant (popularly known as spurges) is one of the largest recognized genus of flowering plants with about 2,000 species. The morphological diversity in this genus includes geophytes, herbs, shrubs, under storey and canopy trees, and an array of succulent and xerophytic forms. Despite the vast vegetative variation, the entire genus is united by a distinctive inflorescence that looks superficial like a typical dicot flower (Horn *et al*., 2012).

Seven hundred and twenty three species of *Euphorbia* are found in Africa (Carter and Eggli, 2003). Thirty four of these were described for West Africa by Hutchinson and Dalziel (1958). Twenty four widely distributed species were deposited in major herbaria in Nigeria. Some of the species have medicinal, cultural and economic importance, for example as purgatives, diuretic agents, manufacturing of gun powder and in fireworks. Also, because they accumulate

steroids and triterpenoids, they are industrially important chemical sources of insecticides, steroids, pesticides, antibiotics, expectorants and sedatives, cyto-toxins, remedies for cancers, tumors and warts.

In Nigeria, several cases of poisoning with severe gastroenteritis, vomiting and diarrhea when Euphorbia plant was taken in orally have been reported. An uncommon but useful species is *Euphorbia kamerunica* Pax. It is a succulent species used in folk medicine as drugs or as raw materials for medicinal preparations in Nigeria. Fai and Fagade (2005) reported poisoning of streams with *Euphorbia kamerunica* when capturing fishes. Also, antimicrobial activities of the plant had been reported by many authors such as; Ogunnusi and Oso (2014).

Therefore, the present study was undertaken in order to contribute more data on the morphology, phyto-chemistry, antimicrobial activities and leaf anatomy of *Euphorbia kamerunica*. These features will be useful in differentiating the plant from other related species. This research is a complementary study for pharmacognostic drug research.

Materials and methods

Morphological (vegetative and floral) assessments of all available herbarium and fresh specimens of *Euphorbia kamerunica* in Nigeria (Table 1) were carried out. Descriptive terminologies employed were based on Hutchinson and Dalziel (1958). All measurements were made (where possible) on similar parts and at comparable developmental stages either with thread, meter rule, dissecting microscope or magnifying hand lens (X10). Statistical analysis of means, standard deviation, standard error and variance were done using Microsoft Excel. All necessary drawings and photographs were taken using Motic Image Plus version 2.0ML fitted to Olympus XSZ-N 107 binocular microscope in Botany Research Laboratory, University of Lagos, Akoka, Lagos.

Foliar epidermal morphology was studied using both fresh and herbarium materials. Methods followed the approach of Faboyede (2015). Leaf architectural terminologies followed Dilcher (1974). Dried Leaves were boiled in soapy water for about ten to twenty minutes. Both fresh and the already boiled leaves were soaked in domestic bleach (5% sodium hypochlorite) until suitable for separation into upper and lower epidermises. The two surfaces were cleaned with camel hair brush and labeled accordingly. These were rinsed in distilled water, stained in 1% safranin and dehydrated in 50%, 60%, 70%, 80%, 90% and absolute ethanol. They were mounted in glycerine on slides and examined under the microscope at x160 and x640 magnifications.

Thirty randomly selected epidermal cells and stomata were measured using a micrometer eye-piece. Twelve micro characters were examined on each sample. They include: (i) length of epidermal cells (ii) width of epidermal cells (iii) cell wall pattern (iv)type of stomata (v) length of stomata (vi) width of stomata (vii) presence or absence of trichomes (viii) type of trichomes (Table 2)

Statistical analysis of means and variances were carried out using Microsoft Excel. Charts were used to depict the major quantitative features. Photomicrographs of all major characters were taken using Motic Image Plus version 2.0ML fitted to Olympus XSZ-N 107 binocular microscope in Botany Research Laboratory, University of Lagos.

Phyto-Chemical studies

Preparation of plant materials

Fresh stems of the plant were weighed and dried in the oven at 50^0C for about 6 days. The weight was recorded after drying. The dried sample was crushed into uniform powder using pestle and mortal. The aqueous and methanolic extracts of the powder obtained were prepared (using soxhlet extractor for at least 10 hours or until complete extraction had taken place). These were further evaporated to dryness using vacuum rotary evaporator. Qualitative chemical tests for bioactive compounds were performed on the extracts using standard methods as documented by Egwaikidi *et al*. (2009) with some modifications. Table 3 shows the result of the phyto-chemical screening,

Antimicrobial studies

The microorganisms (bacteria and fungi) used for this study were isolates obtained from the Microbiology laboratory of the Department of Biological sciences, Crawford University, Igbesa. Ogun State. Nigeria.

Antimicrobial Sensitivity Test

Antibacterial and antifungal activities of the stem extracts of *Euphorbia kamerunica* against four pathogenic bacteria and three fungi were investigated by the agar disc diffusion method. Each purified extracts were dissolved in dimethyl sulfoxide (DMSO) and stored at 4°C. All the extracts were screened for their antibacterial and antifungal activities against four bacteria; *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae* and three fungi; *Candida albicans, Aspergillus niger* and *Fusarium oxysporum*.

0.1 ml of the bacteria (10^6 CFU/ml) was introduced into the Petri dishes and 15 ml of Mueller Hinton Agar distributed into Petri dishes. Also, 0.1 ml each of the fungi was introduced into the Petri dishes and 15 ml of Sabouraud Dextrose Agar was distributed. 7mm wells were cut into the agar using sterile cork borer and 0.2 ml of the extracts (*Euphorbia kamerunica*) of different concentrations (6.25, 12.5, 25, 50, and 100 mg/ml) were introduced into the wells. Control experiments were carried out under similar condition using Gentamycin (10 µg/ml) for antibacterial activities and Nystatin for antifungal activities as standard drugs. The zones of inhibition of growth around the discs were measured after 18 to 24 hours of incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C in order to determine the sensitivities of the microorganisms to the extracts (*Euphorbia kamerunica*). Values ≤ 8 mm were considered as not active against microorganisms.

RESULTS AND DISCUSSION

The result of the morphological study revealed specific features (Table 1; Plate 1a-d) that are in accordance with the information provided by Hutchinson and Dalziel (1958). *Euphorbia kamerunica* is a cacti-form Euphorbia with green stems that are 3-6 angled. The spines were either solitary or paired. The leaves were simple and decussately arranged. The leaf shape were obovate, margins were entire while the base were cuneate. This authenticates the plant materials used for this study. Also these may serve as reliable morphological features of *Euphorbia kamerunica* that scientists can look for while working on the plant.

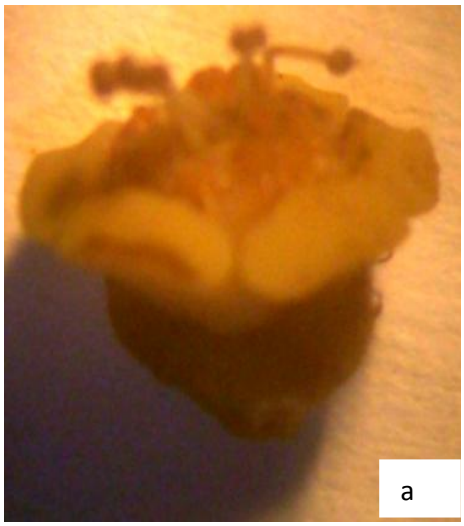
Sometimes, there may be challenging questions on the use of morphological features in the identification of plants. This study recognized the fact that anatomic features had proved useful in some taxonomic studies, for example; Ayodele and Olowokudejo (2006); Akinnubi, *et. al*.(2013). In the present study, unique micro- morphological features were observed and therefore suggested as good taxonomic features of *Euphorbia kamerunica*. Table (2) shows both qualitative and quantitative features of the leaf epidermis of *Euphorbia kamerunica*. Qualitative features are more preferred over quantitative features. This is due to the likely effects of environmental factors on quantitative features.

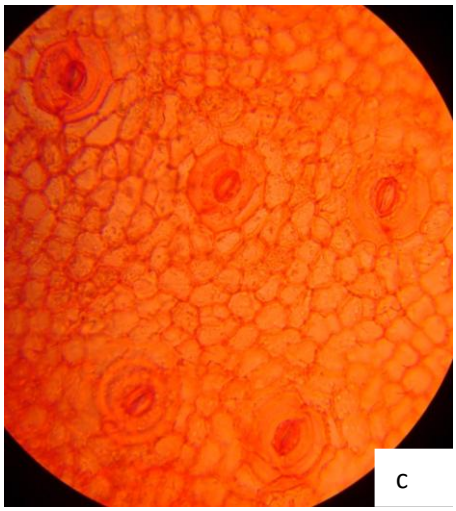
Table 1: Gross Morphology of fresh samples of *Euphorbia kamerunica* Pax

Vegetative Morphology														**Floral Morphology**					
Stem				**Leaves (Mean ± SE) (cm)**															
Nature	**Shape**	**Spines**	**Indumentum**	**Types**	**Arrangement**	**Shape**	**Leaf Length**	**Leaf width**	**Margin**	**Color**	**Base**	**Apex**	**Petiole**	**Location**	**Arrangement**	**Involucres**		**Fruit**	**Gland**
Cacti form (tree)	Angled-3-6	Paired/Solitary	Along the angles	Simple	Decussate	Obovate	0.60 ± 0.12	0.20 ± 0.01	Entire	Green	Cuneate	Emarginated	Ab	Intercalary	Single	Yellow	Bract-Like	Capsule	Oval	Four	Yellow

Table 2: Leaf Epidermal micro-morphological features on *Euphorbia kamerunica* Pax

Qualitative features								Quantitative features							
Anticlinal wall pattern		Type of Stomatal complex		Trichomes		Surface ornamentation		Abaxial Surface {Min(Mean±SE)Max}				Adaxial surface {Min(Mean±SE)Max}			
								Epidermal Cell		Stomata		Epidermal Cell		Stomata	
Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Length (µm)	Width (µm)	Length (µm)	Width (µm)	Length (µm)	Width (µm)	Length (µm)	Width (µm)
Straight	Straight	Paracytic	Paracytic	Ab	Ab	Ab	Ab	35.00 (37.98±0.26) 47.50	15.00 (19.43±0.15) 22.50	50 (63.28±0.38) 70	35.00 (46.15±0.31) 55	20.00 (21.63±0.17) 25.00	25.00 (37.50±0.4)47.50	25.00 (27.25±0.21)29.01	28.05 (29.98±0.03)31.05




c


d

Plate1: Morphological features of *Euphorbia kamerunica* showing:
a. single inflorescence b. a capsule c. microscopc features of the leaf d. stem

Out of the 14 phyto-chemicals investigated, 13 were present in the methanolic stem extracts of the plant at varying concentrations while cyanogenic glycoside was absent (Table 4). The phyto-chemicals present are: tannins and phenolic compounds, phlobatannins, alkaloids, anthraquinones glycosides, cardiac glycosides, flavonoids, terpenes, saponins, carbohydrates, monosaccharides, proteins, reducing sugars and steroids.

Table 3: Phyto-chemical agents present in methanolic extracts of the stem of *Euphorbia kamerunica*

S/N	Phyto- chemicals	Methanolic extract
1	Alkaloids	++
2	Cyanogenic glycosides	-
3	Anthraquinones glycosides	+
4	Carbohydrates	+
5	Cardiac glycosides	+
6	Flavonoids	+++
7	Monosaccharides	+
8	Proteins	+
9	Reducing sugars	+
10	Saponins	++
11	Steroids	+
12	Phlobatinins	+
13	Tannins and phenolic compounds	++
14	Terpenes	+++

The bioactive compounds responsible for the inhibitory effects recorded in Table 3 were detected in the phyto-chemical screening, some of which were reported in literature as antimicrobial constituents (Alzoreky and Nakahara, 2003; Igoli *et. al.* 2005)

The methanolic extract of the dried stems of this plant gave varying degrees of antimicrobial activities (Table 4). Similar results were obtained for the activities of the stem extracts of the plant on both bacteria and fungi used. The highest activity (30.5± 0.5mm) was recorded on *Staphylococcus aureus* at 100mg/ml. This was higher than the effects of the antibiotic Gentamycin. There were no significant differences between the activities of Gentamycin and 100mg/ml of the extract on *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Also, for the fungi, the extract had greater effect on *Candida albicans* than the antibiotic Nystatin at 100mg/ml. There were no significant differences between the effects of the extract on *Fusarium oxysporum* and that of the antibiotic Nystatin at 100mg/ml. However, the antibiotic was more effective than the stem extracts on *Aspergillus niger* at 100mg/ml. Similar results had been obtained previously on *Euphorbia species* (Annapurna *et al.* (2004), Kumara (2011) and Ogunnusi and Oso (2014).

Table 4: Antimicrobial Activity of Crude Methanolic Extract of *Euphorbia kamerunica* (stem)

Zones of Inhibition (mm) ± S.D with Different Concentration (mg/ml)									
S/N	Organisms	100	50	25	12.5	6.25	Negative control	NYST	GENT
1	*Escherichia coli*	17.5 ± 2.5a	12.5 ± 0.5b	12.0± 3.0b	8.5 ± 0.0c	7.5 ± 0.0c	7.0± 0.0c	NT	15.0 ± 0.0a
2	*Klebsiella pneumoniae*	16.0± 2.0a	11.5± 1.0b	8.0± 0.0c	7.5± 0.0c	7.0± 0.0c	7.0± 0.0c	NT	18.0 ± 0.0a
3	*Staphylococcus aureus*	30.5± 0.5a	22.0 ± 2.0b	15.0±1.0c	13.0± 1.50c	7.0 ± 0.0d	7.0 ± 0.0d	NT	14.0 ± 0.0c
4	*Pseudomonas aeruginosa*	21.5 ± 0.5a	15.5 ± 0.5b	15.0 ± 1.0b	14.5 ± 0.5bc	7.0 ± 0.0c	7.0 ± 0.0c	NT	23.0 ± 0.0a
5	*Candida albicans*	23.0 ± 2.0a	22.5 ± 1.5a	17.0 ± 0.0b	9.0 ± 0.0c	9.0 ± 0.0c	7.0 ± 0.0d	23± 0.0a	NT
6	*Aspergillus niger*	12.0 ± 1.0b	11.5 ± 0.5b	8.0 ± 0.0c	7.0 ± 0.0c	7.0 ±0.0c	7.0 ± 0.0c	18.0 ± 0.0a	NT
7	*Fusarium oxysporum*	20.0 ± 2.0a	15.0 ± 1.0b	13.5 ± 1.5b	8.0 ± 0.0c	7.0 ± 0.0c	7.0 ± 0.0c	17.0 ± 0.0b	NT
Note: Values in the same row followed by the same letter are not significantly different (p>0.05) from each other; Diameter of cork borer = 7.0 mm; NT = Not Tested, GENT = Gentamycin, NYST =Nystatin									

Conclusion

The antimicrobial effects of the stems of *Euphorbia kamerunica* established in this result have lent credence to its ethno-botanical usage in treating infections especially Bacteria and Candida. However, the plant should be consumed in small doses since it was found to have toxic effects on streams of water as described by Fai and Fagade (2005). Also, microscopic and macroscopic features of the leaves and flowers of *Euphorbia kamerunica* provided by this research will be useful in the identification process when carrying out pharmacological studies. This study therefore suggests the need for detailed investigations on phyto-chemical and pharmacological properties of *Euphorbia kamerunica* in order to isolate the bioactive compound(s) and investigate the antimicrobial activities against a wider range of pathogenic microorganisms.

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