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COMPARATIVE CHEMOTAXONOMIC STUDIES ON SROTI (*Mallotus oppositifolius* (GEISELER)) MULL AND VELVET BUSH WILLOW (*Mallotus alternifolius*) MERR

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ABSTRACT
Comparative morphological, anatomical and phytochemical studies on two species of Euphorbiaceae (M. oppositifolius and M. alternifolius) found in Niger Delta were studied.. The morphological study was done using direct observation, characteristics studied were the leaf length, width, length of petiole and internode; M. oppositifolius and M. alternifolius are unisexual and are mesophytes. The leaves are simple, branching is erect and crown type. Phyllotaxy is opposite in M. oppositifolius and alternate in M. alternifolius. In M. oppositifolius, the leaf is 2 ± 2.3 long and 8.1 ± 2.2cm wide; petiole is 7.9 ± 2.4cm long, internode 3.7 ± 1.1cm long whereas in M. alternifolius, the leaf is 6. 1 ± 0.7cm long and 7.8 ± 1.8cm wide; petiole is 2.0 ±0.6 long, internode 7.8 ± 1.8cm long. They are perennial shrubs and both species have reticulate venation. The anatomy was done using free hand sectioning, the adaxial and abaxial surfaces of the leaf showed anomocytic type of stomatal arrangement, revealed the presence of both living cells (collenchymatous and parenchymatous cells with vascular bundles). Phytochemical screening was carried out using Trease and Evans method. The result of the phytochemical studies revealed the presence of bioactive constituents tannin, flavonoids, phlobatannins, anthraquinones and cardiac glycosides in the two species while alkaloids, cyanogenic glycosides and saponins were found to be absent in both species. M. alternifolius contains higher crude percentage of the bioactive compounds than M. oppositifolius.
Keywords: Morphological, Anatomical, Phytochemical, Mallotus Spp.

INTRODUCTION

Mallotus oppositifolius (Geiseler) Mull. belongs to the family Euphorbiaceae (Sierra *et al*., 2010) it is a dicotyledon plant which belongs to the genus *Mallotus (* Slik *et al.,* 2003). *M. oppositifolius* is a perennial shrub or tree which can be found from Senegal to Ethiopia, Angola, Mozambique and Madagascar. It is also widely distributed in Ghana and Nigeria. It grows up to 6 to 13cm tall (Kamgang *et al.,* 2006). The young shoots and densely stellate-hairy; older twigs almost glabrous, often purplish brown. Leaves are opposite and simple (Adekunle and Ikumapayi, 2006). petiole is long and short in each pair, 2.5 – 11cm long when long and 0.5-2cm long when short, slightly thickened at the both ends; stipules are long, soon falling; blade is broadly ovate to oblong ovate, 3-18-21cm x 2-13cm, unequal in size in each pair; base shallowly cordate to rounded or truncate, margins almost entire or more or less deeply toothed or lobed, 3 veined from the base, sparingly stellate-hairy to almost glabrous, sparingly gland dotted, also with simple hair beneath. Inflorescence a terminal or axially raceme; male inflorescene up to 10-15cm long, female one up to 10-18cm long, bracts 0.5-1.5mm long, triangular, each 1-5 flowered.

Mallotus alternifolus Merr. belongs to the family Euphorbiaceae (Harinantenaina *et al*., 2013). It is a dicotyledon plant which belongs to the genus *Mallotus*. *M. alternifolius* is a perennial shrub or small tree which can be found in India and South China to New Guinea (Fadeyi *et al*., 1987). It grows up to 15 (-21) m tall, dbh up to 30cm; bole fluted, twisted, twisted to slanting; crown branching freely wide spreading to rounded pyramidal. Outer bark smooth to fissured, lenticellate, mottled indumentum sparse to dense, whitish to cream. Branches lenticellate, glabrescent, glanddotted on young parts. Stipules early triangular to ovate, 1.8-5 by 0.7-1.5mm, margin entire to irregular, apex acute, hairy abaxially.

Mallotus oppositifolius held a place in natural medicine (Fabricant and Farnsworth, 2001). Is a shrub that is commonly found in drier types of forest secondary growth throughout the West, African region. Locally it is known as 'Sroti' in Ewe or 'Strataduca in Akan (Burkill, 1985). Economically, *Mallotus oppositifolius* twig is used as chewing sticks for cleaning the teeth (Okwu and Ekeke, 2003.) The stem is used as yam stakes. In Nigeria the leaves are taken by the Hausas in cold infusion to expel tapeworm, while the decoction is a vermifuge in Ivory Coast (Duke, 1992). *Mallotus altternifolius* is use in the treatment of haemorrhage, expelling of tapeworms, analgesic, antifungal and urological disorders (Fabricant and Farnsworth, 2001).

Morphological features are of great taxonomic importance but cannot always be used solely in grouping plants to particular taxa. Stace (1980) suggested that the internal parts of a plant are less affected by environment; they are useful in taxonomic delimitation of taxa. This is in line with the works of Fahn (1990). The use of anatomical features in taxonomy has increased in the last two years due to the availability of sophisticated light and electron microscope, most in particular the transmission electron microscope. The incorporation of anatomical data with findings from studies of gross morphological and cytology enables those making revisions of the classification of plants to produce more natural systems.

Micro morphological features including epidermal features are broadly exploited for importance taxonomic studies. Fahn and Shimony (1996) suggest the type of stomata present and their frequency as well the nature of their taxonomy. The arrangement of subsidiary cells according is the most valuable taxonomic character of the stomata (Stace, 1980). These subsidiary cells established the different morphological type of stomata, which are dependable taxonomic tools.

In Nigeria, many indigenous plants are used as spices food or medicine (Amberkar *et al*, 2011). A great number of these plants are traditionally noted for their medicinal and pesticide properties (Okwu and Ekeke, 2003). It is widely accepted that fruits and vegetables have many healthful properties (Okwu, 2004). There is considerable amount of epidemiological evidence revealing an association between those who have a diet rich in fresh fruits and vegetables and a decrease risk of cardiovascular diseases and certain forms of cancer.

These plants often exhibit a wide range of biological and pharmacological activities, such as anti-inflammatory, anti-bacterial and anti-fungal properties extracts from the roots, barks seeds and fruits of these plants are used in the preparation of syrups (Holfe *et al*, 1999). And infusions in traditional medicine as cough suppressant and the treatment of liver cirrhosis and hepatitis.

It is generally assumed that the active constituents contributing to these protective effects are the phytochemicals, vitamins and minerals phytochemicals are present in a variety of plants utilized as important components of both human and animal diets (Oliver, 1986). Thus, the objectives of the study are therefore aimed at considering: the comparative morphological, anatomical, and phytochemical investigations of *Mallotus alternifolus* and *Mallotus oppositifolius.*

MATERIALS AND METHODS

Foliar Studies

The leaves were carefully observed for peculiar characteristics. Ten mature leaves from the middle portion of the plant were collected from the Botanical Garden of the University of Port Harcourt. The length and width measurements of the leaves were taken using a 30cm metre rule. The values were subjected to statistical analysis. The following parameters; mean $\left(\overline{X}\right)$ standard deviation (S.D) and the coefficient of variation (C.V) were computed.

Epidermal Studies

The leaf epidermis was studied from freshly harvested materials. The surface to examine was placed on a glass plate and flooded with sodium hypochlorite domestic bleach for 2 minutes. The materials were carefully scraped using a razor blade (Okoli, 1992). The clear epidermal layer obtained was in distilled water and stained with 1% safranin for 1 minute or more and mounted temporarily in glycerine. The epidermis was carefully placed so as to avoid distortion of features. The preparations were of objective lens microscopic magnifications of X40, X10 and X40.

The type of stomata was identified and the stomatal indices computed following the method of Metcalfe and Chalk, (1950).

Anatomical Studies

Fresh specimens were used for the anatomical studies. The leaves and stems were collected from the middle portion of the plant. The young stems and roots were collected and hand-sections were made following the method of Okoli (1992). The middle portion from a mature leaf of about 3cm length and stem of about 5cm was cut with a sharp razor blade to produce a smooth surface. The razor blade was used to cut thin sections (1 cell thick) by making horizontal cuts close to the surface. The first section was discarded; subsequent sections were placed in water held in a glass. Very good sections were collected then mounted temporarily on microscope slides, stained with 1% of safranin and one drop of a mountant (glycerine) was applied about the middle of the section and covered with a clean cover slip and viewed under the microscope. Photographs were also taken using a digital camera.

Phytochemical Studies: The leaves of each species studied were sun dried for 72 hours (3 days) and weighed. Fifty grams (50g) of the leaves were macerated in 96% ethanol using a pestle and a mortar. The extract was thereafter filtered and evaporated to dryness using a rotary evaporator set at 45^0C to constant weight and later, an exhort extraction machine. Residue yields were noted and a portion was used for the phytochemical screening following the method of Trease and Evans (1989) and Harborne (1977).

RESULTS

The results of the phytochemical screening in *Mallotus oppositifolius* and *Mallotus alternifolius* revealed the presence of tannins, flavonoids, cardiac glycosides, anthraquinones, phlobatannins while alkaloids, saponins, cyanogenetic glycoside and terpenes were absent in both species (Table 1).

The results of the quantitative studies showed that *Mallotus alternifolius* contain higher amount of phytochemicals than *Mallotus oppositifolius* (Table 2).

Table 1: Results of Qualitative Screening of *Mallotus oppositifolius and Mallotus alternifolius*

Test	***Mallotus oppositifolius***	***Mallotus alternifolius***
Alkaloids		
i) Dragendroff's reagent	-	-
ii) Mayer's reagent	-	-
Saponins		
i) Frothing test	-	-
ii) Fehling test	-	-
Tannins		
i) Ferric chloride test	+ + +	+ + +
ii) bromine water test	+ + +	+ + +
Flavonids		+
i) Shinoda reduction test	+	
Cardiac glycoside		
i) Salkowski test	+	+
ii)Keller-kiliani test	+	+
iii) Lieberman's test	-	-
Anthraquinones		
i) Borntrager's test (free)	+	+
ii) Combined antraquinone	+	+
Phlobatannins		
Hydrochloric acid test	+	+
Cyanogenetic glycoside	-	-
Terpenes		
Chloroform and Conc. Sulphuric acid	-	-

Key: +++ = High concentration
++ = Moderate concentration
+ = Trace concentration
- = Absent

Table 2: Result of Quantitative Analysis of *Mallotus oppositifolius* and *Mallotus alternifolius* leaf.

Test	***Mallotus oppositifolius(%)***	***Mallotus alternifolius(%)***
Tannins	12.01	14.00
Flavonoids	5.41	7.00
Saponins	0.1	0.16
Terpenes	0.75	1.12
Cardiac glycoside	2.04	2.10

Foliar Morphology: *Mallotus oppositifolius* leaves are heart shape to broadly ovate or less deeply toothed or lobed. Phyllotaxy is opposite. The leaf is 12cm ±2.3 long and 8.1cm ± 2.2cm wide, petiole is 7.9cm ± 2.4cm long, venation is reticulate while *Mallotus alternifolius* has leaves that are entire to irregular apex, blade ovate to obovate. Phyllotaxy is alternate. The leaf is 6.1cm ± 0.7cm and 7.8cm ± 1.8cm wide, petiole is 2.0cm ± 0.6cm long, venation is reticulate (Table 3).

Table 3:Measurement of leaf parameters of *Mallotus oppositifolius* and *Mallotus alternifolius*

Mallotus oppositifolius				***Mallotus alternifolius***		
	Mean $(\bar{x})$	S.D	C.V	Mean $(\bar{x})$	S.D	C.V
Length of leaf	12	2.3	19.3%	6.1	0.7	11.6%
Width of leaf	8.1	2.2	27.3%	7.8cm	1.8	23%
Length of petiole	7.9	2.4	30.4%	2.0	0.6	29.6%
Length of internode	3.7	1.1	30.1%	5.9	1.9	32.1%

Cauline Morphology: The stem of *M. oppositifolius* is woody with a series of internodes. Branching is erect. The internode is 3.7cm ± 1.1cm long. The fruit is a short hairy and gland dotted 3 seeds. The seeds are smooth, shiny, grayish and olive brown. Reproduction is by seeds. In *M. alternifolius,* the stem is woody with series of internodes. Branching is crown type. The internode is 7.8cm ± 1.8cm long. Fruit is an echinate, lobed capsule, purple tingled to dark red glabrous to hairy, densely gland-dotted. Seeds glossy roughly globose.

Leaf Anatomy: Leaf of *M. oppositifolius* and *M. alternifolius* are uniseriate made of a single tier of cell both on
the upper and lower epidermis. The epidermis consist of cells, 2-3 layers, 2-3 parenchyma cells, 3-4 layers of collenchyma cells and clearly differentiated vascular bundles (Plate 6a & 6b).

Stem Anatomy: The stem epidermis of *M. oppositifolius* and *M. alternifolius* were characterized as follows; epidermis were 2-3 layers of cells, 2-3 layers of sclerenchymatous cells, 2-3 layers of collenchymatous cells, 3-4 sizes of parenchymatous cells preceded by a clearly differentiated vascular bundles and pith (Plate 4a & 4b)

Petiole Anatomy: The petiole of *M. oppositifolius* was characterized as follows; epidermis 2-3 layers of cells, 3-4 layers of collenchymatous cells, 2-3 layers of sclerenchymatous cells, 3-4 sizes of parenchyma cells and clearly differentiated vascular bundles and pith whereas in *M. alternifolius* epidermis 1-2 layers of cells, 2-3 layers of collenchymatous cells, 2-3 layers of sclerenchymatous cells, 3-4 cell sizes of parenchymatous cell (Plate 7a & 7b).

Root Anatomy: The root of *M. oppositifolius* was characterized as follows; root hairs, 2-3 layers of sclerenchymatous cells, cortex, preceeded by a clearly differentiated vascular bundles and pith whereas in *M. alternifolius* 1-2 layers of sclerenchymatous cells, cortex, preceeded by a clearly differentiated vascular bundles and pith (Plate 5a & 5b)

Epidermal characteristics: On the abaxial surface of *M. oppositifolius*, there are trichomes and numerous stomata with subsidiary cells. The cells have an irregular outline without intracellular spaces and mostly isodiametric. The taxon exhibits anomocytic type of stomatal arrangement with stomatal index of 23% for the abaxial surface and 19% for the adaxial surface. Stomatal are abundant on the abaxial and adaxial surfaces. There are 4-6 epidermal cells the guard cells (Plate 2a & 3a). In *Mallotus alternifolius, o*n the abaxial surface, there are numerous stomata with subsidiary cells. The cells have an irregular outline without intracellular spaces and mostly isodiametric. The shape of the adaxial surface was the same as the abaxial. The taxon exhibits anomocytic type of stomata arrangement with stomatal index of 16% for the abaxial surface and 15% for the adaxial surface. Stomata are abundant on the abaxial than on the adaxial surface. There are 4-6 epidermal cells around the guard cells (Plate 2b & 3b).

Plate 1a: Vegetative and floral features of *M. oppositifolius*

Plate 1b: Vegetative and floral features of *M.alternifolius*

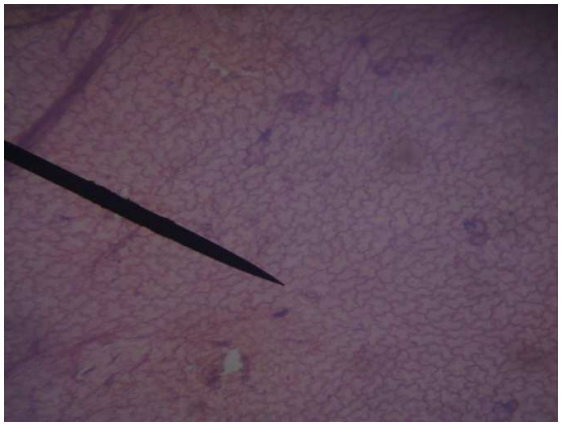

Plate 2a: Abaxial surface of *M. oppositifolius* showing anomocytic type of stomata (X40)

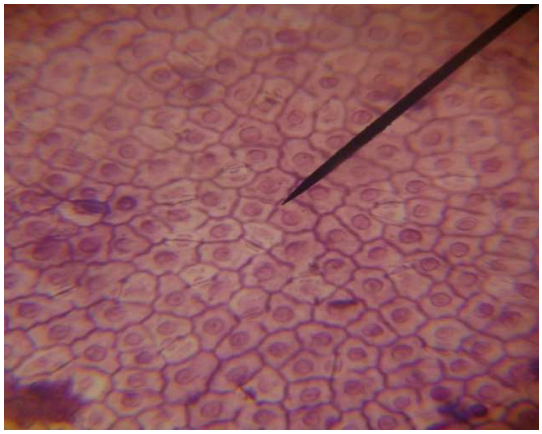

Plate 2b: Abaxial surface of *M.alternifolius* showing anomocytic type of stomata (X40)

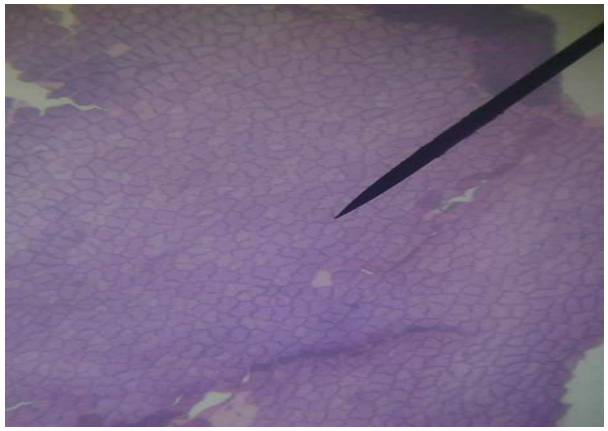

Plate 3a: adaxial surface of *Mallotus oppositifolius* anomocytic type of stomata tomata (x40)

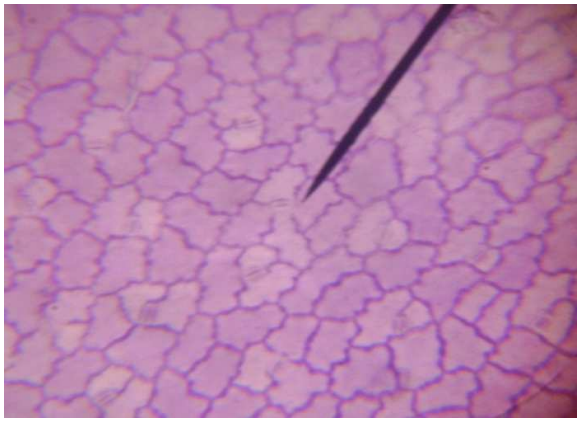

Plate 3b: adaxial surface of *Mallotus alternifolius* anomocytic type of ta (x40)

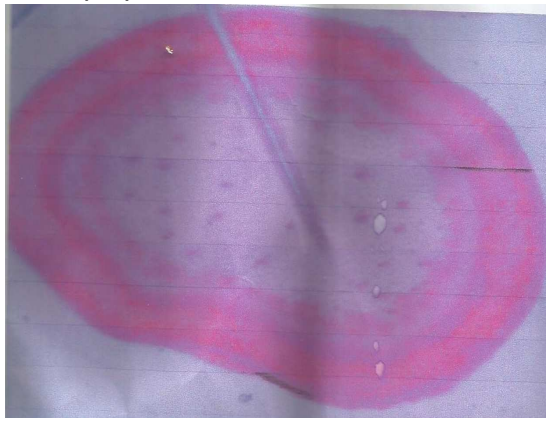

Plate 4a: Transverse section of stem of *M. oppositifolius* (x10

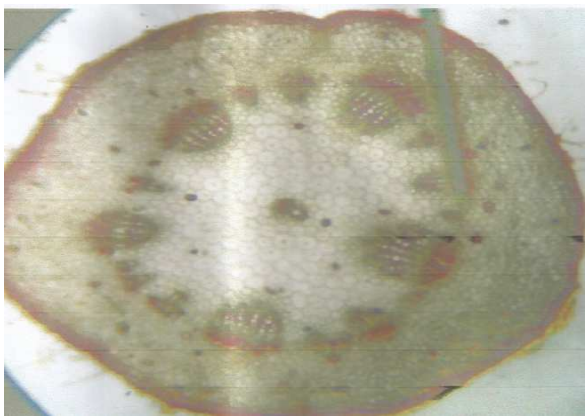

Plate 4b: Transverse section of stem of *M. alternifolius* (x10)

Plate 5a: Transverse section of the root of *M. oppositifolius* (x10)

Plate 5b: Transverse section of the root of *M. alternifolius* (x10)

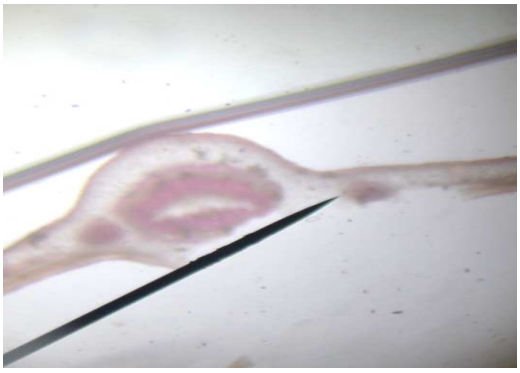

Plate 6a: Transverse section of leaf of *M. oppositifolius* (x10)

Plate 6b: Transverse section of leaf of *M. alternifolius* (x10)

Plate 7a: Transverse section of the petiole of *Mallotus Mallotus oppositifolius* (x10)

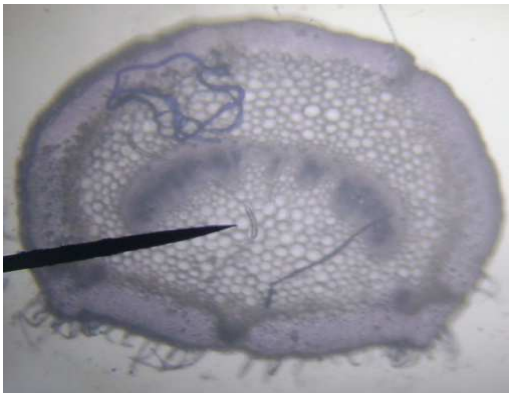

Plate7b: Transverse section of the petiole of *alternifolius* (x10)

DISCUSSION

Morphological and anatomical features show that *Mallotus oppositifolius* and *Mallotus alternifolius* are dicotyledons with highly evolved characters and belong to the genus *Mallotus* (Slik *et al,* 2003). *Mallotus oppositifolius* is found in West Africa and Madagascar while *Mallotus alternifolius* is found in India and south China. This fact is in line with the report of Burkil (1985) who showed that *Mallotus oppositifolius* is a useful plant in West Africa. The pattern of distribution of these species showed that they are mesophytes.

Morphology of the plant species is among the most important criteria that can be successfully used to make useful taxonomic conclusions about species. Stace (1980) had earlier highlighted or extensively utilized morphological characters in delimiting taxa. Leaf morphology can be successfully distinguished between. Therefore the leaf morphology of *Mallotus oppositifolius* and *Mallotus alternifolius* are of diagnostic value. The floral morphology is also of taxonomic importance. A summary of the morphological characters have been provided in this study. The phyllotaxy of *Mallotus oppositifolius* is opposite while *Mallotus alternifolius* is alternate. Branching is erect and crown type.

Epidermal characters have been exploited in taxonomic study of plant Cell, stomatal characteristics, stomatal index and frequency are of taxonomic importance (Abubakar and Yunusa, 1998; Timmerman, 1927).

Anatomical features are of great taxonomic importance, since they are less affected by the environment (Stace, 1980). The vascular system of leaf and stem are also of taxonomic interest and value.

Phytochemical studies show that *Mallotus alternifolius* is have the highest amount of tannin content 14.00% then *Mallotus oppositifolius* 12.01%.

CONCLUSION

These comparative studies of the morphology, anatomy and phytochemistry of *Mallotus* species have shown that these lines of evidence are of taxonomic importance in Euphorbiaceae and this research work has provided information on certain aspects of morphology, anatomy and phytochemistry of *Mallotus oppositifolius* and *Mallotus* alternifolius, tending support to similar conclusion by other investigators of shrubs. It is therefore strongly recommended that more research should be carried out on the quantitative phytochemistry and cytology of these species to establish a relationship between the various characters.

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