

## Abstract

Plant leaf parameters such as stomatal index, stomata type, leaf microscopic features, and leaf powder microscopy are important taxonomic parameters used to properly identify species and to differentiate one species from another and these are vital keys in the pharmacognostic study of medicinal plants because leaf parameters play a vital role in distinguishing crude drugs even when they are fragmented or powdered. Also, extractive index, retention factor, ash value, and phytochemical parameters are vital in the standardization of crude drugs for the establishment of pharmacopeia and monographs. Therefore, the study seeks to validate the use of the phytochemistry and molecular systematics among Asteraceae, Euphorbiaceae, and Fabaceae to support their circumscription and therefore evaluated the pharmacognostic profile of *Desmodium velutinum*, *Synedrella nodiflora* and *Mallotus oppositifolius* folklorically used in South East Nigeria using standard laboratory protocols. The result of the leaf microscopy showed that *Desmodium velutinum*, *Synedrella nodiflora*, and *Mallotus oppositifolius* all had irregular shaped leaf epidermis, and stomata was present only in the abaxial surface respectively. The result showed both *D. velutinum* and *S. nodiflora* powder showed the presence of lignin, starch, cystoliths, tannin, and Oil body while calcium oxalate was absent whereas, *Mallotus oppositifolius* showed the presence of lignin, starch, tannin, and Oil body while calcium oxalate, and cystoliths were absent. Also the result of the TLC showed that *M. oppositifolius* had 3 visible spots with retention factors (RF) of 0.363, 0.425, and 0.5 in that order respectively, *D. velutinum* had 4 visible spot with retention factor (RF) of 0.425, 0.5, 0.550 and 0.66 in

that order respectively while *S. nodiflora* had 6 visible spot with retention factor (RF) of 0.255, 0.363, 0.438, 0.538, 0.600 and 0.688 in that order respectively. The result also showed that methanol exhaustible extracted the phytochemicals in the crude dried plant. The acute toxicity studies showed that *Desmodium velutinum*, *Synedrella nodiflora* and *Mallotus oppositifolius* individually had LD<sub>50</sub> greater than 5000mg/kg. This study validates the claim that leaf parameters such as stomatal index, stomata type, leaf powder microscopy are important parameters used for the identification of crude drugs.

Key words: Pharmacognostic, Acute Toxicity, *Desmodium velutinum*, *Mallotus oppositifolius*, *Synedrella nodiflora*, leaf parameters.

## Introduction

Though the use of herbs as crude drugs is as old as man's existence on earth, its proper identification is very important to its continued benefits. Herbal drugs with high and effective therapeutic activity usually witness a surge in demand and this therefore sometimes leads to its adulteration, replacement and abuse by the herbal practitioners and marketers of the herbal drug leading to consumers disappointment with numerous fatal consequences [1] and even mortality. The increased demand and use of herbal drugs represent a substantial proportion of the global drug market [2-8] due to its perceived efficacy, acceptability and safety. Adulteration of drugs with different materials is often done by traditional medicine practitioners in order to increase the economic gain and this can lead to public health challenges as a result of varying levels of toxicities. There is continued emphasis on proper identification, collection, isolation and screening of plants for biological activities in order to authenticate their safety and therapeutic claims. Accurate identification, collection and preparation of herb for evaluation and for therapeutic administration are of high importance in drug discovery and development.

Whereas it is generally believed that most herbal preparations are safe for consumption, some herbs and certain herbal formulations could be toxic with undesirable side effects [9] Inaccurate identification or collection of plants for therapeutic activity can be catastrophic and may even lead to waste and mortality. Problems of improper

identification of crude drugs, adulteration and toxicities can be solved by proper **pharmacognostic** studies of medicinal plants which is essential in drug standardization and formulation. This thereby will reduce concerns of inaccurate identification, collection and toxicities.

The leaf parameters such as stomatal index, stomata type, leaf microscopic features and leaf powder microscopy are important taxonomic parameters used to properly identify species and to differentiate one species **from** another.

The stomata apart from their roles in transpiration and gaseous exchange are very vital tools found very useful as diagnostic tools in plant taxonomy and systematic [10]. Also, the leaf micro-characters such as the stomatal type, stomata size, types of cell wall thickness, stomata index and occurrence are useful in taxonomic distinction and recognition in the flowering plant families [11] Leaf parameters are also a vital tool for distinguishing crude drugs even when they are fragmented or powdered.

Therefore, the study seeks to validate the use of the phytochemistry and molecular systematics among Asteraceae, Euphorbiaceae and Fabaceae to support their circumscription.

*Desmodium velutinum*, *Synedrella nodiflora* and *Mallotus oppositifolius* are essential botanicals used throughout **Southern** Nigeria for the treatment of various diseases conditions ranging from infections, wounds, ear ache, ulcer and fever and therefore this study tried to evaluate the **pharmacognostic** profile of these three Nigerian plants.

*Desmodium velutinum* (*D. velutinum*) belongs to the botanical family Fabaceae. The plant is generally called Ikeagwani and the leaves are used to control nonspecific diarrhoea among the people of the **South East** Nigeria. Extracts of *Desmodium velutinum* are used traditionally in some disease conditions particularly headache, fever and diarrhoea [12]. The squeezed leaves of *D. velutinum* is locally used for diarrhoea while the stem and root are chewed to relieve toothache [12]. *D. velutinum* is a perennial, erect or semi-erect shrub or sub-shrub, up to 3 m high. The branches are often dark red or yellow-brown when young, velutinous and short hooked-hairy.

*Synedrella nodiflora* (L.) (*S. nodiflora*) Gaertn is of the botanical family Asteraceae, ethnobotanically known for wound healing. It is a small, annual weed of cultivation native to America, found in the plains of India and in the Andamans also. The leaves are used as a poultice for sore rheumatism and juice of the leaves is used for earache [13]. In Ghana the infusion of leaves of *S. nodiflora* is taken as laxative; leaf sap is used in Congo for mouth affections and is rubbed on gums for tightening.

*Mallotus oppositifolius* (Geiseler) Mull. Arg. (Euphorbiaceae) is a predominant edible shrub in the South East Nigeria where it is commonly identified as Ukpo [14]. It is used in Nigerian folk medicine for the treatment of common infections caused by bacteria, fungi pathogens and for the treatment of wounds and ulceration. It has been reported that *M. oppositifolius* exerts antifungal properties [15-16] against pathogens of public health importance. This present study evaluated the anatomical and chemical profile of *Desmodium velutinum*, *Synedrella nodiflora* and *Mallotus oppositifolius* found in South East Nigeria.

## **Materials and methods**

### **Test Plant Collection**

The three plants studied are of public health importance and their selection for studies is because of their therapeutics (biological) and economic importance. The study compared the pharmacognostic, and phytochemical profiles of Asteraceae, Euphorbiaceae, and Fabaceae. Fresh leaves of *M. oppositifolius*, *Desmodium velutinum*, and *Synedrella nodiflora* were collected from their natural habitat around Anambra State South Eastern Nigeria. The plant was properly identified by a plant taxonomist Mr. Felix Nwafor at the Department of Pharmacognosy and Environmental Medicine University of Nigeria Nsukka and the voucher specimen was prepared and deposited at the herbarium department of University of Nigeria Nsukka with Voucher number PCG/UNN/0336/Fabaceae

PCG/UNN/0337/Euphorbiaceae, PCG/UNN/0338 Asteraceae. The plant leaves were air-dried at room temperature (25 °C) for 14 days to obtain the powder for phytochemical and physicochemical parameters.

### **Fresh Leaf Microscopy**

Leaf microscopy to determine the types of epidermal cells, stomata type, stomata size, stomata density, presence of trichome, vein islet number, and stomata index was on both the adaxial and abaxial surfaces of the leaves were carried out using the methods of [17]. The transverse section (TS) of the leaf was made using a Reichert sledge microtome following the procedures of [18-19].

### **Powder Microscopy / Chemo-microscopy**

The leaves were dried under shade and pulverized with local mortar and pestle. Chemo-microscopy was conducted on the powders to determine the presence of starch, calcium oxalate crystals, and lignified vessels using standard laboratory procedures according to [20].

### **Extractive Value**

The extractive value for methanol, water, and n-hexane was determined by the method of [20]

### **MOISTURE CONTENT**

The moisture content of the three plants was determined by the method of [20].

### **Total Ash Value**

The total ash, water-soluble ash, and acid-soluble ash value were determined by the method of [20].

## **Phytochemical Screening.**

The qualitative phytochemical analysis was done using the method of [21-24] to determine the presence of secondary metabolites or active components in *Mallotus oppositifolius*, *Desmodium velutinum*, and *Synedrella nodiflora*. Estimation of the amount of phytochemicals present was assayed by using standard procedures described by [24-26].

## **Acute Toxicity Studies**

The acute toxicity test to determine the LD<sub>50</sub> as an index of safety of the extract was done in 2 phases as described by [27] for *M. oppositifolius* as described by [28]. Briefly, nine animals (rats) were randomly allocated into 3 groups of 3 rats each. Animals in groups 1, 2, and 3 were given 10, 100, and 1000 mg/kg body weights respectively of the extract through the oral route. Animals were therefore monitored for signs of toxicity and mortality for 2 days (48 hours). Signs of toxicity and pathological findings observed were recorded appropriately. All the animals survived so the extract and were further subjected to acute toxicity tests with higher doses in the second trial. In the second trial, 4 animals were randomly allocated to 4 groups of one animal each. Animals in groups 1, 2, 3, and 4 were given 1200, 1600, 2900, and 5000 mg/kg respectively of the extract.

## **Result and Discussion**

## Leaf Microscopy and Leaf Characteristics

The result of the fresh leaf microscopy is presented in Plate 1 to 9 and Figure 1 while the leaf powder is presented in Figures 10-12 and Table 1. Leaf of the plant was studied for leaf type, epidermal cell type, stomata type, stomata density, epidermal cell number, stomata index, stomata length, stomata width, stomata size. Palisade ration and the powder of the ground leaf were evaluated for powder microscopy to evaluate the presence of lignin, starch, calcium oxalate, tannins, etc.

### Qualitative Leaf Microscopy *Desmodium velutinum*, *Mallotus oppositifolius* and *Synedrella nodiflora*

The result of the leaf microscopy is presented in plate 1 to 9. The result showed that the upper (adaxial) and lower (abaxial) leaf epidermal surface of *Desmodium velutinum* are irregular in shape with undulated anticlinal cell walls on both the adaxial (upper) and abaxial (lower) leaf surfaces. The leaf is hypostomatic which implied that stomata only occur on the lower surface of the leaf. The stomata are paracytic which implied that two subsidiary cells are positioned parallel to the guard cells. There is also the presence of unicellular, non-glandular trichome with the bulbous base while in *Mallotus oppositifolius* the result showed that the adaxial and abaxial leaf epidermal surface showed that *Mallotus oppositifolius* has irregular shaped epidermal cell wall with undulated anticlinal cell walls on both the adaxial (upper) and abaxial (lower) leaf surfaces. Also, the result showed that *Mallotus oppositifolius* leaf

is hypostomatic which implied that the stomata only occur on the lower surface of the leaf. The result also showed that the stomata of *Mallotus oppositifolius* is anomocytic which implied that Subsidiary cells are absent around the stomata but the guard cells are directly surrounded by epidermal cells. Also, the Transverse section of the leaf of the three showed upper epidermis, pith, phloem and spongy mesophyll. *Synedrella nodiflora* leaves has irregular shaped undulated anticlinal cell walls on both the adaxial (upper) and abaxial (lower) leaf surfaces. The leaf is hypostomatic (stomata only occur on the lower surface of the leaf. The stomata are anomocytic which implied the absence of Subsidiary cells and the guard cells are directly surrounded by epidermal cells. Also, there is the presence of unicellular, non-glandular trichomes with conical base.

Table 1: Result of leaf powder microscopy of the crude drugs

Parameter	<i>D. velutinum</i>	<i>M. oppositifolius</i>	<i>S. nodiflora</i>
Lignin	Present	Present	Present
Starch	Present	Present	Present
Calcium oxalate	Absent	Present	Absent
Cystoliths	Present	Absent	Present
Tannins	Present	Present	Present
Oil body	Present	Absent	Present

Result of Leaf Microscopy Assays of *Desmodium velutinum*, *Mallotus oppositifolius* and *Synedrella nodiflora*

Leaf Microscopy Assays of *Desmodium velutinum*, *Mallotus oppositifolius* and *Synedrella nodiflora*.

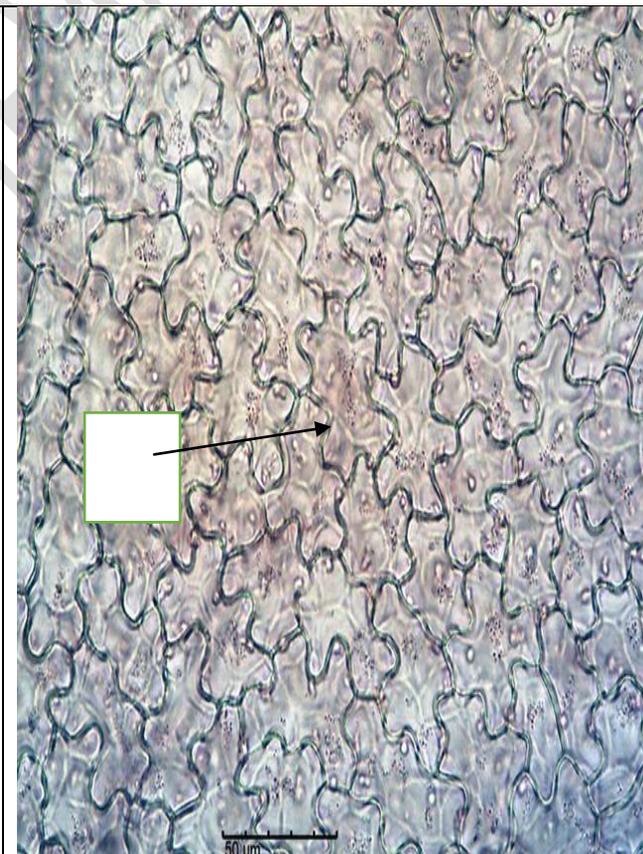
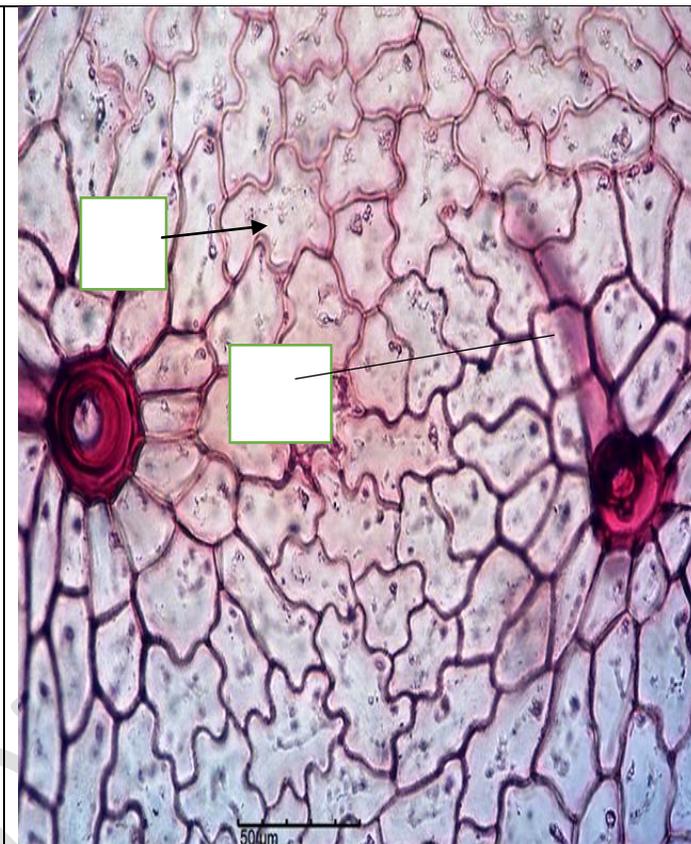
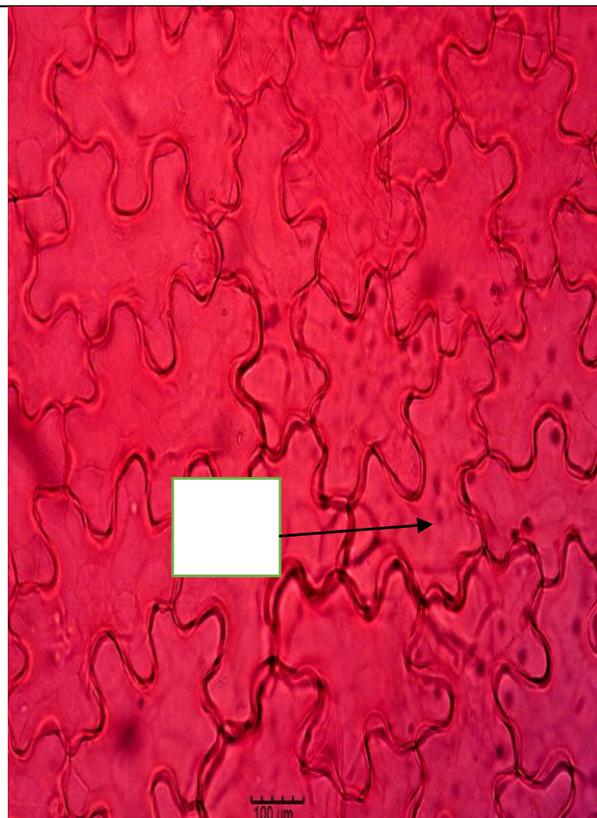


Plate 1: Adaxial surface of the leaf of *S. nodiflora* showing irregular epidermal cells (IEC) with undulated/wavy walls. Stomata absent

Plate 2: Adaxial surface of the leaf of *D. velutinum* showing irregular epidermal cells (IEC) with undulated/wavy walls and presence of unicellular unglanular trichomes (UUT). Stomata absent

Plate 3: Adaxial surface of the leaf of *M. oppositifolius* showing irregular epidermal cells (IEC) with undulated/wavy walls. Stomata absent

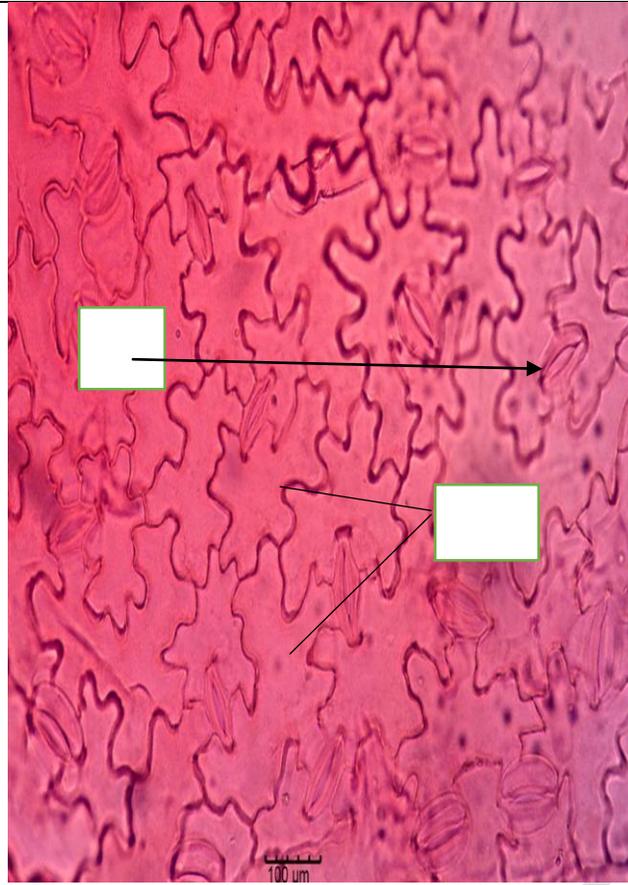


Plate 4: Abaxial surface of the leaf of *S. nodiflora* showing anomocytic type of stomata (AS) and irregular epidermal cells (IEC)

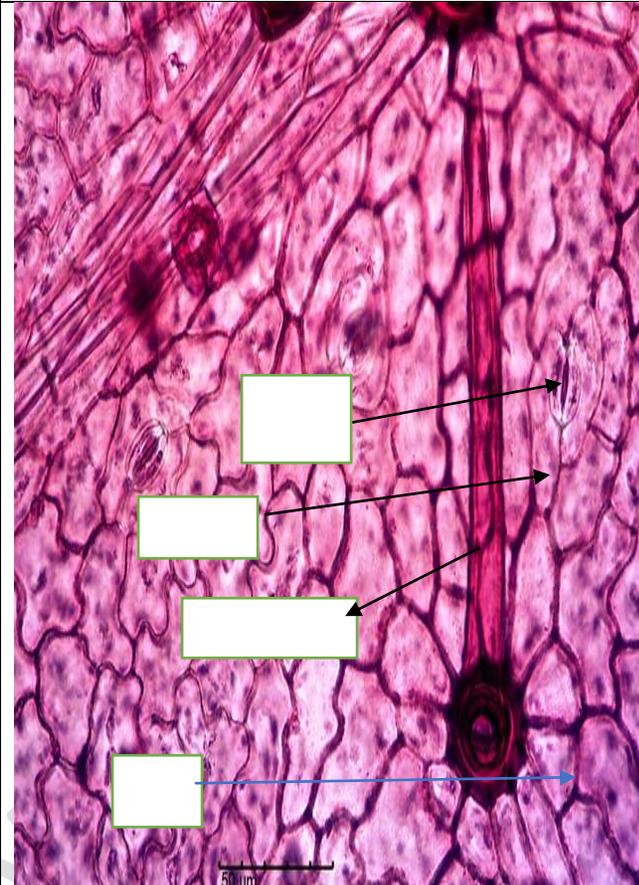


Plate 5: Abaxial surface of the leaf of *D. velutinum* showing paracytic type of stomata (PS), trichome (T), subsidiary cells (SC) and irregular epidermal cells (IEC)

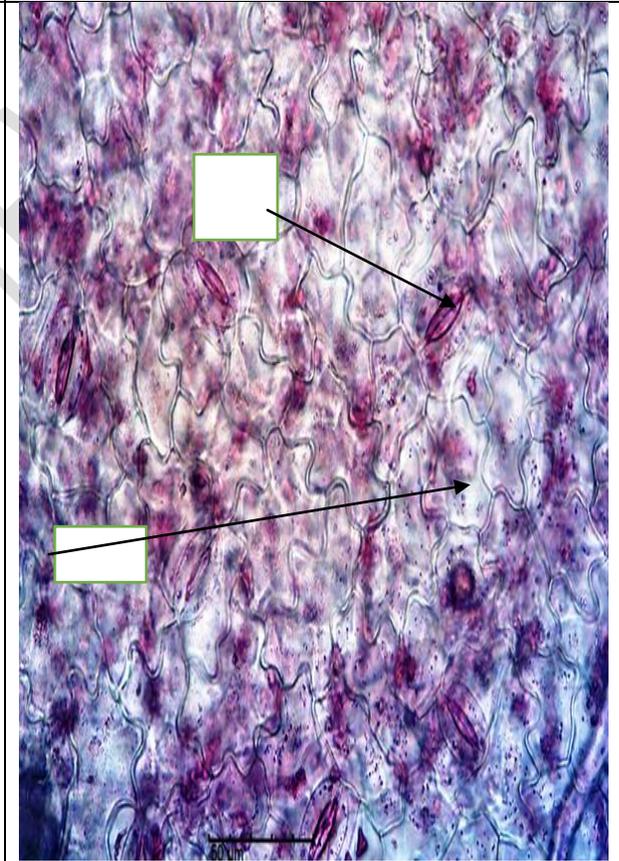


Plate 6: Abaxial surface of the leaf of *M. oppositifolius* showing anomocytic type of stomata and irregular epidermal cells

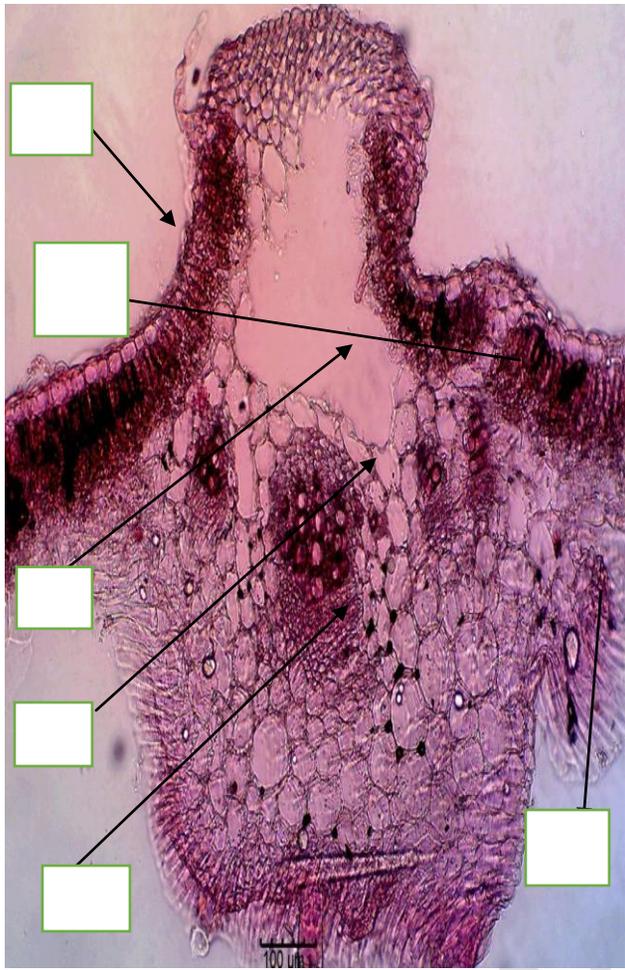


Plate 7: Transverse section of the leaf of *S. nodiflora* showing Upper epidermis (UE), spongy mesophyll (SP), Phloem (P), Xylem (X), Pith (Pi) and lower Epidermis (LE).

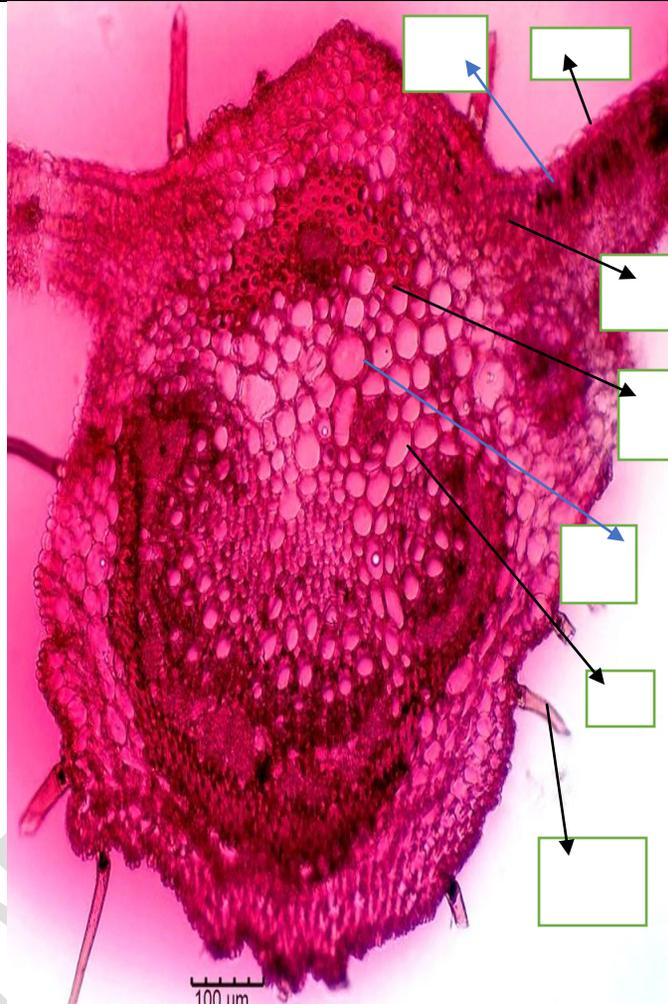


Plate 8: Transverse section of *D. velutinum* the leaf of showing Upper epidermis (UE), Palisade Mesophyll (PM) spongy mesophyll (SP), Phloem (P), Xylem (X), Trichome and Pith (Pi)

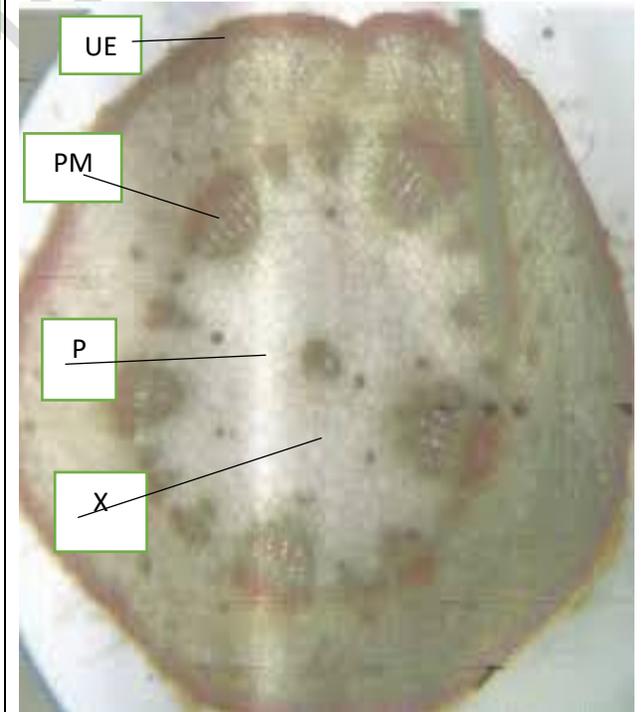


Plate 9: Transverse section of *D. velutinum* the leaf of showing Upper epidermis (UE), Palisade Mesophyll (PM) Phloem (P), Xylem (X) and Pith (Pi)

### **Chemomicroscopy of *D. velutinum*, *M. oppositifolius* and *S. nodiflora***

The result of the Chemomicroscopy is presented in plates 10 to 13. The result showed that the Chemomicroscopy of *S. nodiflora* showed the presence of lignin, starch, cystoliths, tannins, and oil body while crystal of calcium oxalate was absent in the leaf powder sample (Plate 10). Also, the result showed that the Chemomicroscopy of *D. velutinum* showed the presence of linin, starch, cystoliths, tannins and oil body, a crystal of calcium oxalate, trichomes, and a strand of fibre in the leaf powder sample (Plate 11). Furthermore, the result showed that the chemomicroscopy of *M. oppositifolius* showed the presence of linin, fiber, starch, tannins, and crystal of calcium oxalate while oil body, and cystoliths were absent in the leaf powder sample (Plate 12).

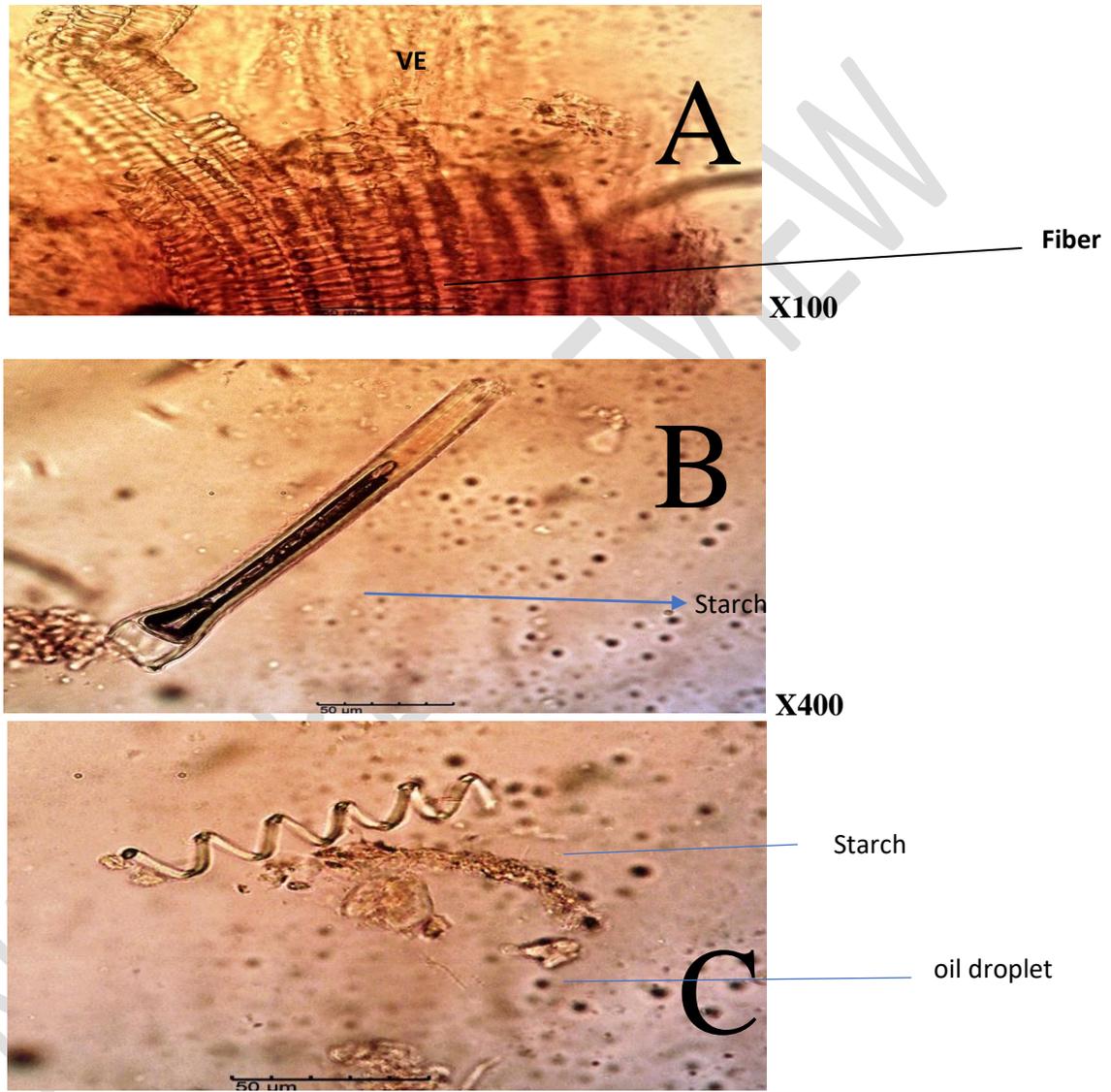
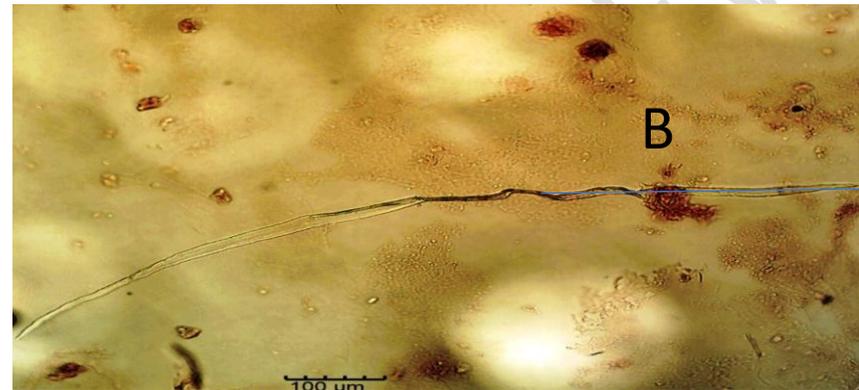


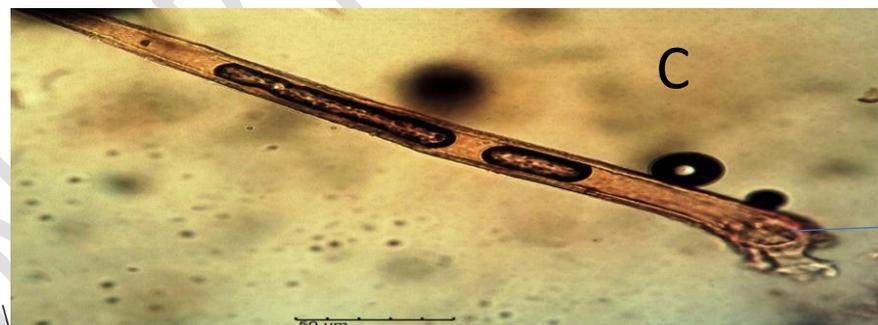
Plate 10: Chemomicrograph of the powdered leaf of *S. nodiflora* (A showed fiber strand, B showed starch while C showed oil droplet)



Crystal of calcium oxalate



Strand of fibre



Trichome

Plate 11: Chemomicrograph of the powdered leaf of *D. velutinum* (A showing crystal of calcium oxalate, B showed strand of fibre while C showed a unicellular covering trichome impregnated with rod-shaped cystoliths).

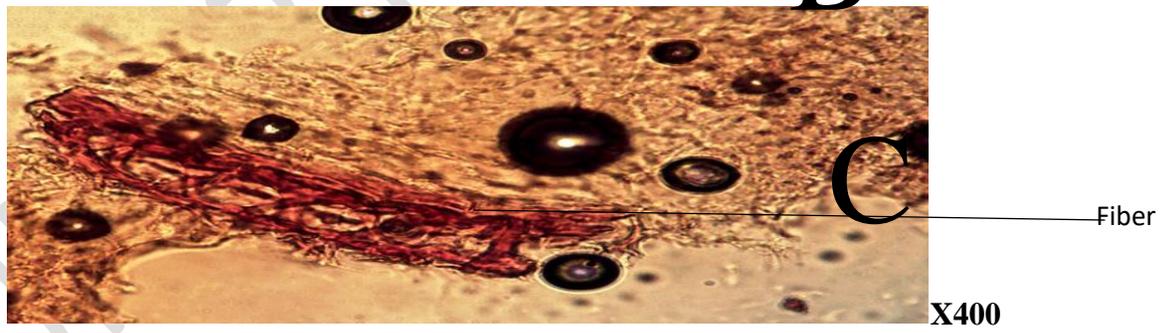
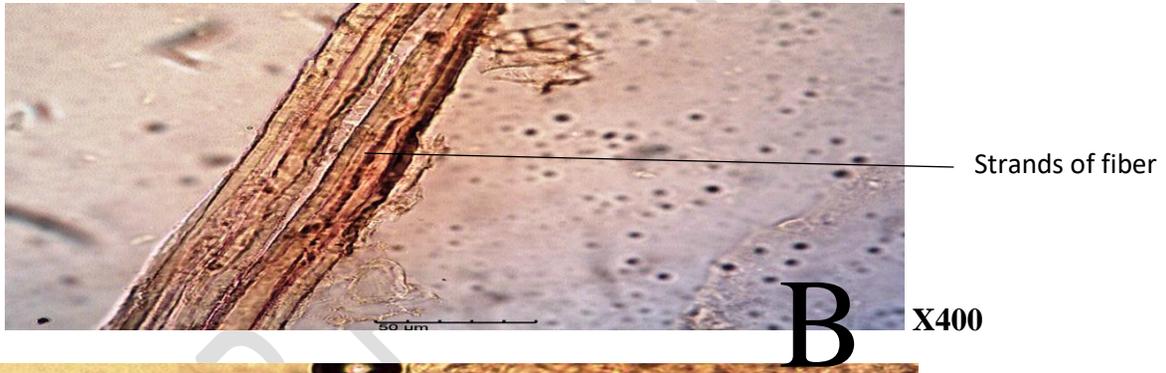
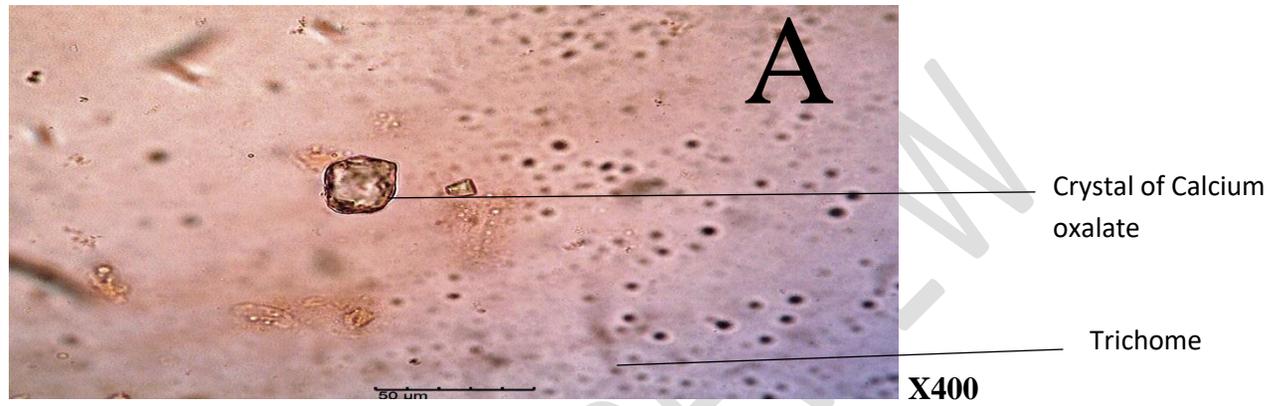


Plate 12: Chemomicrograph of the powdered leaf of *M. oppositifolius* (A showed trichome and crystal of calcium oxalate, B showed fragment of fiber while C showed fiber)

## **Quantitative Leaf Characteristics**

The result of the quantitative leaf characteristics is presented in Figure 1.0 as follows:

### **Epidermal cell number**

The result of the epidermal cell number is presented in Figure 1.0. The result showed that *D. velutinum* (76) had the highest number of epidermal cells on the surface of the leaf, followed by *M. oppositifolius* (66.25) while *S. nodiflora* (46.75) had the least number of epidermal cells.

### **Palisade ratio**

The result of the palisade ratio is presented in Figure 1.0. The result showed that *S. nodiflora* had a significantly high palisade ratio of 11.5 followed by *D. velutinum* (9.25) while *M. oppositifolius* had a significantly lower palisade value of 8.0.

### **Stomata Density**

The result of the stomata density is presented in figure 1.0. The result showed that *S. nodiflora* had a significantly high stomata density of 97 mm<sup>-2</sup> followed by *M. oppositifolius* (56 mm<sup>-2</sup>) while *D. velutinum* had a significantly lower stomata density of 22 mm<sup>-2</sup>.

### **Stomata Index**

The result of the Stomata Index is presented in Figure 1.0. The result showed that *S. nodiflora* had a significantly high stomata index of 26 % followed by *M. oppositifolius* (13%) while *D. velutinum* had a significantly lower stomata index of 4.7 %.

### **Stomata Length**

The result of the Stomata Length is presented in Figure 1.0. The result showed that *M. oppositifolius* had a significantly bigger Stomata length of 28.98 $\mu\text{m}$ , followed by *S. nodiflora* (26.3  $\mu\text{m}$ ) while *D. velutinum* had a significantly smaller stomata length of 20.27  $\mu\text{m}$ .

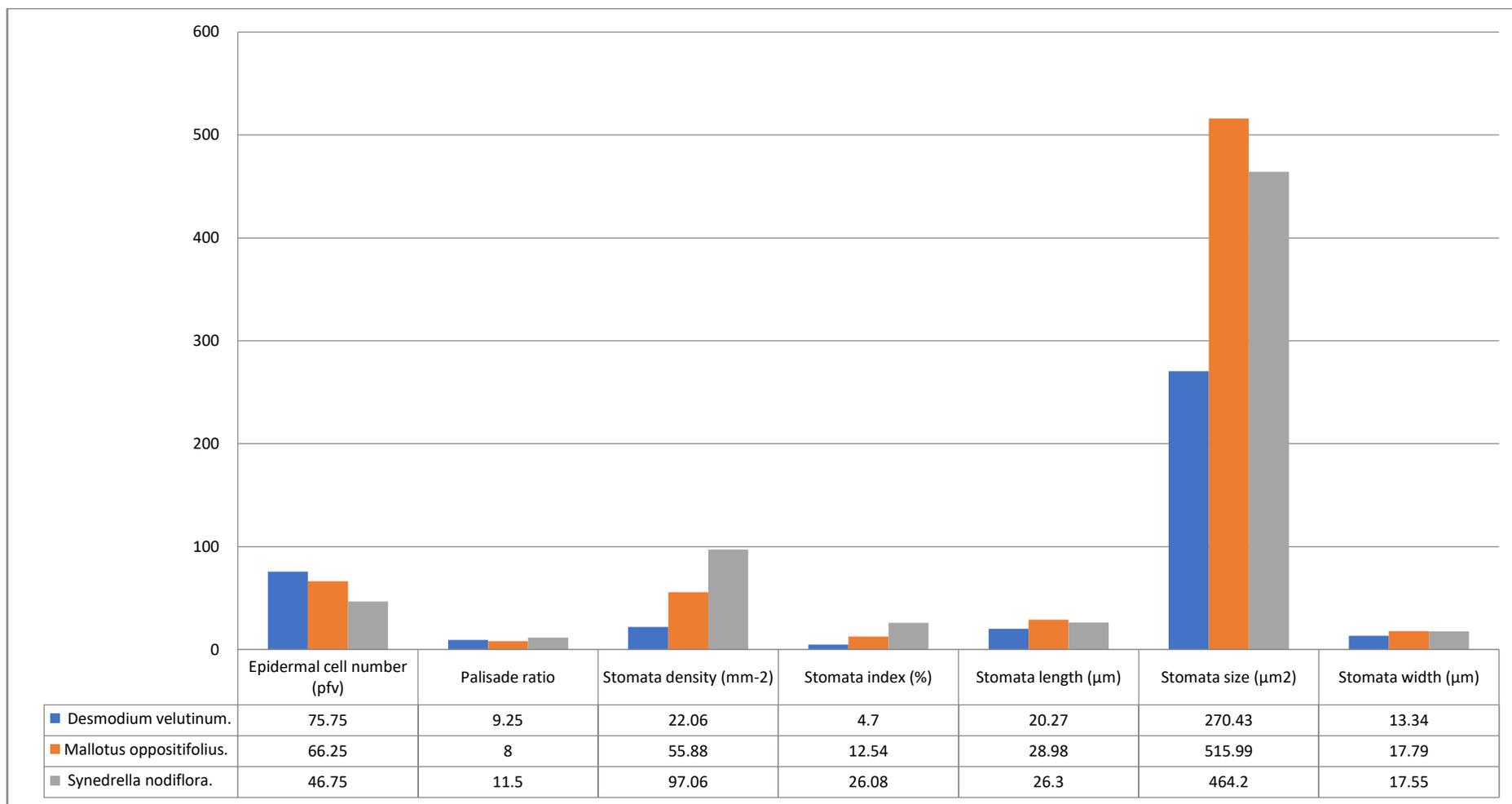
### **Stomata Size**

The result of the Stomata Size is presented in Figure 1.0. The result showed that *M. oppositifolius* had a significantly larger Stomata size of 516  $\mu\text{m}^2$ , followed by *S. nodiflora* (464  $\mu\text{m}^2$ ) while *D. velutinum* had a significantly smaller stomata size of 270  $\mu\text{m}^2$ .

### **Stomata width**

The result of the Stomata width is presented in Figure 1.0. The result showed that *M. oppositifolius* had a significantly bigger Stomata width of 17.79 $\mu$ m, followed by *S. nodiflora* (17.55  $\mu$ m) while *D. velutinum* had a significantly smaller stomata width of 13.34  $\mu$ m.

UNDER PEER REVIEW



**Figure 1 : Qualitative Characteristics of *D. velutinum*, *M. oppositifolius* and *S. nodiflora* leaves microscopy of the crude drugs.**

## Physicochemical Assays

The ash value, extractive value, and the RF values of the crude drugs was evaluated for *D. velutinum*, *M. oppositifolius* and *S. nodiflora* and are presented in Table 2 to Table 4.

### Ash Value

The results of the ash value were presented in Table 2. The result showed that total ash was a significantly higher in *D. velutinum* (16 %) followed by *M. oppositifolius* (13.6%) but was least in *S. nodiflora* (6.7%). Water soluble ashes were also a significantly higher in *D. velutinum* (10 %) followed by *M. oppositifolius* (6.6%) but was least in *S. nodiflora* (3.8%). The result further showed that acid insoluble ash was also a significantly higher in *D. velutinum* (7.0%) followed by *M. oppositifolius* (6.4%) but was least in *S. nodiflora* (3.8%). Water loss on drying was however the same (0.9%) across the three crude plants.

**Table 2: Ash Value of *M. oppositifolius*, *D. velutinum* and *S. nodiflora***

SN	Parameter	Values (Percentage)		
		<i>D. velutinum</i>	<i>M. oppositifolius</i>	<i>S. nodiflora</i>
1	Total Ash %	16.010 ± 0.058 <sup>c</sup>	13.633 ± 0.058 <sup>a</sup>	6.713 ± 0.006 <sup>b</sup>
2	Water soluble ash %	10.167 ± 0.058 <sup>c</sup>	6.617 ± 0.058 <sup>b</sup>	3.833 ± 0.058 <sup>a</sup>
3	Acid Insoluble ash	7.033 ± 0.058 <sup>c</sup>	6.467 ± 0.058 <sup>b</sup>	3.833 ± 0.058 <sup>a</sup>
4	Water loss on drying	0.990 ± 0.010 <sup>a</sup>	0.940 ± 0.000 <sup>a</sup>	0.940 ± 0.000 <sup>a</sup>

Note: Mean with the same alphabet are not statistically different while mean with different alphabets are statistically different.

## Extractive Value

The result of the extractive value of *D. velutinum*, *M. oppositifolius* and *S. nodiflora* using water, ethanol, methanol, and hexane was presented in Table 3. The result showed that greater yield of extract for *D. velutinum* was obtained with water ((10.8%) followed by methanol (9.6%), and ethanol (7.2%) but was least in hexane (2.2%).

The highest extract yield for *M. oppositifolius* was obtained using methanol (10.6%) followed by aqueous extract (9.8%) and ethanol (5.9%) but was least with hexane. Also, the result further revealed that methanol (15.3%) gave a greater yield of crude extract for *S. nodiflora* followed by aqueous (13.6%) and ethanol (2.9%) while hexane (2.60) had the lowest yield.

**Table: 3 : Extractive Values of *M. oppositifolius*, *D. velutinum* and *S. nodiflora***

SN	Solvent	Values (Percentage)		
		<i>D. velutinum</i>	<i>M. oppositifolius</i>	<i>S. nodiflora</i>
1	Water %	10.833 ± 0.058 <sup>d</sup>	9.7667 ± 0.058 <sup>c</sup>	13.633 ± 0.058 <sup>c</sup>
2	Methanol %	9.633 ± 0.058 <sup>c</sup>	10.567 ± 0.058 <sup>d</sup>	15.267 ± 0.058 <sup>d</sup>
3	Ethanol %	7.233 ± 0.058 <sup>b</sup>	5.900 ± 0.100 <sup>b</sup>	2.900 ± 0.100 <sup>ab</sup>
4	Hexane %	2.233 ± 0.058 <sup>a</sup>	3.403 ± 0.058 <sup>a</sup>	2.600 ± 0.100 <sup>a</sup>

## Thin Layer Chromatography of *M. oppositifolius*, *D. velutinum* and *S. nodiflora*

The result of the thin layer chromatography using methanol and ethyl acetate solvent in the ratio of 6:4 for *M. oppositifolius*, *D. velutinum* and *S. nodiflora* is presented in Table 4 as follows: *M. oppositifolius* had 3 visible spots with retention factor (RF) of 0.363, 0.425 and 0.5 in that order respectively, *D. velutinum* had 4 visible spots

with retention factor (RF) of 0.425, 0.5, 0.550, and 0.66 in that order respectively while *S. nodiflora* had 6 visible spots with retention factor (RF) of 0.255, 0.363, 0.438, 0.538, 0.600, and 0.688 in that order respectively.

**Table 4: RF Values of *M. oppositifolius*, *D. velutinum* and *S. nodiflora***

SN	RF Values		
	<i>D. velutinum</i>	<i>M. oppositifolius</i>	<i>S. nodiflora</i>
No of visible Spot	4 spots	3 spots	6 spots
1	0.425	0.363	0.255
2	0.500	0.425	0.363
3	0.550	0.500	0.438
4	0.663	-	0.538
5	-	-	0.600
6	-	-	0.6875

( Solvent = Methanol +ethyl acetate ) @ ratio of 6: 4

### Phytoconstituents

Phytochemical constituents of *D. velutinum*, *M. oppositifolius*, and *S. nodiflora* studied were investigated for the following metabolites: alkaloid, flavonoids, anthraquinones, steroids, terpenoids, proteins, carbohydrates,

anthocyanins, saponins, tannins and reducing sugar. The results of phytochemical analysis were presented in both Tables 5 and 6.

The qualitative analysis was presented in Table 5 while the quantitative analyses were presented in Table 6.

### Qualitative Phytochemical Analysis

The result of the qualitative phytochemical is presented in Table 5.0. All the phytochemicals tested were present in honey however, the result further showed the presence of alkaloids, glycosides, flavonoids, tannins, phlobatanins, saponins, proteins, carbohydrates, and anthocyanins across the three plants *D. velutinum*, *M. oppositifolius*, *S. nodiflora*. Anthraquinones and steroids were present in *M. oppositifolius* but were absent in *D. velutinum* and *S. nodiflora* while terpenoids was present in *D. velutinum* but absent in both *M. oppositifolius* and *S. nodiflora*. The result further showed the presence of reducing sugar in *S. nodiflora* but was absent in *D. velutinum* and *M. oppositifolius*

**Table: 5 The Qualitative Phytochemical constituents of *M. oppositifolius*, *D. velutinum* and *S. nodiflora***

SN	Bioactive compound	<i>D. velutinum</i>	<i>M. oppositifolius</i>	<i>S. nodiflora</i>
1	Alkaloids	Present <sup>++</sup>	Present <sup>+</sup>	Present <sup>+</sup>
2	Glycosides	Present <sup>+</sup>	Present <sup>+</sup>	Present <sup>+</sup>
3	Flavonoids	Present <sup>++</sup>	Present <sup>++</sup>	Present <sup>++</sup>
4	Tannins	Present <sup>+++</sup>	Present <sup>+</sup>	Present <sup>+++</sup>
5	Phlobatanins	Present <sup>++</sup>	Present <sup>++</sup>	Present <sup>++</sup>
6	Saponin	Present <sup>++</sup>	Present <sup>+++</sup>	Present <sup>++</sup>
7	Steroid	<b>Absent</b> ( )	Present <sup>++</sup>	<b>Absent</b> ( )
8	Proteins	Present <sup>++</sup>	Present <sup>++</sup>	Present <sup>++</sup>
9	Terpenoids	Present <sup>+</sup>	<b>Absent</b> ( )	<b>Absent</b> ( )

10	Carbohydrates	Present <sup>++</sup>	Present <sup>+</sup>	Present <sup>++</sup>
11	Anthraquinones	Absent <sup>(-)</sup>	Present <sup>+</sup>	Absent <sup>(-)</sup>
12	Anthocyanins	Present <sup>++</sup>	Present <sup>+</sup>	Present <sup>++</sup>
13	Reducing Sugar	Absent <sup>(-)</sup>	Absent <sup>(-)</sup>	Present <sup>++</sup>

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### Qualitative Phytochemical Analysis

The result of the pharmacologically important quantitative phytochemical assay is presented in Table 6 and it showed that *D. velutinum*, *M. oppositifolius*, *S. nodiflora* contained varying values of these therapeutically important phytochemicals.

The result showed that *D. velutinum* has the highest content of Alkaloid (13.7), and Tannins (17.85) followed by *M. oppositifolius* (7.97 & 17.72) and *S. nodiflora* (5.46 & 11.573) respectively in that order while honey has the least content of Alkaloids (0.224) and Tannins (0.67) respectively.

Flavonoid content was highest in *D. velutinum* (60.29), followed by *M. oppositifolius* (28.21) while *S. nodiflora* was the least (2.75). Saponin was highest in *M. oppositifolius* (0.24) followed by *D. velutinum* (0.23) respectively while *S. nodiflora* (0.14) had the least. Steroids content was higher in *M. oppositifolius* (0.077) followed by *D. velutinum* (0.049) but was absent in *S. nodiflora*. Terpenoids were absent in *M. oppositifolius* and *S. nodiflora* but were present in *D. velutinum* (0.48). Carbohydrates were highest in *D. velutinum* (36.49) followed by *M. oppositifolius* (33.79) while in *S. nodiflora* (21.11) it was the least. Reducing sugar was highest in *D. velutinum* (6.84) and *S. nodiflora* (2.01) respectively but was least in *S. nodiflora* (1.65) respectively.

**Table: 6 The Quantitative Phytochemical constituents of *M. oppositifolius*, *D. velutinum* and *S. nodiflora***

SN	Bioactive compound	<i>D. velutinum</i>	<i>M. oppositifolius</i>	<i>S. nodiflora</i>
1	Alkaloids	13.740 ± 0.052 <sup>d</sup>	7.797 ± 0.023 <sup>c</sup>	5.457 ± 0.006 <sup>b</sup>
2	Flavonoids	60.290 ± 0.017 <sup>d</sup>	28.213 ± 0.029 <sup>c</sup>	2.747 ± 0.006 <sup>a</sup>
3	Tannins	17.850 ± 0.017 <sup>d</sup>	17.723 ± 0.029 <sup>c</sup>	11.573 ± 0.029 <sup>b</sup>
4	Saponins	0.228 ± 0.002 <sup>b</sup>	0.239 ± 0.003 <sup>c</sup>	0.143 ± 0.004 <sup>a</sup>
5	Steroid	0.049 ± 0.002 <sup>a</sup>	0.077 ± 0.000 <sup>b</sup>	-
6	Terpenoids	0.483 ± 0.001 <sup>a</sup>	-	-
7	Carbohydrates	36.486 ± 0.029 <sup>c</sup>	33.793 ± 0.046 <sup>b</sup>	21.113 ± 0.029 <sup>a</sup>
8	Reducing sugar	6.837 ± 0.029 <sup>c</sup>	2.013 ± 0.029 <sup>b</sup>	1.65 ± 0.017 <sup>a</sup>

Note: Mean with the same alphabet are not statistically different while mean with different alphabets are statistically different.

### Acute toxicity studies

The result of the acute toxicity studies showed that LD<sub>50</sub> of *M. oppositifolius*, *D. velutinum* and *S. nodiflora* were above 5000mg/kg as presented in Table 7. These implied that the three plants are relatively safe.

**Table 7. Acute toxicity test of *M. oppositifolius*, *D. velutinum* and *S. nodiflora***

Phase	Dose (Mg/kg)	No of Animals used	Death Ratio		
			<i>D. velutinum</i>	<i>M. oppositifolius</i>	<i>S. nodiflora</i>
Phase 1	10	3	0/3	0/3	0/3
	100	3	0/3	0/3	0/3
	1000	3	0/3	0/3	0/3
Phase 2	1200	1	0/1	0/1	0/1

1600	1	0/1	0/1	0/1
2900	1	0/1	0/1	0/1
5000	1	0/1	0/1	0/1

Note: 0/3 and 0/1 means that no animal died when 3 and 1 animals were used respectively:

The Result implied that the LD<sub>50</sub> of *M. oppositifolius*, *D. velutinum* and *S. nodiflora* is above 5000mg/kg body weight

## Discussion

The findings showed wide variation across all the parameters studied across all the three-families and species studied. Whereas, *D. velutinum* (Fabaceae) had the least stomata size (length and width), stomata density and stomata index, it has the highest number of cells, and this could be an adaptation to conserve water in the species of the family. *M. oppositifolius* (Euphorbiaceae) on the other hand had the highest size (length and width), it has the least palisade ratio while *S. nodiflora* (Asteraceae) had the highest density and stomata index and this could be a key in their adaptation to different environmental stress.

The differences reported in the quantitative parameters of the leaf (stomatal size, cell wall thickness and stomata index) could be attributed to their physiological responses to a number of environmental factors and these differences could be usefully adopted as diagnostic characters in their delimitation at the family genus and specie level [29-30]. hence very useful in the identification of the plants.

## Conclusion.

The present showed that the pharmacognostic, physicochemical, phytochemical, and toxicological details of the *D. velutinum*, *M. oppositifolius*, *S. nodiflora* leaf are useful in laying down standardization and pharmacopeia parameters. Therefore, we conclude that pharmacognostic parameters using stomata stomatal parameters revealed

interesting variations among the three families studied and this implied that this could be a strong diagnostic tool for the classification and identification of the crude drugs. This is a vital tool in the classification and identification of the crude drugs.

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