PHARMACOGNOSTIC STUDIES OF THE LEAVES OF *Pseuderanthemum carruthersii* (Seem.) Guillauminvar. *carruthersii* (ACANTHACEAE)

Abstract

Background: *Pseuderanthemum carruthersii* var. *carruthersii*, belongs to the family of Acanthaceae. It is used in ethnomedicine as pain relief and for the management of anti-hypertension, cancers and antimicrobial. The aim of this study was to evaluate the pharmacognostic parameters of the leaves of *P. carruthersii* var. *carruthersii*.

Methods: The leaves were collected, identified, air dried, pulverized, weighed and subjected to evaluation parameters using standard procedures. GC-MS analysis was also carried out on the dichloromethane extract of the leaf.

Results: The results showed that the stomatal distribution was hypostomatic with diacytic stomatal type. Stomatal index was 23.12% and the stomatal number was 60 (69.9±2.65)86. Flow rate of the leaf was poor with the bulk volume of 32.30±1.04, tapped volume of 21.8±0.76, bulk density of 0.32±0.02, tapped density of 0.46±0.02, angle of repose 42.65°, Carr's index of 31.06% and Hausner's ratio of 1.45±0.03. The chemomicroscopy and fluorescence analyses revealed characteristic features for the drug. The water-soluble, methanol-soluble, ethanol-soluble extractive values were 20.67%$^{w}/_{w}$, 11.67%$^{w}/_{w}$ and 11.33%$^{w}/_{w}$ respectively. The moisture content of the leaf powder was 9.84%$^{w}/_{w.}$ The total ash, acid-insoluble and water-soluble ash values were 6.72%$^{w}/_{w}$, 1.34%$^{w}/_{w}$ and 16.17%$^{w}/_{w}$ respectively. The GC-MS analysis revealed the presence of 11 chemical constituents with phytochemicals such as hexanoic acid, benzoic acid, undecenoic acid, heptanoic acid and Eicosane which are of useful pharmaceutical importance.

Conclusion: These data obtained from the pharmacognostic studies provides information about the identity, purity and quality of *P. carruthersii* var. *carruthersii*.

KEYWORDS: Abaxial, Diacytic, Hypostomatic, Microscopy and *Pseuderanthemum carruthersii*

1. INTRODUCTION

Pseuderanthemum carruthersii (Seem.) Guillaumin var. carruthersii belongs to the family Acanthaceae. The species is an ornamental plant, widely and commonly cultivated throughout the tropics [1]. *Pseuderanthemum* is traditionally defined as a pantropical genus with about 60 species both in the old and New world tropics; the genus belongs to the tribe Justicaeae of the subfamily Acanthoideae [2]. This species is highly morphologically variable and as such different varieties are recognized, although they may be more appropriately be referred as cultivars since the variation is mainly seen in cultivated plants but not found in the wild [1]. *Pseuderanthemum carruthersii* is a soft-wooded shrub that reaches up to 1.5 m tall. Cystoliths are present on the surface of the plant. The leaves are ovate to elliptic, up to 17 cm long and often variegated. The leaf margin is grossly and irregularly crenate, with an acute apex and attenuate base. Flowers cluster in cymules aggregated into racemoid or paniculate cymes, with minute bracts. The calyx is 3-5 mm long, divided almost to the base into five narrowly triangular lobes. The corolla is white to pink or pale purple, with a tube that is 1-1.3 cm long and lobes that measure approximately 1 × 0.5 cm. The two lobes in the upper lip are erect and the three in the lower lip are deflexed. There are two erect stamens, held under the upper lip. The anthers are bithecous, oblong and rounded [3]. Further description by Smith (1991) and Stone (1970). Var. carruthersii encompasses both green-leaved wild plants and a cultivated form

with ovate, coarsely wrinkled or reticulate-surfaced leaves with a prominent yellow zone in the central area of the blade [4] and [5] The literature proposes the plant of *P. carruthersii* possesses important bioactive compounds. Three nitrogen-containing compounds, including uracil, adenine, indole 3-carboxaldehyde were isolated from the leaves and six phenylethanoids, such as, darendoside B, verbascoside, isoverbascoside, martynoside, leucosceptoside A and jionoside D were isolated from the root. Two lignans, pseuderesinol, pseuderanoside and a new triterpene, pseuderanic acid were isolated from the dried root of *Pseuderanthemum carruthersii* (SEEM.) GUILL. var. atropurpureum (BULL.) FOSB. (Acanthaceae), together with ten known compounds, including five lignans, eudesmin, magnolin, syringaresinol, episyringaresinol, 1-hydroxysyringaresinol and five triterpenes, squalene, oleanolic acid, lupeol, betulin, betulinic acid.

The leaf extract from this plant have been found to contain some fatty compounds, iridoids, phenylethanoids and flavonoids, which have shown high cytotoxic activity against a breast cancer cell line [6]. Root extracts composed of lignans and triterpenes have also shown moderate cytotoxic activity for the same cell line [7]. *P. carruthersii* has been used in Vietnamese traditional medicine to heal wounds due to its anti-inflammatory properties and the Ati Negrito indigenous people in the Philippines (Guimaras Island) use the leaves as an anti-pyretic poultice [7, 8]. It is also used to treat high blood pressure, diarrhoea, wounds, arthritis, tumours and diabetes.

Figure 1: *Pseuderanthemum carruthersii* (Seem.) Guillaumin var. carruthersii in its natural habitat

Scientific classification of *Pseuderanthemum carruthersii* according to Angiosperm Phylogeny Group System [9] (APG, 2016).

Kingdom	-	Plantae
Clade	-	Spermatophytes
Clade	-	Angiospermae
Clade	-	Dicotyledone
Clade	-	Scrophulariales
Family	-	Acanthaceae
Genus	-	*Pseuderanthemum*
Species	-	*Pseuderanthemum carruthersii*
Variety	-	*Carruthersii*
Synonym	-	*Pseuderanthemum reticulatum*
Common name	-	False eranthemum

MATERIALS AND METHODS

Collection, Identification and Preparation of plant material

The leaves of the plant were collected from University of Uyo Town campus, Akwa Ibom State in January 2021 and identified by Dr. Imoh I. Johnny of the department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria and the sample deposited in the University of Uyo Pharmacy Herbarium with the voucher specimen number UUPH 10(a) for reference purpose. The fresh leaves of the plant were air-dried, pulverized and packed in a well labeled dry container.

Microscopy Evaluation of Leaf

The standard median portion of the well expanded matured leaf was obtained. Microscopical examinations of the transverse section was made, the Epidermis of both adaxial and abaxial surfaces were also made by placing the leaf on a glass slide. The samples were irrigated with water and scraped gently with a sharp razor blade and the loose cells from the epidermis were washed away with water and the desired epidermis was reached. The epidermal peels were further cleared with sodium hypochlorite and rinsed gently with water. The epidermal peels were stained with aqueous solution of safranin-O for (five) 5 minutes and 10% glycerol as mountant. The stained samples were mounted on a binocular microscope. Photomicrographs were taken from good preparations using the Olympus CX21 binocular microscope fitted with an MD500 amscope microscope eyepiece camera. Measurements were done at ×10 while ×40 for photomicrographs [10].

Quantitative Microscopy of the Leaf

Quantitative microscopy parameters such as leaf constant studies namely stomatal length, width, guard cell length, width, stomatal number, stomatal index, epidermal cell length, width, epidermal cell number and epidermal cell thickness were carried out using standard procedures.
All measurements were made using a calibrated ocular micrometer and 10 microscopic fields chosen at random were used and data presented as mean ± Standard Error of Mean (SEM).

Stomatal Index Determination

The stomatal index (S.I) was determined according to Metcalfe and Chalk [11, 12].

The sample (quantitative microscopy) was placed under the microscope and the stomatal index was determined using the formula;

$$S.I=\frac{S}{E+S} X100$$

Where S = Number of stomata per unit area

E = Number of epidermal cells in the same area

Evaluation of Powders

Micromeritic Analysis

The flow property was determined using standard methods [13]. Which constitutes;

Bulk Density and Tapped Density

The weight of 10 g of dried powdered leaf was weighed into 100 ml measuring cylinder and the volume occupied was noted as the bulk volume (Vb). The cylinder was gently tapped repeatedly to obtain a constant volume noted as the tapped volume (Vt). Bulk density was calculated using the formula below;

$B\rho = M / Vb$

$Tv = M / Tv$

Where Bρ = Bulk density
M = Mass of powder
Bv = Bulk volume of powder
Tρ = Tapped density
Tv = Tapped volume

Hausner's Ratio and Carr's index

Hausner's ratio a function of interparticle friction was calculated using the formula
Hausner's ratio = Tp/Bp
While *Carr's index* = Tp - Bp/Tp × 100

Where; Tp = Tapped density
Bp = Bulk density.
Angle of repose(θ) = Tan^{-1} (Heap height of powder / Radius of heap base)

Chemomicroscopic Analysis of Leaf Powder

Powdered leaf was examined for its chemomicroscopic properties namely mucilage, lignin, starch, oils, calcium carbonate and calcium oxalate crystals using standard procedures [14] Killedar, 2014.

Fluorescence Analysis of Leaf Powders

The fluorescent analysis of dried leaf powder was carried out using standard method [15, 16].

Physico-chemical Evaluation of Leaf Powders

The physicochemical parameters such as moisture content, ash values (total ash, acid-insoluble ash and water-soluble ash values), soluble extractive values such as ethanol, methanol and water-soluble extractive values were performed according to the official method prescribed by the WHO guidelines on quality control methods for medicinal plant materials [12].

Gas Chromatography and Mass Spectrometry (GC-MS) Analysis

The GC-MS was used in the analysis, including a fused silica column packed with Elite-1 and the components were separated using helium as the carrier gas at a constant flow rate of 1ml/min. Methanol leaf extracts of 2 µL of the *P. carruthersii* var. carruthersii was used for GC-MS analysis [17]. The sample extracts were injected into the instrument and detected. During the 23rd minute of the GC extraction process, the oven was maintained at a temperature of 290°C for two (2) minutes. Mass spectra were obtained at 70eV, a scan interval of 0.5s and fragments from 40 to 440Da. The relative percentage (%) amount of each component was calculated by comparing its average peak area to the total areas.

Identification of Phytocomponents

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute of Standards and Technology (NIST), having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The retention time, compound name, molecular weight and percentage area of each component of the dichloromethane leaf extract of *P. carruthersii* was determined and recorded.

Results

Table 1: Microscopic Features of *P. carruthersii* var. *carruthersii* and Standard Error of Mean (SEM)

Leaf Surface	Abaxial	Adaxial
Epidermal cells wall pattern	Irregular	Polygonal
Stomatal distribution	Hypostomatic	Absent
Stomatal morphology type	Diacytic	Absent
Stomata length (µm)	14.90(18.03 ± 0.75)20.59	Absent
Stomata width (µm)	9.88(12.32 ± 1.31)20.19	Absent
Stomatal pore length (µm)	11.19(12.75 ± 0.33)14.13	Absent
Stomatal pore width (µm)	1.21(1.73 ± 0.15)2.50	Absent
Stomatal index	23.12%	Absent
Stomatal number	60(69.9±2.65)86	Absent
Guard cell width (µm)	3.59(6.64 ± 0.71)11.55	Absent
Guard cell length (µm)	12.34(15.11±0.86)18.11	Absent
Epidermal cell number	198(232.40 ± 10.87)298	279(364.70 ± 16.30)414
Length of epidermal layer (µm)	36.35(35.94 ± 5.49)43.42	28.53(37.23±2.16)39.64
Width of epidermal layer (µm)	14.68(16.57 ± 0.47)19.27	13.63(19.63± 1.24)22.42
Epidermal cell thickness (µm)	1.22(1.73 ± 0.10)2.12	0.50(1.13 ± 0.14)1.79

Trichome type	Glandular	Glandular
Anticlinal wall pattern	Undulate	Straight

Values are represented as mean of ten replicate (10) ± SEM

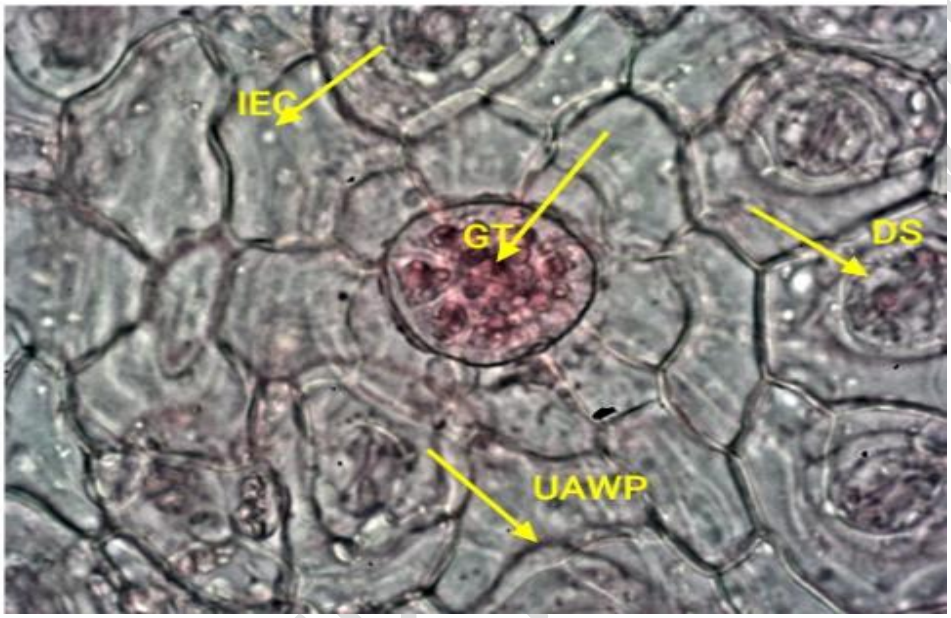


Figure 2 Abaxial Surface of *P. carruthersii* showing: IEC (Irregular Epidermal Cell); GT (Glandular Trichome); DS (Diacytic Stomata) and UAMP (Undulate Anticlinal Wall Pattern) Magnification 400

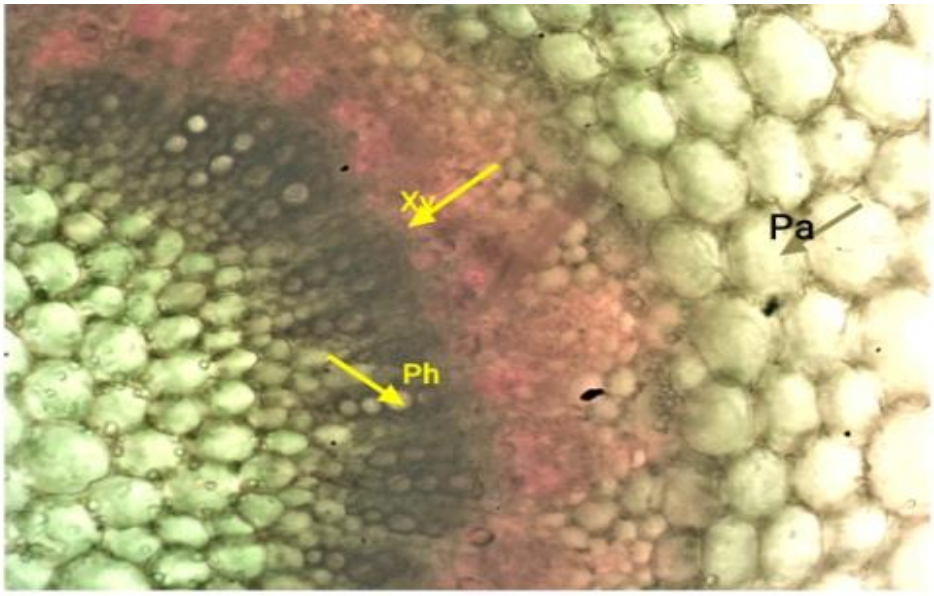


Figure 3 Petiole of *P. carruthersii* showing: Ph (Phloem) and Xy (xylem) Magnification 1003

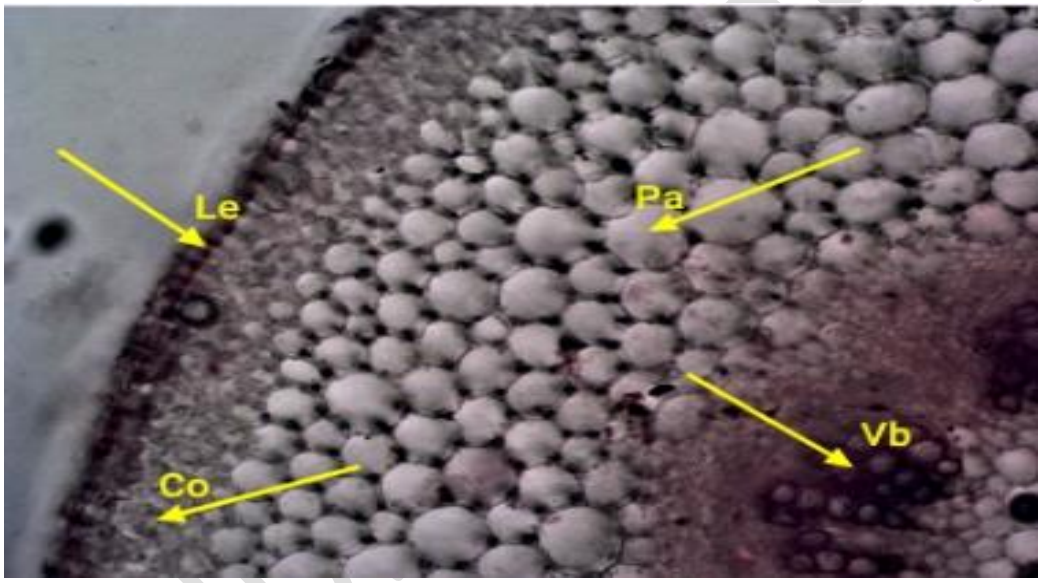


Figure 4: Transverse section of *P. carruthersii* showing Co (Collenchyma); Vb (Vascular bundle);Pa (Parenchyma) and Le (Lower Epidermis) Magnification 100

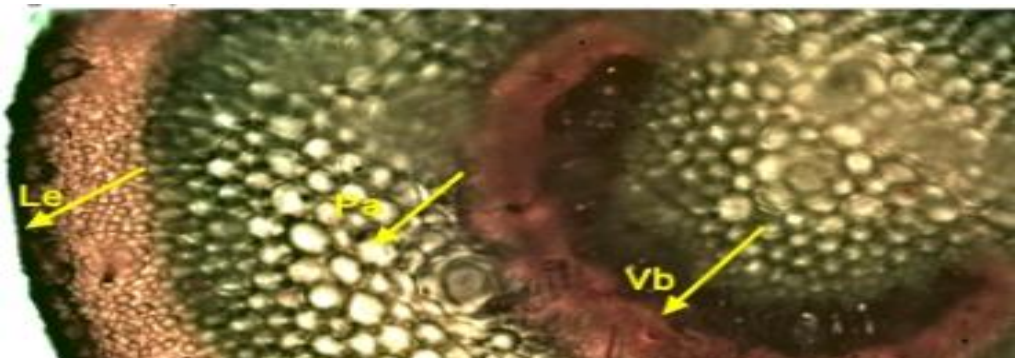


Figure 5: Cross section of the petiole of *P. carruthersii* showing Co (collenchyma); Vb (Vascular bundle); Pa (Parenchyma) and Le (Lower Epidermis) Magnification 40

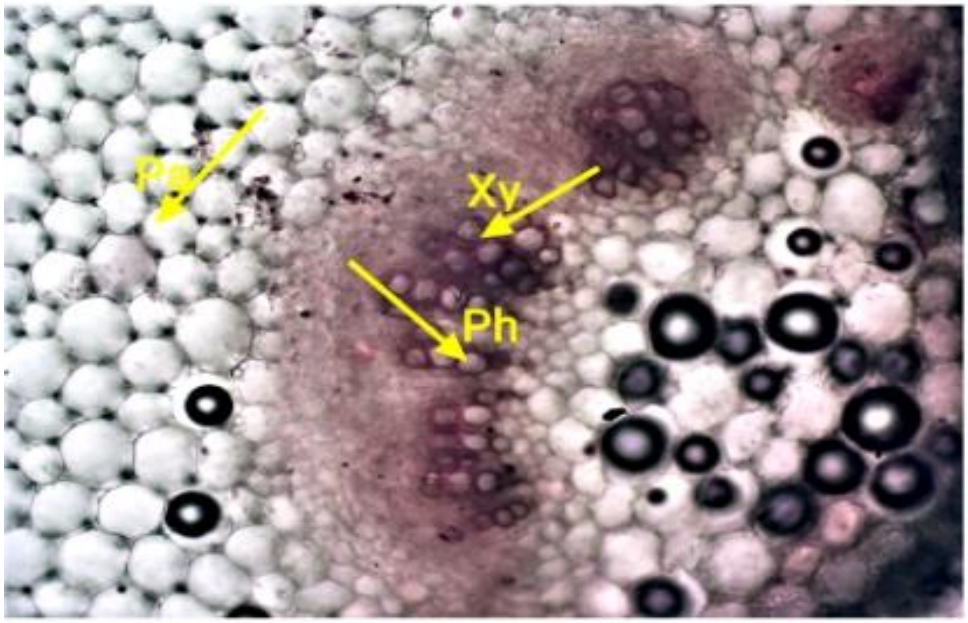


Figure 6: Transverse section of *P. carruthersii* showing Pa (Parenchyma); Xy (Xylem) and Ph (Phloem) Magnification 100

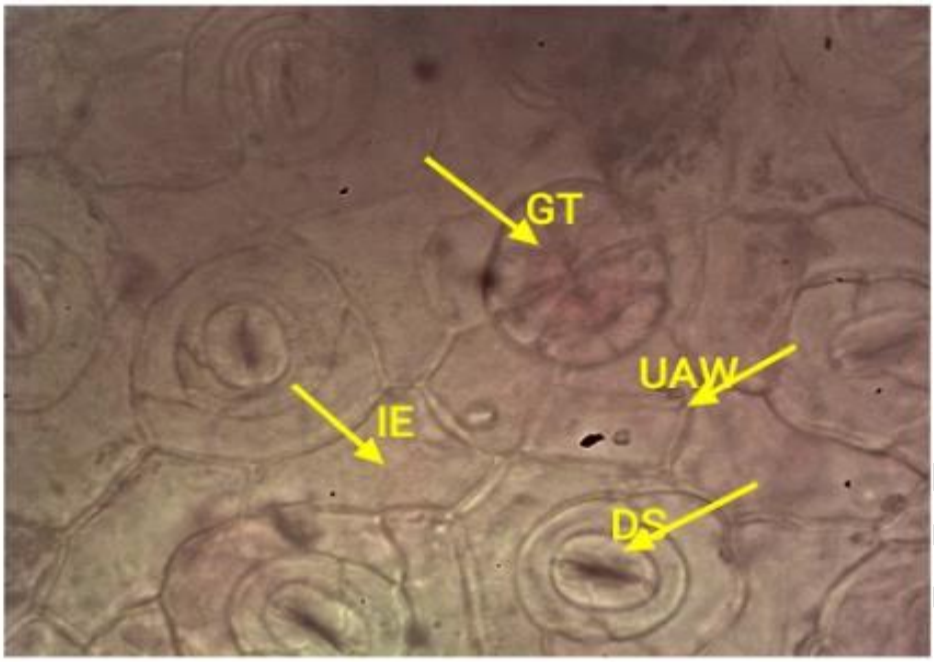


Figure 7: Powdered leaf analysis of *P. carruthersii* showing GT (Glandular Trichomes); UWA (Undulate Anticlinal Wall Pattern); IE (Irregular Epidermal Cell) and DS (Diacytic Stomata) Magnification 400

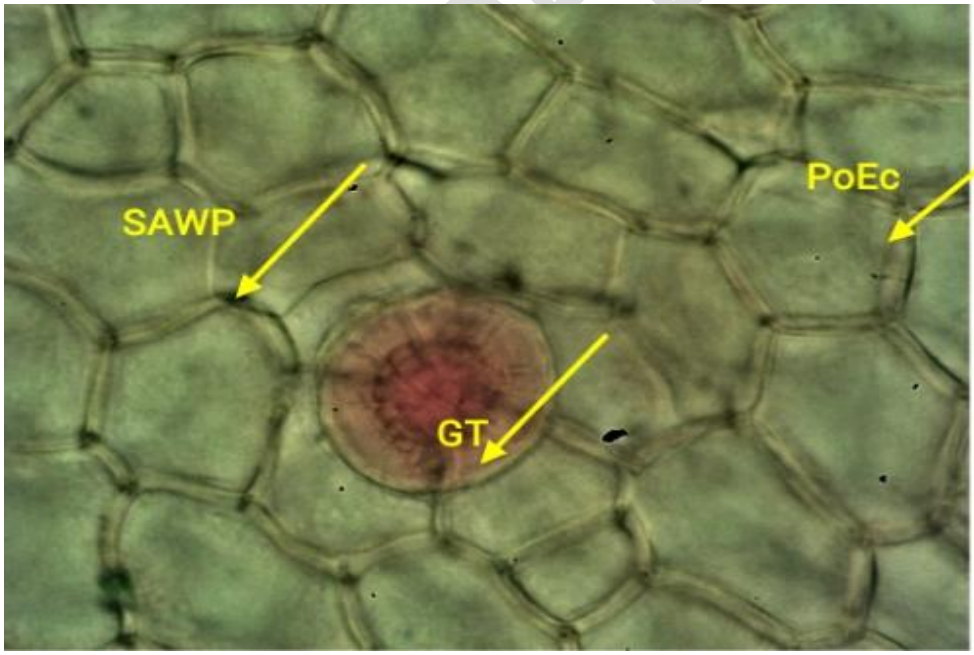


Figure 8: Adaxial surface of *P. carruthersii* showing SAWP (Straight Anticlinal Wall Pattern); GT (Glandular Trichome) and PoEc (Polygonal Epidermal Cell) Magnification 100

Table 2: Micromeritic Properties of Powdered Leaf of *P. carruthersii* var. carruthersii

Parameters	Results
Bulk Volume (ml)	32.30±1.04
Tapped Volume (ml)	21.80±0.76
Bulk Density (g/ml)	0.32±0.01
Tapped Density (g/ml)	0.46±0.02
Hausner's Ratio	1.45±0.03
Diameter (cm)	6.84±0.04
Angle of Repose (°)	42.65
Carr's Index (%)	31.06±1.58
Height of Heap (cm)	3.15±0.33
Flow Time (sec)	14.06±0.38
Flow Rate (g/sec)	0.71

Values are represented as mean of three replicates (3) ± SEM

Table 3 Chemomicroscopy of *P. carruthersii* var. carruthersii leaf powder

Parameters	Qualitative test	Observation	Inference
Mucilage	Ruthenium red	Pink colouration	Present
Lignin	Phloroglucinol + HCL	Red colouration	Present
Calcium oxalate	Chloral hydrate + 80% HCL	Shining crystal	Absent
Starch	Iodine	Blue-black colouration	Present
Cellulose	Iodine + 66% sulphuric acid	Blue colouration	Present
Protein	1% Picric acid	Brown colouration	Present

Table 4: Fluorescence Analysis of *P. carruthersii* var. *carruthersii* leaf powdered extracts

Extract	Daylight	UV-365nm
Water	Grey	Grey
Methanol	Green	Pink
Ethanol	Green	Pink
Dichloromethane	Green	Pink
N-hexane	Yellow	Pink
Ethyl acetate	Green	Pink

Table 5: Physicochemical Properties of *P. carruthersii* var *carruthersii*

Parameter	Value	Percentage (% w/w)

Moisture content	0.197 ± 0.007	9.84
Total ash value	0.403 ± 0.008	6.72
Acid-insoluble ash value	0.127 ± 0.102	1.34
Water-soluble ash value	0.323 ± 0.017	16.17
Water-soluble extractive value	0.207 ± 0.007	20.67
Methanol-soluble extractive value	0.117 ± 0.009	11.67
Ethanol-soluble extractive value	0.113 ± 0.003	11.33

Values are represented as mean of six replicates (6) ± SEM for moisture content and total ash.
Values are represented as mean of three replicates (3) ± SEM for acid-insoluble and water-soluble ash values.
Values are represented as mean of three replicates (3) ± SEM for extractive values.

Table 6: Phytochemical composition of methanol leaf extract of *P. carruthersii* var *carruthersii* by GC-MS analysis

S/N	Retention time	Compound name	Molecular formula	Molecular weight	Area %
1	5.057	Benzoic acid	$C_7H_6O_2$	122	6.395
2	5.246	Benzoic acid, 1-methyl propyl ester	$C_{11}H_{14}O_2$	178	13.199
3	5.435	Hexadecenoic acid, Z-11-	$C_{16}H_{30}O_2$	254	6.242
4	6.814	Eicosane, 10-butyl-10-propyl-	$C_{27}H_{56}$	380	9.501
5	6.911	E-2-Tetradecen-1-ol	$C_{14}H_{28}O$	212	5.469
6	7.020	Trans- -ocimene	$C_{10}H_{16}$	136	5.482
7	7.123	Hexanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)-	$C_{16}H_{28}O_2$	252	16.255
8	7.460	Ethoxyacetaldehyde diethylacetal	$C_8H_{18}O_3$	162	8.584
9	7.495	Heptanoic acid, 2-hydroxy-6-methyl-4-[(t-butoxycarbonyl)amino]3-vinyl-,t-butyl ester	$C_{19}H_{35}NO_5$	357	11.403
10	7.569	10-undecenoic acid, octyl ester	$C_{19}H_{36}O_2$	296	12.209
11	10.916	2-Tridecenal, (E)-	$C_{13}H_{24}O$	196	5.260

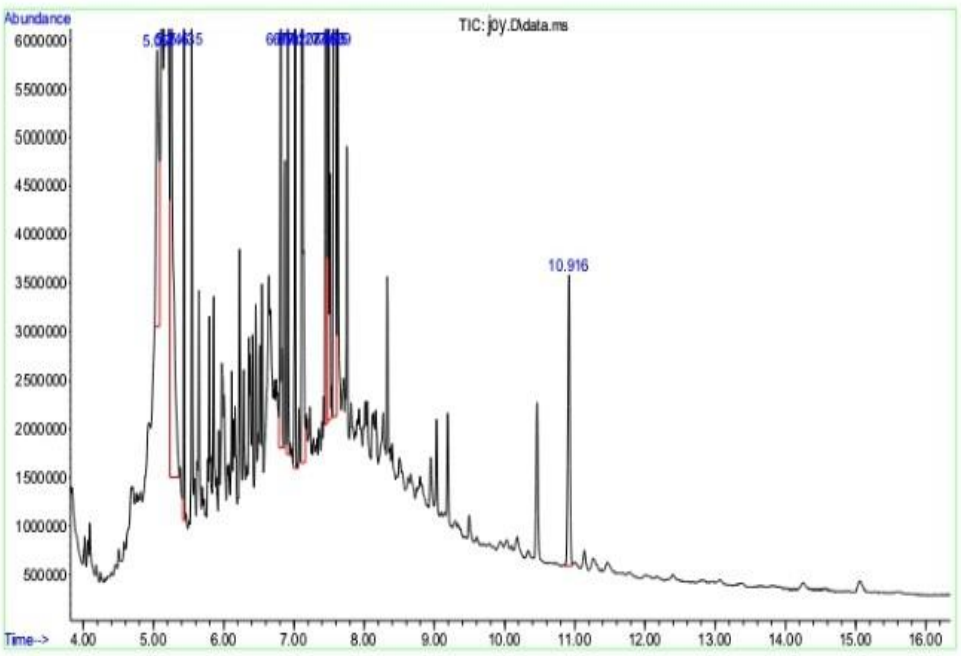
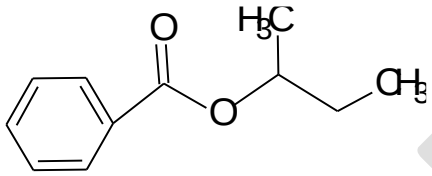
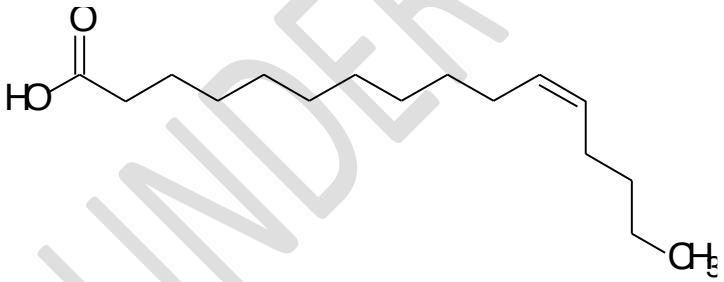


Figure 9: Extract GC-MS Scan

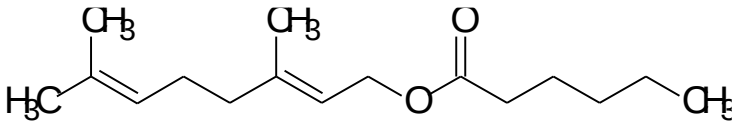
Fig 10. CHEMICAL STRUCTURES OF HIGHLIGHTED COMPOUNDS



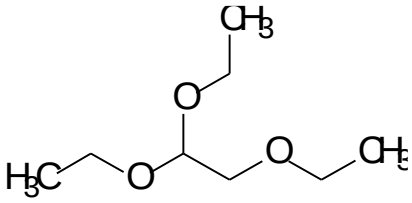
Benzoic acid, 1-methyl propyl ester



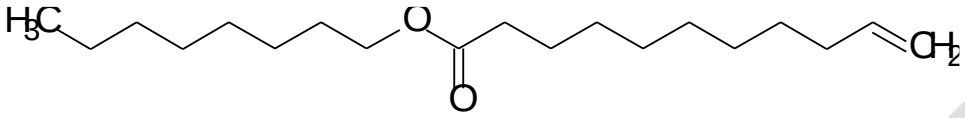
Hexadecenoic acid, Z-11-



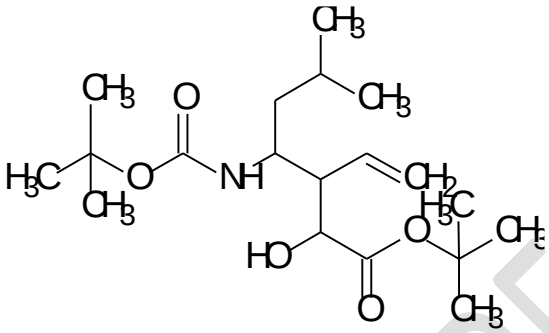
Hexanoic acid, 3,7-dimethyl 2,6-octadienyl ester, (E)



Ethoxyacetaldehyde diethylacetal



10-Undecenoic acid, octyl ester



Heptanoic acid, 2-hydroxy-6-methyl-4-[(t-butoxycarbonyl)amino]-3-vinyl-, t-butyl ester

4. Discussion

The results obtained from the microscopy of *P. carruthersii* var *carruthersii* leaf in table 1 and figure 1 to 11 showed polygonal epidermal cell shape on adaxial surface and irregular epidermal cell shape on abaxial surface with straight anticlinal wall pattern on adaxial surface and undulate anticlinal wall pattern on abaxial surface. Stomata were only found in abaxial surface and the type of stomata found was diacytic. The presence of the stomata on the abaxial surface is important as it

acts as a preventive mechanism against water loss, since the abaxial surface is not directly exposed to solar radiation [18]. Both surfaces also recorded glandular trichomes. The stomatal index of the abaxial surface recorded 23.12%. The stomatal index is significant because it does not vary with external factors, hence it is employed in plant identification.

The micromeritic properties showed the flow characteristics of the powdered leaf. Carr's index was 31.06% which indicates poor flow, Hausner's ratio was 1.452 indicating poor flow and the angle of repose was 42.65^0 indicating a passable flow property [13]. The micromeritic properties help to characterize, standardize the formulation properties of powdered herbal drug, in order to determine its suitability for formulation into solid dosage form.

Chemomicroscopy evaluation recorded the presence of lignin, starch, cellulose, mucilage, protein but calcium oxalate crystals were absent. This confirms the pharmacological potency of this plant. The fluorescence analysis of the powdered drug treated with methanol, ethanol, ethyl acetate, n-hexane, dichloromethane and water when observed in daylight and long UV light (365nm) showed different colour changes as a result of the chemical interactions between the solvents and the phytochemicals in the leaf.

For the soluble-extractive values, the water-extractive value was found to be 20.67%$^w/_w$, methanol-extractive value was 11.67%$^w/_w$ and ethanol-extractive value was 11.33%$^w/_w$. This indicates that water is the most suitable solvent for extraction of constituents of this plant.

The moisture content of *P. carruthersii* var *carruthersii* leaf as recorded in table 6, was 9.84%$^w/_w$ and this value is within the recommended range of 8-14%$^w/_w$ for vegetable drugs [12]). This is an indication that the powdered leaf can be stored for a long period with less probability of microbial attack [19].

The total ash value of *P. carruthersii* leaf was 6.72%$^w/_w$ and this value is within the accepted limit indicated in the European pharmacopoeia, 2007 [22] (not exceeding 14%$^w/_w$). Ash value gives the information about the purity, quality of any drug and give information relative to its adulteration with inorganic matter [19, 20].

The acid insoluble ash value of *P. carruthersii* var. *carruthersii* leaf was 1.34%$^w/_w$ and this value is within the accepted limit indicated in the European Pharmacopoeia, 2007 (not exceeding 2%$^w/_w$) [22]. The water soluble ash value of *P. carruthersii* leaf was 16.17%$^w/_w$.

The obtained spectrum of GC-MS analysis of methanol extract of the leaves of *P. carruthersii* var. carruthersii is shown in the results. The spectrum detected a total of 11 phytochemicals with 5 prominent peaks having higher retention time. The major components that characterized these prominent peaks include hexanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)- (16.255%), benzoic acid, 1-methyl propyl ester (13.199%), 10-undecenoic acid, octyl ester (12.209%), heptanoic acid, 2-hydroxy-6-methyl-4-[(t-butoxycarbonyl) amino] 3-vinyl-, t-butyl ester (11.403) and Eicosane, 10-butyl-10-propyl- (9.501%).

Many of these identified constituents are known to possess several pharmacological activities. Hexanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)- is known to possess several pharmacological properties such as antifungal, anti-inflammatory, anticancerous, anti-depressant, antibacterial, antioxidant, antiseptic, anti-dysentary and anti-diabetic properties [23]. Benzoic acid, 1-methyl propyl ester is known for its antimicrobial properties [23]. 10-undecenoic acid, octyl ester is used in the treatment of skin problems and it also possesses antifungal properties. Heptanoic acid, 2-hydroxy-6-methyl-4-[(t-butoxycarbonyl) amino]3-vinyl-, t-butyl ester are useful as angiotensin converting enzyme (ACE) inhibitors and as antihypertensives where it acts as a vasodilator and it also possesses properties such as anti-inflammatory, anti-arrhythmic and anti-obesity effects [23]. Eicosane, 10-butyl-10-propyl- is known to show anti-inflammatory, analgesic and antipyretic effects.

Conclusion

The result obtained from the pharmacognostic and taxonomic studies provide information about the identity, quality and purity of *P. carruthersii*. The data collectively might be used to provide information for further studies of *Pseuderanthemum carruthersii* (seem.) Guillaumin leaf.

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