STUDIES ON VARIOUS TAXONOMIC, PHARMACOGNOSTIC AND PHYTOCHEMICAL PROPERTIES OF *Sanchezia speciosa* L.

ABSTRACT

An investigation was carried out to evaluate the various taxonomic, pharmacognostic and phytochemical standards to ensure the identity, purity, safety and efficacy of the medicinal plant, *S. speciosa*. Various observations were recorded which included microscopy, micromeritics, fluorescence, chemomicroscopy, soluble extractive values, moisture contents and phytochemical analysis. The results indicated that the epidermal shapes were both polygonal on the adaxial and abaxial surfaces. Stomatal distribution was amphistomatic with anisocytic and diacytic stomata on the adaxial and abaxial surface, respectively. Stomatal index was 21.47% on the adaxial surface and 15.43% on the abaxial surface. Micromeritics results for the leaf powder were bulk volume of 25.33± 0.408, tapped volume of 19.00± 0.00, bulk density of 0.40± 0.006, tapped density of 0.53± 0.021, Hausner's ratio of 1.34± 0.037, Carr's index of 25.00± 2.550 and angle of repose of 43.07°. The micromeritics indicated a passable flow. The chemo microscopy indicated presence of lignin, mucilage and starch. Fluorescence properties showed different colours under different ultraviolet lights. The water-soluble, methanol-soluble, ethanol-soluble extractive values were 14.7% $^{w}/_{w}$, 4.3% $^{w}/_{w}$ and 5.0% ^{w}/w, respectively. Moisture content was 8.5% $^{w}/_{w}$. Total ash, acid-insoluble and water-soluble ash values were 20.0 % $^{w}/_{w}$, 2.0% $^{w}/_{w}$ and 4.2% $^{w}/_{w}$, respectively. GC-MS of methanol extract revealed the total of 39 phytochemicals with over 5 prominent peaks having higher area% which include hexadecanoic acid methyl ester (2.64%), 9,12,15-octadecatrienoic acid methyl ester (4.77%), phytol (9.98%), 9,12,15 octadecatrienoic acid (12.97%), Stigmasterol (2.00%) and Squalene (28.16%). Other compounds are glycerin (5.83%), n-hexadecanoic acid (2.85%) and 9 octadecenamide (2.18%). Many of them possess good pharmacological properties. The data generated from the present study would help to authenticate *S. speciosa* and also affirm its folklore use in traditional medicine which has potential for further development into drug product.

Keywords: Amphistomatic, GC-MS, micromeritics, pharmacognostic, *Sanchezia speciosa*, phytochemicals.

INTRODUCTION

Sanchezia speciosa L., is a genus of the plant family Acanthaceae. It is an evergreen semi-woody shrub, erect up to 2.5 m tall with quadrangular stems of green or yellowish purple colour. The leaves are 0.52 cm long, petiole winged at the base, simple, opposite, oblong elliptic brusquely pointed at the apex of 10-25 cm length and 4-8 cm breadth, intense glossy green with white or pale yellow nervations and waved crenate margins. The inflorescences are 20-40 cm long erect terminal spikes,

with yellow or pink quadrangular rachis, with 1-3 cm long internodes and persistent ovate red orange bracts, up to 4 cm long, subtending three or more unilateral hermaphroditic flowers. The singular flowers present an about 2.2 cm long calyx with five 1.6 cm long and 0.5 cm broad lobes, tubular yellow or orange corolla, 4-5 cm long with five 0.4-0.6 cm long twisted lobes. The semi woody evergreen shrub featuring bright green to purple-coloured smooth stems and large dark green leaves usually grow up to 6.5 feet in height though it can sometimes reach 10 feet as well. It rarely produces yellow flowers that are short-lived.

The plant contains alkaloids, glycosides, flavonoids, triterpenoids, carbohydrates, steroids, phenolics compounds, saponins and Tannins. The plant also contains Quercetin 3-0-a-l-rhamnopyranoside, Quercetin 3-0-b-D-galactopyranoside, 3.b-Sitosterol, 3-0-b-D glycopyranoside, 3-methyl-1H-benz indole-4,9-dione. The plant has anti-oxidative and anti-inflammatory properties. It also has anti-cancer, antifungal, insecticidal and antibacterial activities. Considering these facts in view, the present study was aimed at investigating the pharmacognostic and taxonomic parameters to aid in the identification and safe use of this drug.

Sanchezia speciosa

MATERIALS AND METHOD

Collection, Identification and Preparation of the Plant Material

The plant, *S. speciosa* was collected from Idoro road, Uyo Local Government Area. Akwa Ibom state, Nigeria in March, 2022 and identified by Dr Johnny I. Imeh of Department of Pharmarcognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo with herbarium identification number**:**UUPH No.1(f). The fresh plant material was air-dried, pulverized and packed in a dry container, well labelled and used when needed.

Anatomical Studies

Microscopy Evaluation of Leaf

The standard median portion of the well expanded matured leaf was obtained. Microscopical examinations of the Epidermis of both adaxial and abaxial surfaces were made by placing the leaf on a glass slide. The sample was irrigated with water and scraped gently with a sharp razor blade till 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 5 minutes and stained with 10% glycerol. 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**.**

Quantitative Microscopy of the Leaf

Quantitative microscopy parameters such as leaf constant studies namely stomatal length and width, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number, 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).

RESULTS

Microscopic Evaluation of Leaf

Table 1: Qualitative and Quantitative micro-morphological characters of *S. speciosa*

Leaf surface	**Abaxial**	**Adaxial**
Stomatal morphology type	Anisocytic and dicytic	Anisocytic and Anomocytic
Stomatal distribution	Amphistomatic	Amphistomatic
Stomatal length(µm)	41.49(60.22 ± 4.56)76.05	29.14(23.43 ± 1.06)18.77
Stomatal width(µm)	28.60(45.52 ± 2.81)	13.60(16.20 ± 0.56)18.91
Stomatal pore length(µm)	11.88(20.17 ± 1.93)29.00	5.81(9.46 ± 0.55)11.44
Stomatal pore width(µm)	4.75(6.86 ± 0.70)10.08	2.37(2.96 ± 0.17)3.67
Stomatal number	64(61.25 ± 0.66)58	56(68.10 ± 2.46)77
Stomatal index	15.43%	21.47%
Length of epidermal	81.25(102.24 ± 5.24)107	23.49(34.23 ± 2.92)55.09

layer(µm)		
Width of epidermal layer(µm)	33.00(44.25 ± 2.53)57.85	15.50(23.70 ± 1.74)36.09
Thickness(µm)	2.38(5.04 ± 0.52)7.13	1.16(2.12 ± 0.17)2.60
Epidermal cell shape	Polygonal	Polygonal
Epidermal number	327(335.60 ± 2.500)348	241(249 ± 2.09)262

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

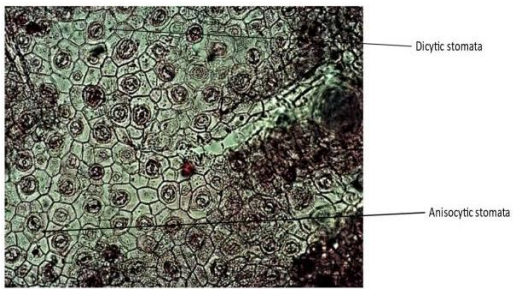


Fig. 1: *S. speciosa* Abaxial surface ×10

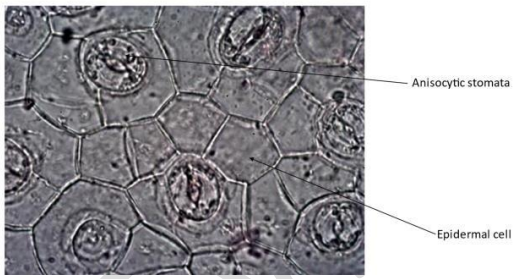


Fig. 2: *S. speciosa* Adaxial surface showing Anisocytic stomata ×40

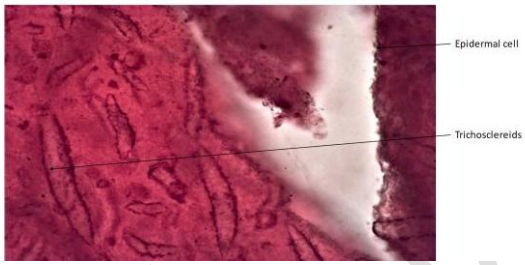


Fig. 3: *S. speciosa* leaf powder Showing Trichosclereids×10

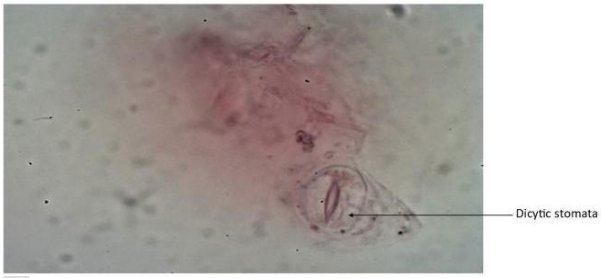


Fig. 4: *S. speciosa* leaf powder showing diacytic stomata ×40

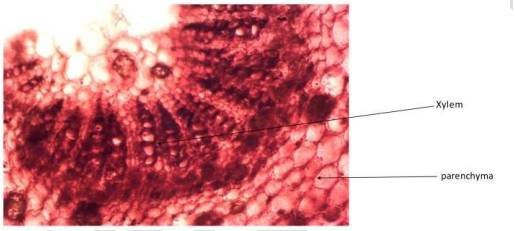


Fig. 5: *S. speciosa* leaf Petiole showing vascular bundles and parenchyma ×10

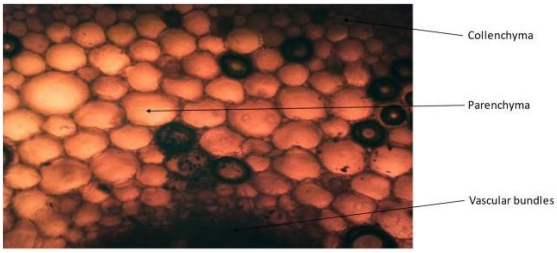


Fig. 6: *S. speciosa*leaf petiole showing Collenchyma, Parenchyma and Vascular bundle ×10

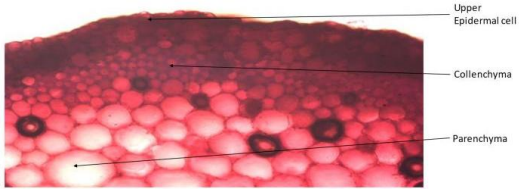


Fig. 7: *S. speciosa* leaf petiole showing upper epidermis and Collenchyma ×10

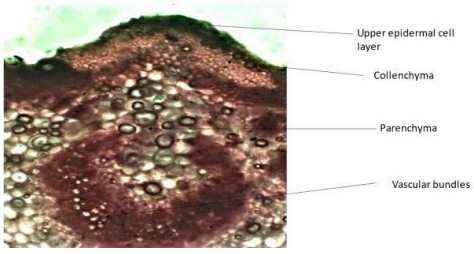


Fig. 8: T.S of Epidermis, Collenchyma, Parenchyma and Vascular bundles ×40

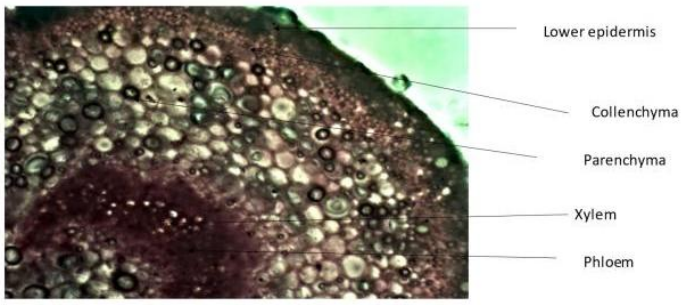


Fig. 9: T.S showing lower epidermis, Phloem and Xylem tissues ×40

Table 2: Micromeritic Properties of *S. speciosa* Powdered Leaf

PARAMETERS	RESULTS
Bulk volume (ml)	25.33±0.408
Tapped volume(ml)	19.00±0.00
Bulk density(g/ml)	0.40±0.006
Tapped density (g/ml)	0.53±0.021
Flow rate(g/s)	1.72±0.198
Angle of repose (^{o})	43.07
Hausner's ratio	1.34±0.037
Carr's index (%)	25.00±2.550
Diameter of heap (cm)	6.423±0.046
Height of heap(cm)	2.33±0.147

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

Table 3: Chemo microscopy of *S. speciosa* Leaf

Constituents	Observation	Inference
Mucilage	Pink coloration	Mucilage present
Lignin	Red coloration	Lignin present
Starch	Blue-black coloration	Starch is present
Oils	No pink coloration	Oil absent
Calcium oxalate	Calcium oxalate crystals not seen	Calcium oxalate absent

Table 4: Fluorescence analysis of *S. speciosa* leaf powder

Extract	Ordinary light	UV-365nm	UV-253.7nm
Water	Purple	Brown	Maroon
Methanol	Light green	Deep pink	Red
Ethanol	Green	Pink	Light yellow
DCM	Green	Pink	Orange
N-hexane	Brown	Orange	Red
Ethyl Acetate	Light green	Light red	Light red

Table 5: Water-soluble Extractive Value, Ethanol-soluble Extractive Value, Methanol-soluble Extractive Value and standard Error of Mean for Leaf Powders of *S. speciosa*.

Parameters	Weight (g)	Percentage (% $^{w}/_{w}$)
Water-soluble extractive value	0.1467 ±0.004	14.7
Ethanol-soluble extractive value	0.05±0.007	5.0
Methanol-Soluble Extractive value	0.0433 ±0.004	4.3

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

Table 6: Moisture Content, Total Ash Value, Acid-Insoluble Ash Value, Water-Soluble Ash Value and Standard error of Mean for the leaf of *S. speciosa*

Parameters	Weight (g)	Percentage (% $^{w}/_{w}$)
Moisture content	0.17 ±0.002	8.5%
Total ash	0.40 ±0.007	20.0%
Acid-insoluble ash value	0.04 ±0.00	2.0%
Water-soluble ash value	0.0834 ±0.004	4.2%

Values are represented as mean of eight (8) replicates ± SEM for moisture content and total ash.
Values are represented as mean of four (4) replicates ± SEM for acid-insoluble and water-soluble ash values

Table 7: Phytochemical constituents identified from methanol extract of leaf from *S. speciosa* by GC-MS analysis

S/N	Retention time	Compound name	Molecular formula	Molecular weight	Area %
1.	8.975	Glycerin	$C_3H_8O_3$	92	5.83
2.	13.310	2,6-Difluorobenzoic acid, tridec-2-ynyl ester	$C_{20}H_{26}F_2O_2$	336	0.16
3.	13.498	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	0.09

4.	13.544	Phenol, 2,4,6-tris(1-methylethyl)-	$C_{15}H_{24}O$	220	0.10
5.	13.591	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	0.20
6.	13.702	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7	$C_{11}H_{16}O_2$	180	0.37
7.	14.164	Ethanediamide, n-dodecyl-N'-(2-thiazolyl)-	$C_{17}H_{29}N_3O_2S$	339	1.63
8.	14.739	3-Buten-2-ol, 3-methyl-4-(2,6,6-trimethyl-2-cyc	$C_{14}H_{24}O$	208	0.64
9.	15.199	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabic	$C_{13}H_{20}O_3$	244	1.80
10.	15.267	3-Buten-2-one, 4-(5-hydroxy-2,6,6-trimethyl-1	$C_{13}H_{20}O_2$	208	0.49
11.	15.547	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	0.30
12.	15.985	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4]	$C_{14}H_{22}O_3$	338	1.95
13.	16.178	2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4	$C_{13}H_{18}O_3$	222	0.28
14.	16.256	3-Butylindolizidine	$C_{12}H_{23}N$	181	1.20
15.	16.703	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	1.74
16.	17.129	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.36
17.	17.345	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222	0.28
18.	17.432	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.64
19.	17.648	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	1.88
20.	17.941	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.85
21.	18.426	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284	0.12
22.	19.084	11,14-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	2.80
23.	19.141	9,12,15-Octadecatrienoic acid, methyl ester, (Z,	$C_{19}H_{32}O_2$	292	4.77
24.	19.389	Phytol	$C_{20}H_{40}O$	296	9.98
25.	19.715	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	12.97
26.	19.851	Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetra	$C_{20}H_{34}O$	290	2.97
27.	19.960	Formamide, N,N-dibutyl-	$C_9H_{19}NO$	157	1.86
28.	20.291	Octacosylheptafluorobutyrate	$C_{32}H_{57}F_7O_2$	606	1.74
29.	21.169	6-epi-shyobunol	$C_{15}H_{26}O$	222	0.34
30.	21.430	Cycloheptanone, 3-butyl-	$C_{11}H_{20}O$	168	1.54
31.	21.537	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	2.18
32.	21.840	Phytol, acetate	$C_{22}H_{42}O_2$	338	0.33
33.	22.219	Cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5.al	$C_{29}H_{50}O_2$	430	0.39
34.	23.030	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	0.39
35.	24.833	Stigmasterol	$C_{29}H_{48}O$	412	2.00

36.	24.925	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethy	$C_{32}H_{52}O_2$	468	1.22
37.	25.500	Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tet	$C_{20}H_{34}O$	290	0.88
38.	25.790	Squalene	$C_{30}H_{50}$	410	28.16
39.	25.909	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetram	$C_{20}H_{34}O$	290	0.54

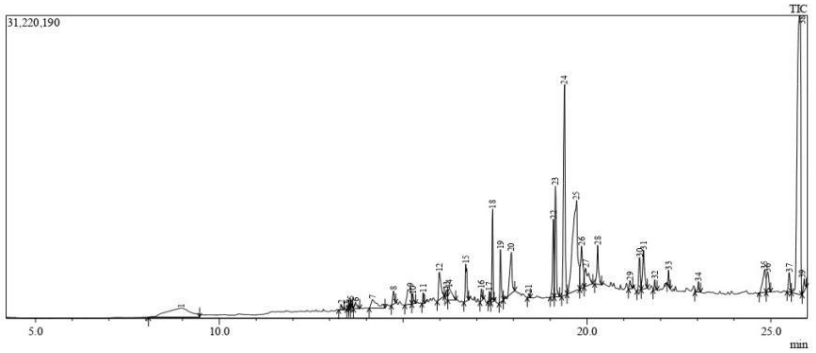
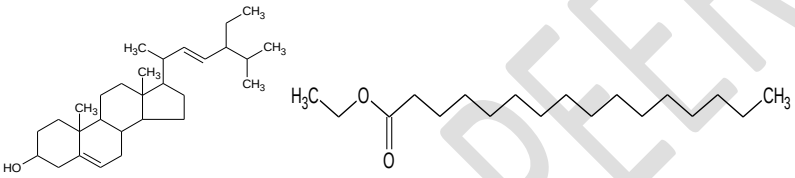


Fig. 10: GC-MS chromatogram of methanol leaf extract of *S. speciosa*



Stigmasterol **Hexadecanoic ethyl ester**

Fig. 11: Structures of some abundant phytochemical constituent in the GC-MSanalysis

DISCUSSION

Commercial supply of crude drugs has been faced with improper identification which has led to the adulteration of the genuine drug, leading to the reduction in the efficacy of the drug. Taxonomic and pharmacognostic standardization is meant for identification and detection of adulterants which represents quality control measures of crude drugs. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such material. The qualitative result in the present study showed that the epidermal cell shapes were both polygonal on the adaxial and abaxial surfaces (Fig. 1 and 2).

Johnny *et al.* (2022) reported the presence of polygonal epidermal cell shape in *C. millenii*a daxial surface as a diagnostic character for its identification.

Stomatal distribution was amphistomatic with anisocytic stomata on both the adaxial and abaxial surfaces (Fig. 1 and 2). Stomatal indices were 21.48% and 15.43% on adaxial and abaxial surfaces respectively (Table 1). Study by Metcalfe and Chalk, (1979) distinguished *Waltheria indica* from other species using the presence of paracytic and anisocytic stomata which was diagnostic. Essiett *et al*. (2010) reported that stomatal index and the guard cell provide values that will serve as parameters for comparison among taxa, which can be useful for identification of the studied taxa. Essiett and Etukudo (2012) in their study on three species of *Acalypha* occurring in Nigeria also reported that variation in stomatal index and guard cell areas are useful diagnostic tools.

The mean stomatal length and width ranged between 23.43µm - 60.22µm and 16.19µm -20.17µm for the abaxial and adaxial surfaces respectively (Table 1).

For the micromeritic studies of the powder, the bulk and tapped densities of the leaf were (0.19 ± 0.00 and 0.27 ± 0.00), Hausner's ratio and Carr's index for the leaf were (1.34 and 25 %) and angle of repose 43.07^0 (Table 2). While the bulk and tapped densities of the stem were (0.40 ± 0.06 and 0.53 ± 0.021g/mL). The angle of repose is related to the free flowability properties of particulate materials in bulk forms, the angle of repose of the powder indicated passable flowability according to the USP standard. In recent uses, the compressibility index and the closely related Hausner's ratio have become simple, fast and popular methods of predicting powder flow characteristics.

The angle of repose is considered to be the most classical technique used for characterizing the flow properties of powders. Angle of repose is a characteristic related to inter-particulate friction or resistance to movement between particles.

Chemo microscopy revealed the presence of cellulose, mucilage, lignin and starch (Table 3). The ethanol-soluble extractive value, methanol-soluble extractive value and water-soluble extractive value were $5.00\%^{w}/_{w}$, $4.3\%^{w}/_{w}$ and $14.67\%^{w}$/w, respectively (Table 5). The water- soluble extractive value indicated the presence of water-soluble matters such as sugars, amino acids and vitamins derived from plants. The ethanol and methanol-soluble extractive values indicate the presence of polar compounds.

The moisture content of $8.7\%^{w}/_{w,}$ obtained was within the African Pharmacopoeial limit of moisture content for vegetable drugs between $8\%^{w}/_{w}$ to $14\%^{w}$/w. Moreover, high moisture content is uneconomical and in the presence of appropriate temperature could lead to enzymatic activation,

hydrolytic reactions and contamination from microbes which may lead to degradation of active constituents. This plant possesses a suitable moisture content hence, it can be stored for a reasonable length of time without sample degradation. The total ash, acid-insoluble ash and water-soluble ash values were 19.8 %w/w, 2%$^{w}/_{w}$ and 4.2 $^{w}/_{w}$ %, respectively (Table 6). Ash values gives estimation about the quality and purity of crude drugs and also gives information relative to its adulteration or contamination with inorganic matter. The total ash values was high which indicated the presence of impurities, this was above the recommended limit of 14%$^{w}/_{w}$ and the acid-insoluble ash value of 2 $^{w}/_{w}$ % was within the limit of the European Pharmacopoeia, 2007 which states that the acid-insoluble ash for crude vegetable drug should not exceed 2% $^{w}/_{w.}$

The Gas Chromatography-Mass Spectroscopy is a vital tool due to its potential to supply suggested qualitative and quantitative information on constituents based on their structural compositions. The GC-MS analysis of the leaves of *Sanchezia speciosa* showed the presence of thirty nine phytochemical constituents (Table 7 and Fig. 10) for the leaf with over 5 prominent peaks having higher area percentage.

The major components that characterized these prominent peaks include hexadecanoic acid methyl ester (2.64%), 9,12,15-octadecatrienoic acid methyl ester (4.77%), phytol (9.98%), 9,12,15-octadecatrienoic acid (12.97%), Stigmasterol (2.00%) and Squalene (28.16%). Also present are important bioactive compounds such glycerin (5.83%), n-hexadecanoic acid (2.85%) and 9-octadecenamide (2.18%).

Many of these identified constituents are known to possess several pharmacological properties. Among the highlighted phytochemicals, research suggests that squalene plays important role in reducing inflammatory mediators and increase energy production. Squalene is also effective as a cancer inhibitor, antitumor, emollient and antioxidant agent in the skin. Phytol shows antitumor activity, research has also shown that Phytol is an important compound with anti-inflammatory and metabolic properties. 9,12,15-octadecatrienoic acid has anti-inflammatory, antioxidant properties and Hexadecanoic acid also has antioxidant activity. Stigmasterol has been shown to have anti-cancer activity, 9-Octadecanamide has been considered as a potential treatment for mood and sleep disorders and glycerin is used in treating many skin conditions, used in soaps, as a laxative and in enemas, it also has antimicrobial activities. Thus, *Sanchezia speciosa* has potential in the development of drugs, however further research is required.

CONCLUSION

The results from the pharmacognostic studies will help in the proper identification, collection and authentication of *Sanchezia speciosa* and its development into a standard herbal drug. The presence of various bioactive constituents confirms the usage of *S. speciosa* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may give birth to a novel drug.

REFERENCES

African pharmacopoeia. 1986. General method of analysis Pharmacopoeia **11**: 121-208.

Bakrim, S., Benkhaira, N., Bourais, I., Benali, T., Lee, L. H., El Omari, N., Sheikh, R. A., Goh, K. W., Ming, L. C. and Bouyahya, A. 2022. Health benefits and Pharmacological properties of Stigmasterol. *Antioxidants (Basel, Switzerland)* **11**(10): 1912.

Barros de Alencar, M. V. O., Islam, M. T., Torres de Lima, R.M., Paz, M. F. C. J., Reis, A. C. D., Ferreira da Mata, A.M.O., Filho, J. W. G. O., Cerqueira, G. S., Ferreira, P.M.P., Sousa, J.M. C., Mubarak, S. M. and Melo- Cavalcente, A.A.C. 2019. Phytol as an anticarcinogenic and antitumoral agent: An invivo study in Swiss mice with DMBA-induced breast cancer. *International Union of Biochemistry and Molecular Biology life* **71**(2): 200-212.

Bertani, E., Chiappa, A., Biffi, R., Bianchi, P.P., Radice, D., Bianch, V., Spampatti, S., Vetrano, I. and Andreoni, B. 2011. Comparison of oral polyethylene glycol plus a large volume glycerine enema with a large volume glycerine enema alone in patients undergoing colorectal surgery for malignancy: a randomized clininical trial. *Colorectal disease* **13**(10): 327-334.

Dalla Valle J. M. 1943. Micromeritrics: The Technology of Fine Particles. 1906- 1958.

Essiett, U. A. and Etukudo, I. S. 2012. Leaf epidermal studies of three species of *Acalypha* Linn. (Euphorbiaceae). *Advances in Applied Science Research* **3**(5): 3185–3199.

Essiett, U. A., Bala, O. N. and Agbakahi, J. A. 2010. Pharmacognostic studies of the leaves and stems of *Diodas candens* Sw in Nigeria. *Archives of Applied Research* **2**(5): 184–198.

European Pharmacopoeia. 2007. *Pharmacopoeial Limits of Crude Drugs.* Strasbourg: Council of Europe, **6**: 124-164.

Flora of India. 2014. Flowers of India, Online resource: *Sanchezia speciosa.* https://www.flowersofindia.net.

Guerrero, R. V., Abarca-Vargas, R. and Petricevich. 2017. Chemical compounds and biological activity of an extract from *Bougainvillea buttiana (*var. rose) Holttum and Standl. *International Journal of Pharmacy and Pharmaceutical Sciences* **9**(3): 42-46.

Gunes, F. 2013. Medical use of squalene as a natural antioxidant. *Journal of Marmara University institute of health sciences* **3**: 221-229.

Huang, Z., Lin, Y. and Fang, J. 2009. Biological and pharmacological activities of Squalene and related compounds: potential uses in cosmetic dermatology. *Molecules* **14**(1): 540-554.

Johnny, I. I., Umoh, U. F., Umoh. R. A., Alozie, M. F., Udobre, A. S., Igboasoiyi, A. C., Bassey, M. E., Andy, N. A., Udo, I. J. and Umoh, O. T. 2022. Pharmacognostic Characterization of *Colamillenii* K. Schum. (Malvaceae). *Asian Journal of Biology* **14**(1): 6-24.

Killedar, G. S., Harianth, N. and Sameer J., Nadaf, S. and Karade, R. 2014. Phytochemical potential of *Memecyclon umbellatum*. Burm. Leaf extracts. *Journal of Drug Delivery and Therapeutics* **4**(2): 30-35.

Kokate, C. K., Purohit, A. P. and Gokhale, S. B. *Analytical Pharmacognosy*, Nirali publication. 2005. **30**:199.

Kokoski, C. J., Kokoski, R. J. and Slama, F. J. 1958. Fluorescence of powdered vegetable drugs with particular reference to development of Ultraviolet Light radiation. *Journal of the American Phharmaceutical Association* **38**:715-771.

Kumar, D., Gupta, J., Kumar, S., Arya, R., Kumar, T. and Gupta, G. 2012. Pharmacognostic evaluation of *Cayratia trifolia* (Linn.) leaf. *Asian Pacific Journal of Tropical Biomedicine* **2**(1): 6 – 10.

Li Q, Rudolph V, Weigl B, Earl A. 2004. Interparticle van der Waals force in powder flowability and compactibility. *International Journal of Pharmaceutics* **280**:77– 93.

Loi, V. D., Tung, B.T., Ha, V .H., and Tuyen, N. M. 2016. Phytochemical and anti-inflammatory effect from the leaf of *Sanchezia speciosa* Leonard growing in Vietnam. *Journal of chemical and Pharmaceutical research* **8**(7): 309-315.

Martirosyan, D., Ashoori, M. R., Mikaeili, A. S., Serani, A., Sussman, I. and Mirmiranpour. 2022. The effect of squalene on cellular energy and inflammation in type 2 diabetes patients. *Dietary Supplements and Nutraceuticals* **1**(2): 16-29.

Mbah, C. C., Builders, P. F., Akuodor, G. C. and Kunle, O. O. 2012. Pharmaceutical Characterization of *Bridelia ferniginea* Benth. (Euphorbiaceae). *Tropical Journal of Pharmaceutical Research* **11**(4): 637-644.

Mechoulam, R., Fride, E., Hanu., Sheskin, T., Bisogno, T., Di Marzo, V., Bayewitch, M., Voge L. and Zvi. 1997. Anandamide may mediate sleep induction. *Nature* **389** (6646): 25–26.

Metcalfe, C. R. and Chalk, L. *Anatomy of the Dicotyledons.* Clarendon Press, Oxford, 1979. **1**(2): 279.

Olofsson, P., Hultqvist, M., Hellgren, L.I. and Holmdahl, R. 2014. Phytol: A Chlorophyll component with anti-inflammatory and metabolic properties: In Jacob, C., Kirsch, G., Slusarenko, A., Winyard, P., Burkholz, T. *Recent advances in redox active plant and microbial product* 345-359.

Phylogeny of *Thunbergia laevis* According to Angiosperm Phylogeny Group(APG) System. 2016.

Rafshanjani, M. A. S., Parvin, S., Kader, M. A. and Sharmin, T. 2015. Preliminary phytochemical screening and cytotoxic potentials from leaves of *Sanchezia speciosa* Hook f. *International Journal of Advances in Scientific Research* **1**(3): 145-150.

Rafshanjani, M. A. S., Parvin, S., Kader, M. A., Saha, M. R. and Akhtar, M. A. 2014. Invitro antibacterial, antifungal and insecticidal activities of ethanolic extracts and its fractionates of *Sanchezia speciosa* Hook. F. *International Research Journal of Pharmacy* **5**(9): 717-720.

Rasanen E, Antikainen O, Yliruusi J. 2003. A new method to predict flowability using a microscale fluid bed. *American Association of Pharmaceutical Scientists Pharmscitech* **4**: 418–424.

Spinler, J. K., Auchtung, J., Brown, A., Boonma, P., Oezguen, N., Ross, C. L., Luna, R. A., Runge, J., Versalovic, J., Peniche, A. and Dann, S .M. 2017. Next- generation Probiotics Targeting *Clostridium difficile* through precursor directed antimicrobial biosynthesis. *Infection and immunity* **85**(10): 303-317.

Teunou, E. and Fitzpatrick, J. J. 1999. Effect of relative humidity and temperature on food powder flowability. *Journal of Food Engineering* **42**: 109-116.

Tung, B. T., Vu Duc, L., Nguyen, T. H. and Nguyen, T. V. 2017. In vitro antioxidant and anti-inflammatory activities of isolated compounds of ethanol extract from *Sanchezia speciosa* Leonard's leaves. *Journal of basic and clinical physiology and pharmacology* **28**(1):79-84.

Umoh, R. A., Umoh, U. F., Johnny, I. I., Umoh, O.T., Anah, V. U., Udoh, A.E., Elijah, A. A., Adefabi, M. A. and Matthew, E A. 2020. Phytopharmacognostic evaluation of the leaves of *Gnetum africanum* Welw (Gnetaceae). *Journal of complementary and Alternative Medical Research* **11**(3): 32-41.