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Pharmacognostic Evaluation of the Leaves of *Thurnbegia laevis* Nees (Acanthaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Thurnbegia laevis Nees. belong to the Acanthaceae family. It is used in folklore medicine for the management of diabetes, piles, joint pains as well as skin related problems. This study aims to evaluate the leaves of the plant by employing the quality control parameters. The leaves of *T. laevis*

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were collected, identified, air-dried, pulverized and stored in glass bottles. Standard procedures were used to carry out microscopy on the fresh leaves, micromeritics, chemomicroscopy, fluorescence analysis, soluble-extractive values, moisture contents and ash values using powdered samples of the leaf. Methanol extract of the leaf was used to conduct GC-MS analysis. The result showed that the leaf was amphistomatic with diacytic stomata on both surfaces. The micromeritic studies showed that the leaf had an angle of repose of 37.47°, Carr's Index of 23.01% and Hausner's ratio of 1.3. Water-soluble, ethanol-soluble and methanol-soluble extractive values were 21%$^{w}/_{w}$, 10%$^{w}/_{w}$ and 11%$^{w}/_{w}$, respectively. Fluorescence analysis of the leaf showed different colours indicating the presence of different phytochemicals. The moisture content, total ash, acid-insoluble and water-soluble ash values were 5.31%$^{w}/_{w}$, 15.18%$^{w}/_{w}$, 2.7%$^{w}/_{w}$ and 5.6%$^{w}/_{w}$, respectively. Chemomicroscopy of the leaf powder showed the presence of mucilage, lignin, starch, cellulose and protein while, oil and calcium oxalate crystals were absent. The GC-MS analysis showed the presence of thirty-five (35) phytochemicals with compounds like n-Hexadecanoic acid, 9,12,15-Octadecatrienoic acid, Vitamin E, Stigmasterol, Supraene and, Benzoic acid. These compounds have antioxidant, anticonvulsant, antiserum, antifungal, hypoglycaemic, antimicrobial, anti-inflammatory and larvicidal activities. The results stated could be used to establish standards for the authentication of the fresh and powdered drug leaf products of *Thunbergia laevis*.

Keywords: GC-MS; micromeritics; micromorphological; pharmacognostic; phytochemicals; Thunbergia laevis.

1. INTRODUCTION

Thunbergia laevis is a plant species of the Acanthaceae family also known as the acanthus family [1]. *Thunbergia laevis* is a perennial twinning plant that creeps unto any medium close to its place of habitat. It is native to India and a great part of Southern Asia; however, it crosses to Australia and Africa. The stem is slightly branched, striated longitudinally with leaves that are opposite and long-stalked. The plant has its white flowers blooming from a bud which gains it the common name, "white trumpet vine" The paste of the leaves of this plant has been found to be useful in the management of hyperglycaemia, treatment of skin problems, back and joint pains, pile and many others [2]. *T. laevis* just as the other species of Acanthaceae family is found mostly in moist and shady habitats in the forest of West India and Nepal. They also grow among the grass lands in Africa, Brazil, Central America and Malaysia [3]. *Thunbergia grandiflora* has a wide range of chemical constituents present is the essential oils of the leaf. The essential (volatile) oils in the leaf contains 3-octanol being the highest with 46%, 3, 7 dimethyl 1, 6-octadien-3-ol (13.22%), 2-methoxy-3-(-2-propenyl) phenol (8.36%) [2]. The leaf contained a total of twenty-seven (27) compounds which amounted to 98.13% of the compounds in the leaf.

Thunbergia laurifolia has been discovered to have hypoglycaemic activity. It is commonly used as herbal medicine in Thailand and other parts of Asia [4]. *Thunbergia laevis* tea has been approved to be enlisted on the Thai National List of Essential Medicines for detoxification of poisons with hypoglycaemic side effects. Although, the investigation carried out by Preechasuk‟s team did not indicate any hypoglycaemic effects on humans (both hyperglycaemic and normoglycaemic humans), *T. laevis*‟ hypoglycaemic effect on animal models cannot be ruled out [5]. However, reports have still been made on the significant hypoglycaemic properties of the leaf [6].*Thunbergia alata* has been investigated and found to contain flavonoids, a group of polyphenolic compounds with a unique benzo- γ - pyrone structure. Kaempferol-3-*O*-(6""-ϱ-coumaroyl) glycoside specifically showed high energy binding score which will enable it achieve its antiviral remedy especially against COVID-19 [7].

Fig. 1. *Thunbergia laevis,* in its natural habitat

2. MATERIALS AND METHODS

2.1 Collection, Identification and Preparation of the Plant Material

Plant sample was collected at the Faculty of Pharmacy, University of Uyo in March, 2021. The plant was identified by Dr. Imoh I. Johnny of department of Pharmarcognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo and voucher specimen have been deposited in the herbarium with the number UUPH 1 (j). The dried leaves were pulverized using Sonik SON/MPR/EE/1314 electric blender, sieved through 350 micron sieve size and stored in airtight bottles to avoid interference from some natural factors such as moisture, microorganisms, humidity etc. before use.

2.2 Microscopy Evaluation of the Leaf

2.2.1 Qualitative microscopy

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 (five) 5 minutes and 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 [8,9].

2.2.2 Quantitative microscopy

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).

2.3 Stomatal Index Determination

The stomatal index (S.I) was determined according to Metcalfe and Chalk [10,11].

using the formula:

The sample 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

2.4 Micromeritics

The flow property was determined using standard methods [12] which constitutes:

2.5 Bulk Density and Tapped Density

The weight of 10 g of dry 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:

$$B\rho = \frac{M}{Vb}$$

$$T\rho = \frac{M}{Vt}$$

Where $B\rho$ = Bulk density
M = Mass of powder
Vb = Bulk volume of powder
$T\rho$ = Tapped density
Vt= tapped volume

Interparticulate porosity was also calculated using the formula below;

$$IP = \frac{\rho T-\rho B}{\rho T * \rho B}$$

2.6 Hausner's Ratio and Carr's index

Hausner's ratio a function of interparticle friction was calculated using the formula

$$\text{Hausner's ratio} = \frac{\text{T}\rho}{\text{B}\rho}$$

While Carr's Index is measured as

$$\text{Carr's index} = \frac{\text{T}\rho - \text{B}\rho}{\text{T}\rho} \times 100$$

Where; $T\rho$ = Tapped density
$B\rho$ = Bulk density.

2.7 Angle of Repose

$$\theta = \text{Tan}^{-1}\left(\frac{\text{Heap height of powder}}{\text{Radius of heap base}}\right)$$

2.8 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 [13].

2.9 Fluorescence Analysis of Leaf Powders

The fluorescent analysis of dried leaf powder was carried out using standard method [14].

2.10 Physico-chemical Evaluation of Leaf Powders

The physicochemical parameters such as moisture content, ash values (total ash, acid-insoluble ash, water-soluble ash), 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 [11,15].

3. RESULTS

The qualitative and quantitative micro-morphological characters for the abaxial and adaxial surfaces of the leaves of *T. laevis* Table 1 and Fig. 2 (A – E) respectively. The transverse section was presented in Fig. 3 (A – D) while Fig. 4 showed the result for the powder analysis. Micromeritic property, chemomicroscopy, fluorescence, extractive values, moisture content and GC-MS analysis were presented in Tables 2, 3, 4, 5, 6 and 7 respectively.

4. DISCUSSION

Many plants have not been evaluated for proper identification even though the first step towards standardization is ensuring the quality of its starting material is by proper authentication. The

Table 1. Microscopic Features of *Thunbergia laevis*

Parameters	Adaxial surface	Abaxial surface
Shape of Epidermal cell	Irregular	Irregular
Anticlinal wall pattern	Sinuous	Sinuous
Stomatal type	Diacytic stomata	Diacytic stomata
Stomatal length(µm)	19.78(24.26±1.01)29.62	20.98(21.42±0.13)22.19
Stomatal width(µm)	7.44(10.16±0.66)13.99	5.20(7.07±0.28)8.21
Stomatal number	138(158±4.85)183	196(205±2.35)217
Stomatal pore length(µm)	11.19(12.23±0.33)13.48	12.92(13.31±0.07)13.60
Stomatal pore width(µm)	2.32(2.45±0.04)2.60	2.15(3.039±0.22)3.82
Stomatal index (%)	29.80(33.4±0.783)36.79	35.50(37.11±0.43)40.03
Epidermal layer number	280(316.1±4.20)328	319(347.7±5.56)376
Epidermal wall pattern	Sinuous	Sinuous
Epidermal cell length(µm)	54.83(77.24±3.68)96.67	40.81(56.08±2.85)73.78
Epidermal cell width(µm)	17.74(23.84±1.48)34.32	13.20(18.08±1.14)
Epidermal wall shape	Irregular	Irregular
Thickness of epidermis(µm)	2.50(3.03±0.22)4.65	3.20(3.86±0.14)4.65
Trichome type	Unicellular covering and multicellular	Unicellular covering and multicellular
Trichome length(µm)	140.96(172.5±5.48)198.99	146.48(267.8±73.32)914.22
Trichome width(µm)	9.93(11.28±0.41)13.99	10.71(19.6±1.39)24.94

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

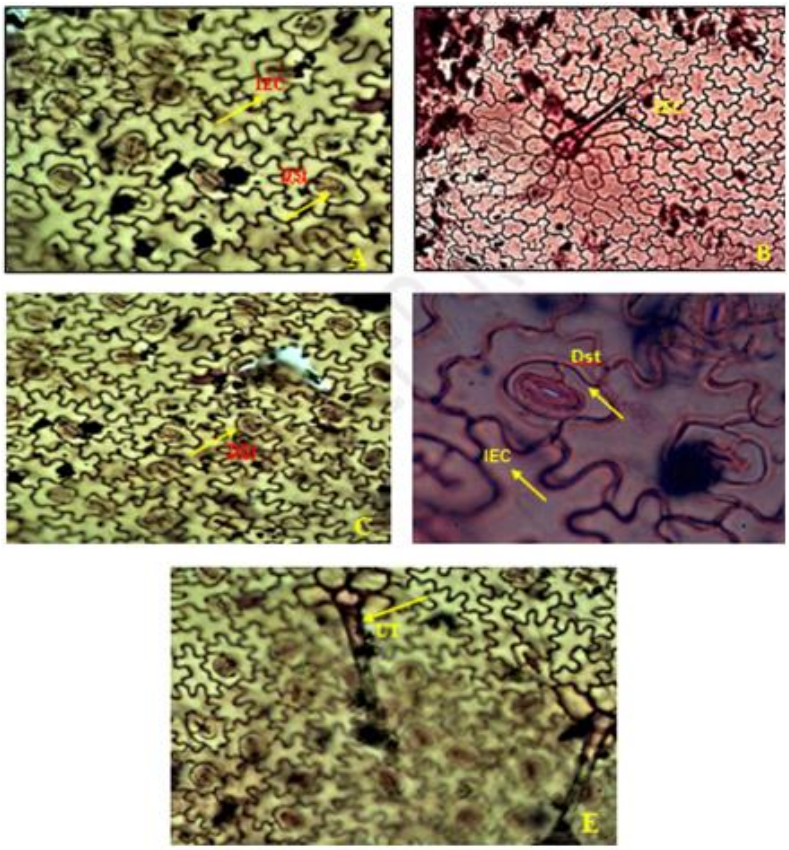

Fig. 2. (A and B Adaxial Surface): Irregular epidermal cell (IEC) × 40: Diacytic stomata × 40 (DSt): (UT) (B): Unicellular trichome (UT) × 10. (C and D Abaxial surface): (C): Diacytic stomata (DSt) × 10: (D): Irregular epidermal cell (IEC) × 40; (E): Unicellular trichome (UT) × 10

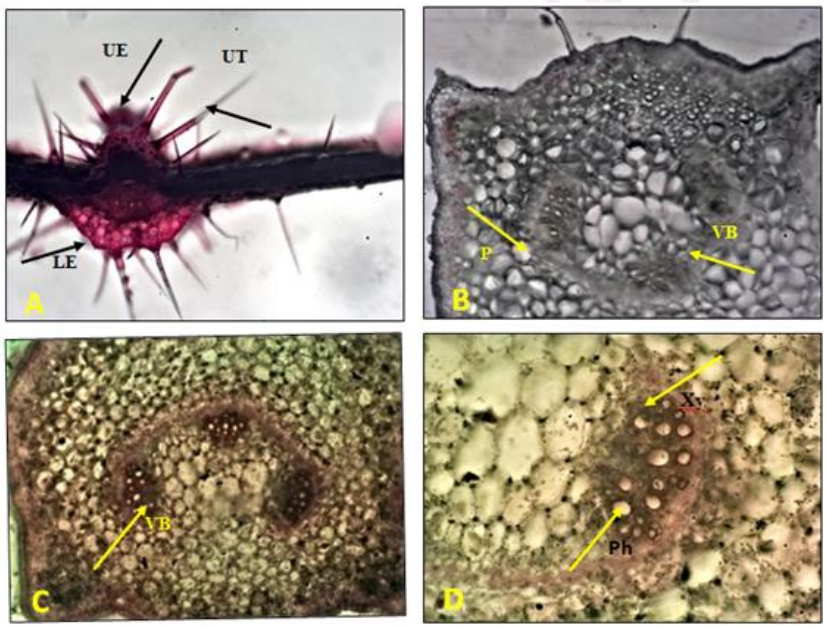

Fig. 3. (A): Transverse section of leaf; (UE) Upper epidermis, Unicellular trichome (UT), Lower epidermis (LE) × 4; (B): Vascular bundle (VB), Parenchyma (P) × 10: (C): Vascular bundle (VB) × 10: (D): Xylem (Xy), Phloem (Ph) × 40

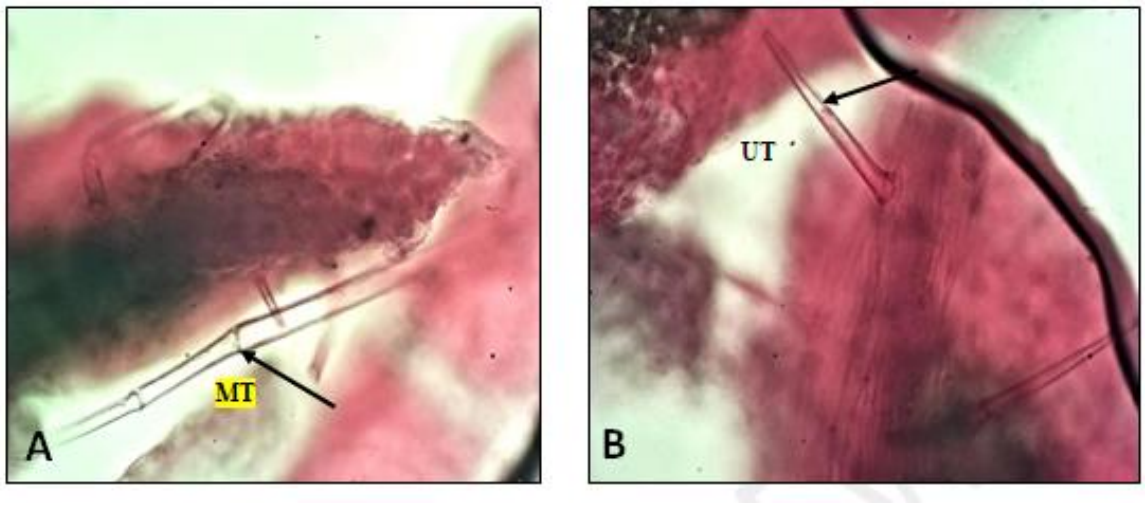

Fig. 4. Powder microscopy (A): Multicellular trichome (MT) × 10: (B): Unicellular trichome (UT) × 10

Table 2. Micromeritic evaluation of powdered leaf of *T. laevis*

Micromeritic parameters	Leaf powder values
Bulk volume (ml)	42.33 ± 1.47
Tapped volume (ml)	32.66 ± 0.40
Bulk density (g/ml)	0.23 ± 0.01
Tapped density (g/ml)	0.30 ± 0.01
Flow rate (g/s)	17.81 ± 0.96
Angle of repose (degrees)	37.47 ± 2.44
Carr"s index (%)	23.01 ± 4.37
Hausner"s ratio	1.30 ± 0.07

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

Table 3. Results for Chemomicroscopy of *Thunbergia laevis* Leaf Powder

Constituents	Qualitative tests	Observations	Inference
Mucilage	Sample + Ruthenium red, viewed under microscope	Sample stained pink	Mucilage present
Lignin	Sample + Phloroglucinol + Conc. HCl viewed under microscope	Sample stained red	Lignin present
Starch	Sample + N/50 iodine, viewed under microscope	Sample stained blue	Starch present
Calcium oxalate crystals	Sample cleared, viewed under microscope	No crystals	Calcium oxalate crystals is absent
Cellulose	Sample + N/50 iodine + 66% sulphuric acid, viewed under microscope	Sample stained blue	Cellulose present
Oils	Sample + Sudan IV, viewed under microscope	No colour change	Oil absent
Proteins	Sample + 1% picric acid, viewed under microscope	No colour change	Protein absent

Table 4. Results for Fluorescence Properties of *T. laevis* Leaf Powder

Extract	Physical observation Colour	UV-365nm Colour	UV-253.7nm Colour
Water	White with brown boundary	Blue	White with blue boundary
Methanol	Light green with deep green boundary	Pink with red boundaries	Green
Ethanol	Light green with deep green boundary	Pink	Light Green
DCM	Deep green with light green boundary	Brown with pink boundaries	Deep green with light green boundary
N-Hexane	Very light green	Pink	Light Green
Ethyl acetate	Light green	Pink	Light Green

Table 5. Water-Soluble Extractive Value, Ethanol-Soluble Extractive Value and Methanol-Soluble Extractive Value for Powdered Leaf of *T. laevis*

Parameters	Weight (g)	Percentage by weight (%$^{w}/_{w}$)
Water-soluble extractive value	0.21	21
Ethanol-soluble extractive value	0.10	10
Methanol-soluble extractive value	0.11	11

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

Table 6. Moisture Content, Total Ash Value, Acid-Insoluble Value and, Water-Soluble Value

Parameters	Weight (g)	Percentage by weight (%$^{w}/_{w}$)
Moisture content	0.1063	5.31
Total ash value	0.3037 ± 0.00	15.18
Acid-insoluble ash value	0.055 ± 0.00	2.7
Water-soluble ash value	0.1125 ± 0.00	5.6

Values are represented as mean of eight (8) replicates ± SEM for Moisture Content and Total Ash Values
Values are represented as mean of four (4) replicates ± SEM for Acid-Insoluble and Water-Soluble Ash Values

Table 7. Phytochemical Composition of Methanol Leaf Extracts of *T. laevis* by GC-MS Analysis

S/N	Retention time	Compound Name	Molecular Formula	Molecular weight	Area Percentage (%)
1	4.587	Glycerin	$C_3H_8O_3$	92	0.85
2	9.415	1-Piperidinethicarboxamide	$C_6H_{12}N_{25}$	114	14.76
3	13.542	Phenol,2,4,6-tris(1- methlethyl)-2,4,6-Triisopropylphenol	$C_{15}H_{24}O$	220	0.16
4	13.584	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	0.27
5	13.696	2(4H)-Benzofuranone,5,6,7,7atetrahydro-4,4,7a-trimethyl-(R)	$C_{11}H_{16}O_2$	280	0.59
6	14.162	Propylphosphonic acid, fluoroanhydride,heptyl ester	$C_{10}H_{22}FO_2P$	224	1.42
7	14.517	3-hydroxy-beta.-damascone	$C_{13}H_{20}O_2$	208	0.34
8	14.751	2,5-Dimethylhex-5-en-3-yn-2- ol	$C_8H_{12}O$	124	0.62
9	15.259	1-{2-[3-(2-Acetyloxiran-2-yl)-1,1-dimethylpropyl]cycloprop-2enyl}ethanone	$C_{14}H_{22}O_2$	222	1.12
10	15.544	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	0.21
111	15.836	Trans-Geranylgeraniol	$C_{20}H_{34}O$	290	0.41

S/N	Retention time	Compound Name	Molecular Formula	Molecular weight	Area Percentage (%)
12	16.451	Oxirane,2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19heneicosapentaenyl)-	$C_{30}H_{50}O$	426	1.09
13	16.725	Phytol, acetate	$C_{22}H_{42}O_2$	338	1.20
14		3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.32
15	17.341	9-Hexadecenoic acid	$C_{17}H_{32}O_2$	268	0.26
16	17.424	Hexadecanoic acid, methyl ester	C17H34O2 $C_{17}H_{34}O_2$	270	1.50
17	17.646	Dibutyl phthalate 1,2Benzedicarboxylic acid	$C_{16}H_{22}O_4$	278	1.92
18	18.011	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	16.37
19	18.423	Isopropyl palmitate	$C_{19}H_{38}O_2$	298	0.20
20	19.077	12,15-Octadecadienoic acid	$C_{19}H_{34}O_2$	294	0.77
21	19.131	9,12,15-Octadecatrienoic acid	$C_{19}H_{32}O_2$	292	2.22
22	19.360	Phytol	$C_{20}H_{40}O$	296	2.84
23	19.746	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O2$	278	21.12
24	19.910	Octadecenamide	$C_{18}H_{36}O_2$	284	7.46
25	21.540	9-Octadecenamide	$C_{18}H_{35}NO$	281	2.46
26	22.211	Vitamin E	$C_{29}H_{50}O_2$	430	3.37
27	22.716	1H-Indene, 1-hexadecyl-2,3-dihydro-1	$C_{25}H_{42}$	342	1.19
28	23.031	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	0.67
29	23.234	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430	0.48
30	24.172	5-Cholestene-3-ol	$C_{28}H_{48}O$	400	1.88
31	24.777	Stigmasterol	$C_{29}H_{48}O$	412	0.89
32	25.417	Obtusifoliol	$C_{30}H_{50}O$	426	0.34
33	25.724	Supraene	$C_{30}H_{50}$	410	10.21
34	25.878	2H-Benzo[f]oxireno[2,3E] benzofuran8(9H)-one	$C_{21}H_{28}N_2O_3$	356	0.14
35	25.992	Benzoic acid	$C_{14}H_{24}O_4Si_2$	312	0.35 100

qualitative microscopic studies of the leaf showed that *T. laevis* was amphistomatic with a diacytic stomata on the adaxial and abaxial surfaces respectively (Fig. 2 A and C) [16]. Both surfaces of the leaf were also seen to possess the unicellular and multicellular trichomes respectively. The adaxial and abaxial surfaces had a stomatal index of 33.4% and 37.11% respectively (Table 1).

The occurrence of more stomata on the abaxial surface is an adaptation to water loss as this signifies a coping strategy to survive drought [17]. Therefore, *T. laevis* had the stomatal number of (205±2.35) with stomatal length (21.42±0.13 µm), stomatal width (7.07±0.28 µm), stomatal pore length (13.31±0.07 µm) and stomatal pore width (3.039±0.22 µm) as reported in Table 1.

Stomatal length, stomatal width, number of stomata, length of trichome and width of trichome were 24.26 µm, 10.16 µm, 158, 172.5µm, 11.28µm respectively. The micromeritic studies of the leaf powder of the research plant showed an angle of repose of 37.47 degrees (which indicates a fair flow because it falls within the range of 36-40 degrees; Hausner"s ratio of 1.3 indicating a passable flow as it is within the range of 1.26-1.34 and Carr"s index of 23.01% indicating passable flow which is between ranges 21%-25%. Micromeritic properties are the most important parameters to be considered in ensuring the quality of tablets. Micromeritic properties indicate the interparticulate resistance between powder particles which in turn influence the processing parameters during manufacturing of formulation [18]. Flow properties of a particle may be affected by the paricle size, moisture content, particle shape, time of storage, etc. the angle of repose, Hausner's ratio and Carr's index are parameters used in the characterisation of flow properties of particles [9]. An angle of

repose is a measure of powder flow. It is used to qualify powder flowability due to its relationship with interparticulate cohesion. A particle flowing at a large angle of inclination will overcome frictional forces while that which flows at an angle of inclination that is not large will not overcome the frictional forces (adhesion/cohesion). The frictional forces affect the particle's flow rate into tableting dies during manufacturing. Hausner"s ratio compares the tapped density of a powder to the bulk density. Powders are in an indirect relationship with interparticulate friction meaning the powders with low interparticulate friction are free-flowing (with ratios less than 1.2) while, powders with high interparticulate friction are less free-flowing (with ratios greater than 1.5). Compressibility index also referred to as Carr's index also compares the bulk density of a powder to the tapped density. It indicates how well powder particles can be compressed into tablet formulations or any other solid dosage form [19]. In alignment with the flow property characterization, the results are said to have passable to fair flow characteristics.

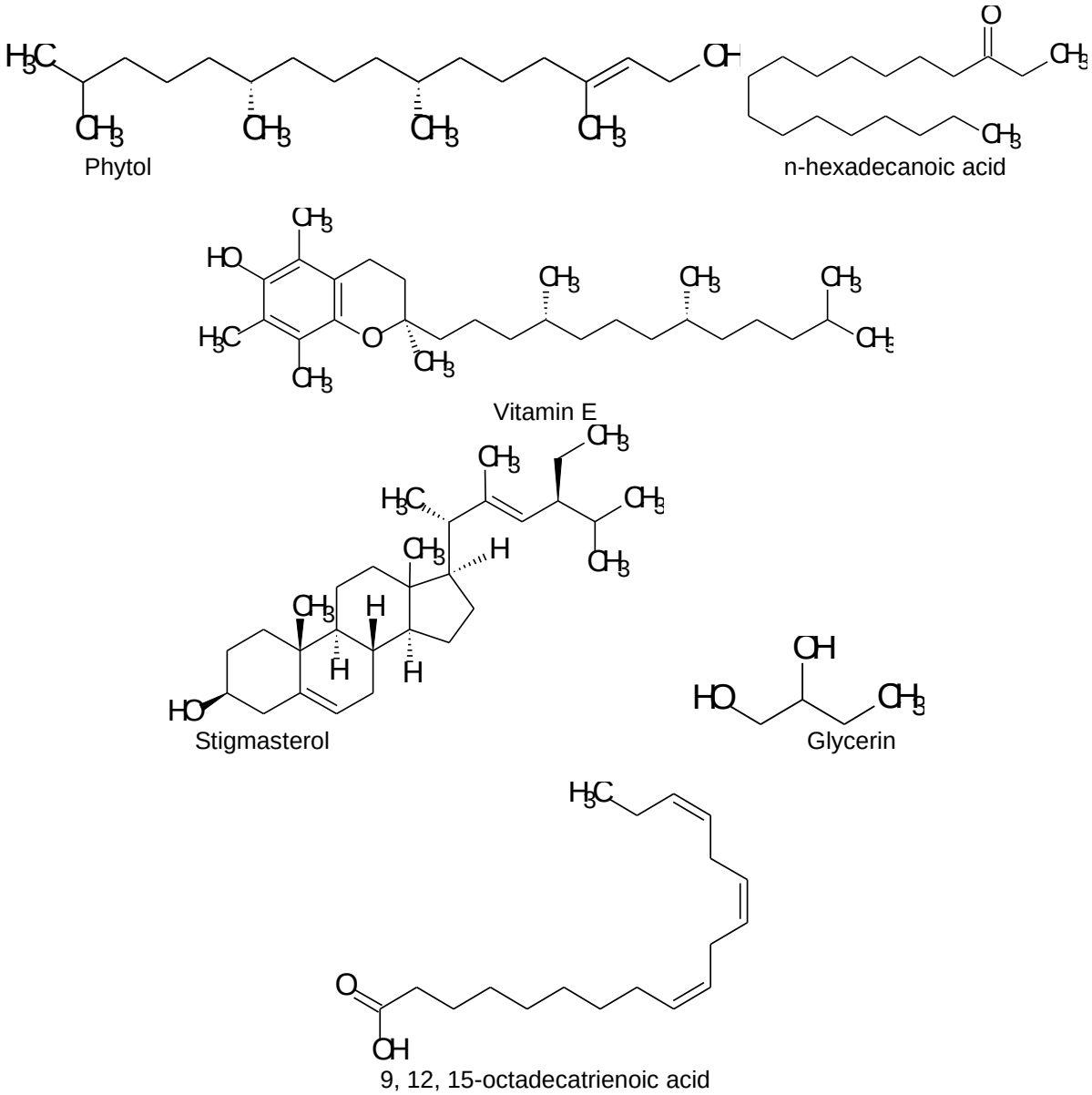

Fig. 5. Structures of some phytochemical constituents in the leaf GC-MS ethanolic extract

Chemomicroscopy study showed the presence of mucilage, cellulose, starch and lignin, while, calcium oxalate crystals, oils and, proteins were absent.

In soluble- extractive values determination, water had the highest extractive value of 21%$^{w}/_{w}$ and ethanol,the lowest extractive value of 10%$^{w}/_{w}$ as seen in Table 4. Extractive values play an important role in the evaluation of crude drugs as the specific constituents based on its solubility in a particular solvent used for its extraction can be estimated [20].

The fluorescence property of the powdered leaf sample was conducted for different solvents like n-hexane, dichloromethane, ethyl acetate, ethanol, methanol and water (according to the eluotropic series). At daylight, low wavelength (253.7nm) and high wavelength (365 nm), the extracts spotted on the TLC plate showed different colours as shown in Table 4 which indicate the presence of different phytochemicals. The intensity of fluorescence is used to identify a particular drug and also differentiate adulterated herbal medicines [21].

The moisture content obtained was 5.31%$^{w}/_{w}$ which does not fall within the African Pharmacopoeia, 1986 recommended range of 8-14%$^{w}/_{w}$. The moisture content is an indication of the herbal medicines‟ shelf life''. High moisture content indicates that the herbal drug has either been incorrectly prepared or inappropriately stored. It could lead to enzymatic activation and hydrolytic reactions as well as proliferation of microbial growth which may lead to degradation and loss of active constituents [20]. This property is most important in plants with hygroscopic characteristic.

The total ash values for the powdered leaf as shown in Table 6 is 15.18%$^{w}/_{w}$. This is above the recommended limit of 14%$^{w}/_{w}$ according to the European Pharamcopoeia [22]. The total ash value measures the degree of purity as well as quality of the crude drug. For a crude drug to be stated a drug of high purity, the ash value should not exceed the limit of 14%$^{w}/_{w}$. The acid-insoluble ash value of the powdered leaf was above the stated limit of 2%$^{w}/_{w}$ by the European Pharmacopoeia, [22] and the value shown in the Table 6 is 2.7%$^{w}/_{w}$.

GC-MS Analysis showed the presence of 35 phytochemicals in the powdered leaf of the sample.

This is contrary to the twenty-seven (27) compounds with 9,12,15-octadecatrienoic acid (Z,Z,Z) appeared highest with a percentage of 21.12% followed by n-Hexadecanoic acid of 16.37% then, 1-piperidinethioca rboxamide of 14.76%. 9,12,15-Octadecatrienoic acid (ODA) is traditionally known for its cardiovascular-protective, anti-cancer, anti-inflammatory and, anti-oxidative properties. ODA reduces risk associated with death from ischemic heart diseases [23]. This is an indication that the leaves of *T. laevis* which contain ODA can be used to manage the above listed conditions. ODA has also been reported to possess activity against breast cancer by inhibiting ER-positive and ER-negative breast cancer cells [24].

According to literature, during molecular docking analysis, n-hexadecanoic acid interacts at a high affinity with DNA topoisomerase-I with free binding energy-6.7kcal. it shows significant cytotoxicity value of 0.8µgmL against human colon cancer cells- HCT-116 cells [17]. This indicates that *T. laevis* leaves which contain n-hexadecanoic acid possess anticancer activities. Also, n-hexadecanoic acid has anti-inflammatory activity as it shows significant inhibitory activity against Phospholipase A_2 [25]. Larvicidal activities against mosquitoes have also been reported in research with n-hexadecanoic acid [26].

However, vitamin E appeared at 3.37%, Stigmasterol at 0.89% and, Glycerin at 0.85%. Vitamin E is an important phytochemical although present in minute quantity. It is known for its antiaging, anti-oxidant, antitumor, cancer preventive, vasodilator, antispasmodic properties. Stigmasterol is reported to possess anti-hepatotoxic, antiviral and even antioxidant properties [27]. Glycerine is used as remedy for skin problems and keeps the skin moist and soft [28].

Phytol which is also an important phytochemical has been reported to possess anti-oxidant and antimicrobial activity [29]. It is also reported to possess anticonvulsant activities and is even more potent than the anticonvulsant activity produce by diazepam [30].

Supraene also called Trans-Squalene has been discovered to have activity over fungi and oomycetes such as *Fusarium circinatum, Cryphonectria parasitica and Phytophthora cijnamomi* [31]. It also has hypoglycaemic activity

by inhibiting the activity of alpha-glucosidase and alpha-amylase [32].

Benzoic acid, also called phenyl formic acid together with its derivatives can be used as food preservatives and flavouring agents [33]. Its derivative 2-hydroxy-4-methoxy benzoic acid is reported to have antiserum action against snake bites [34].

5. CONCLUSION

The data obtained from this pharmacognostic studies would aid in the identification and authentication of the diagnostic features of the plant *Thunbergia laevis*. The research plant *Thunbergia laevis* of the Acanthaceae family with moisture content 5.31%$^{w}/_{w}$ should be included in the African Pharmamcopoeia as an example of exceptional plants with moisture content that does not fall within the recommended range of 8%$^{w}/_{w}$ – 14%$^{w}/_{w}$ like the *Digitalis* leaf with a moisture content of 6%$^{w}/_{w}$.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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