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Pharmacognostic Evaluation, and GC-MS Profiling of *Landolphia dulcis*, Sabine ex G.Don (Apocynaceae) Leaf Methanol Extract

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Abstract

Introduction: Standardization is the investigation and documentation of unique and peculiar characters and properties which identify and separate a plant from others even members of the same family. L*andolphia dulcis*, Sabine ex G.Don belongs to the family Apocynaceae which is known to contain many secondary metabolites and have many pharmacological activities. Standardization refers to the body of information and control necessary to product material of reasonable consistency. Methods of standardization take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, Physicochemical quantitative evaluation (LOD, ash values, extractive values), phytochemical evaluation (e.g. GC-MS), microscopy. Of these, the phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs. This research aimed to provide information on standardization parameters and GC-MS profile of the leaves of L*andolphia dulcis*, Sabine ex G.Don. **Methodology**; The fresh leaf was used for quantitative microscopy studies while a part of the collection was dried under shade, powdered and used for powder microscopy, physico-chemical analysis. Standard pharmacopoeia methods were followed in evaluation of pharmacgnstic parameters. GC-MS, profiling was carried out using standard methods and equipment. **Results**: Physicochemical properties were within normal pharmacopeia limits. Total ash was 7.40 ± 0.02 %, methanol had extractive value of 9.27 ± 0.05 % while water gave an extractive value of 8.91 ± 0.04. Leaf microscopy revealed the presence of prism-shape Calcium oxalates crystals, Stomata index (%) 18.75 ± 0.38, Stomata number 15.25 ± 0.67, and palisade ratio 12.50 ± 0.65. The

leaf has paracytic stomata type which was only present on the dorsal (adaxial) surface. GC-MS result showed the presence of 45 compounds with diverse activities. **Conclusion**: These study have provided basic and fundamental information on the plant, and justification for its safe use in ethno medicine.

Keywords: *Landolphia dulcis***,** GC-MS, pharmacognostic evaluation, Standardization

INTRODUCTION

DESCRIPTION OF *Landolphia dulcis*

Landolphia dulcis is native to West Tropical African countries of Senegal to Nigeria, also found in Central Africa, in particular, Gabon and Angola. The species is a climber capable of growing up to 5m tall as a sarmentose shrub, reach a height of 10m as a liana with stout stem up to 10 m long. Its leaves, the glabrous or pilose petiole is 2-17 mm long; leaf-blade is ovate to obovate in outline, leaflets have a coriaceous surface, leaf apex is emarginate to acuminate while the base is cordate to cuneate. The inflorescence is axillary with 1-3 flower per axil, peduncle is 0.5-5.5 mm long, pedicels are 0.1-3.1 mm long. The flowers are fragrant, the calyx is green, brown or violet, 2-2.9 mm long with 4 or 5 unequal sepals. The corolla is often discolorous and is commonly dark green, yellow, violet, cream or reddish. Fruit is globular, 5-50 seeded, commonly green, orange, yellow or red turning blackish or reddish when cut. Its fruit is edible and eaten by locals. Local medicinal uses include leaf and stem bark extract decoctions used by herbalists as a treatment for healing serious wounds, stomach ulcers, chronic sores, body pains, dysentry, arthritis and kidney pains.

TAXONOMIC CLASSIFICATION OF *Landolphia dulcis*

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Gentianales

Family: Apocyanaceae

Genus: *Landolphia*

Specie: *Landolphia dulcis*

Local name: Katakpo

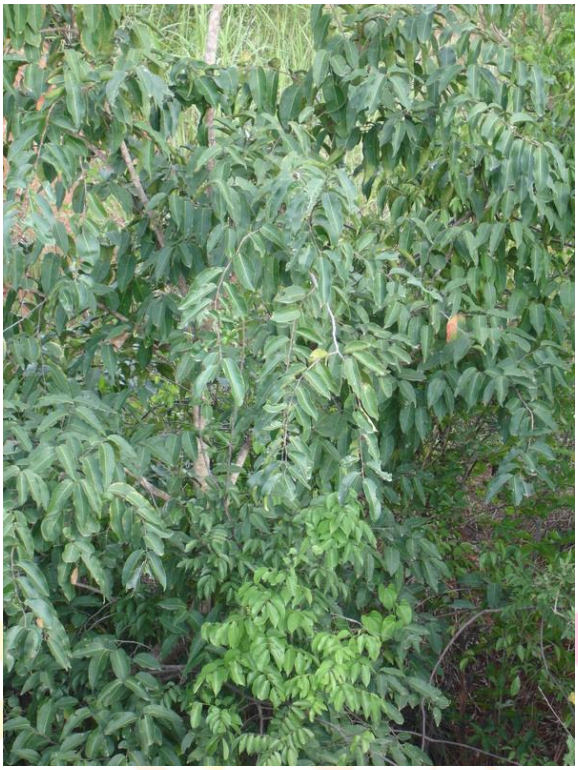

Figure 1: Pictorial representation of *Landolphia dulcis* *Landolphia dulcis* (Sabine ex G.Don) (Apocynaceae)

Pharmacognostic studies guarantees plant identity through organized standardization parameters to ensure quality and safety of use. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. [1] According to [2, 3], standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug.

Physicochemical Analysis

The parameters which are studied are moisture content, ash values, total ash acid-insoluble ash, sulphated ash, alcohol and water soluble extractive values. [4] Ash value gives the standard to judge quality and purity of crude drugs. Extractive values are indicative weights of the extractable chemical constituents of crude drug under different solvent environments. [2,3] Moisture content of crude drug is supposed to be little to support their preservation, enhance product stability and control enzyme systems that might interfere with the chemical composition of the drug and generate compounds with less activity. [4]

Qualitative and Quantitative Chemical Evaluation

Qualitative chemical evaluation covers identification and characterization of crude drug with respect to phytochemical constituent. It employs different analytical technique to detect and isolate the active constituents.

Phytochemicals

Plants produce chemical substances known as phytochemicals (by reason of their plant origin), which are non-structural parts of the plant. Their synthesis entails using biosynthetic pathways to construct complex chemicals (molecules) from simple components. Identification techniques for phytochemicals include; include colorimetric (e.g., flavonoids' yellow colour in an alkaline medium), precipitation (e.g., brick red precipitate from alkaloids

using Draggendorff's reagent), or physical (e.g., foaming by an aqueous saponin solution) characteristics (e.g. blue-black complexes by tannins with ferric ions). Determining the quantity of phytochemicals in a plant or animal material is just as crucial as confirming their existence.

Gravimetric, titrimetric, spectrophotometric, and chromatographic methods (high performance liquid chromatography, HPLC, and gas chromatography (GC)) can all be used for quantitative determination. The breadth and precision (sensitivity) of both qualitative and quantitative determinations are now greater due to recent advancements in analytical techniques and equipment. The study field of complex biological matrices has been significantly expanded by hyphenated analytical approaches, which combine the use of detectors that generate spectrum information with a separation device that is connected online. Among these include the use of TLC, GC-MS, HPLC-UV, HPLC-UV/MS, and CE-MS.

Gas chromatography mass spectroscopy (GC/MS)

A material analysis technique known as gas chromatography mass spectroscopy (GC/MS) uses gas chromatography (GC) equipped with mass spectrometers (MS), which are mass selective detectors. [5,6] Both mass spectrometry and gas chromatography are stand-alone analytical techniques. When gas chromatography and mass spectrometry are combined, the likelihood of an error occurring is reduced and the presence of a certain analyte in the sample is established. [7] As long as the chemicals are sufficiently volatile and thermally stable, a wide range of samples can be analyzed. [8] By comparing each component's mass spectrum to reference libraries holding more than 275,000 distinct spectra, the components are identified. By comparing the entire mass spectrum of unknown peaks with a mass spectra library or database, unknown chemicals can be identified. [9]

Aim of study

This research aimed to provide information on standardization parameters and GC-MS profile of the leaves of L*andolphia dulcis*, Sabine ex G.Don.

Materials and methods

Collection and Identification

PLANT COLLECTION AND IDENTIFICATION

Landolphia dulcis leaf was collected from Obollo afor in Udenu local government of Enugu State, Nigeria on August, 2023. It was authenticated by Mr. Ozioko (taxonomist) of University of Nigeria Nsukka. Herbariun specimens were deposited in the herbarium of the department of pharmacognosy, faculty of pharmaceutical sciences Enugu State University of Science and Technology (FP/Cog/01016).

MATERIALS, GENERAL REAGENTS AND DETECTING REAGENTS

Equipment

Shimadzu ATX224 Analytical Balance, AAS Machine MODEL AA-7000 *(*Shimadzu, Kyoto 604-8511, Japan), spectrophotometer, Model UV 7 UV/Visible Spectrophotometer by Mettler-Toledo Inc., Manual single channel micropipette (Pipet-Lite XL Model, Mettler-Toledo Inc., Columbus, USA), test tubes, beakers, spatula, glass stirring rods, Whatmann No 1 filter paper.

Reagents

Dragendorff's reagent, picric acid, Fehling's solutions A and B, rutin,, Methanol, conc. Sulphuric acid concentrated ammonia (Merck KGa A, Darmstadt, Germany), gallic acid, potassium ferricyanide, NaOH, acetic anhydride, (Reagents, Charlotte, NC 28214,USA) ferric chloride (Xilong Scientific Co., Ltd, China) Na_2CO_3, Zouping Zhijin New Material Technology Co., Ltd Shandong, China), Cholesterol Fissions Chemicals (United Kingdom) atropine and vanillin (Sigma Chemical, USA), diosgenin Xiangyang Wellbeing Pharmchem Co., Ltd, China), linalool (Basf Se, Belgium)

Physico-chemical evaluation was conducted following the standard methods described by [10-12].

using appropriate reagents, and with slight modifications. The following parameters were evaluated: total ash, water-soluble ash, acid-insoluble ash, sulphated ash, moisture content, water soluble extractive, ethanol soluble extractive, foaming index, bitterness value, and swelling index

Gas Chromatography- Mass Spectroscopy (GC-MS) Analysis

The GC-MS analysis carried out using GCMS-QP2010SE SHIMADZU, JAPAN data using Rtx SMS column, 60m x 0.25mm x 0.25µm, helium carrier gas, flow rate = 3.22 ml/minute. Oven Temperature started at 60^{o}C held for 2 minutes, increased to 240^{o}C (rate =12) held for 2 minutes, increased to 290 (rate =12), held for 2 minutes. Injection temperature was 250^{o}C, start m/z = 45.00 end m/z =700.00. Ion Source temperature = 230^{o}C, while Interface Temperature was 250°C. The spectrum of the sample (unknown) was compared with the spectrum of the component stored in the "National Institute Standard and Technique" (NIST) 11 library. The data was analyzed with GC-MS SOLUTION software. The name, molecular weight, molecular formula, and structure of the component were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total area.

Results

PHARMACOGNOSTIC EVALUATION

3.1.1 MACROMORPHOLOGICAL STUDY OF THE LEAF OF *Landolphia dulcis*

Table 1 below shows the result of the macromorphological study of the leaf sample.

Table 1: Macromorphological description of the leaf of *Landolphia dulcis*

S/N	Character	Observation
Organoleptic characters		
1.	Colour	Dark green on the upper surface and light green on the lower surface
2.	Odour	Uncharacteristic
3.	Taste	Slightly bitter

Botanical features		
4.	Leaf type	Glabrous or pilose petiole
5	Leaf blade	Ovate to obovate in outline
6.	Leaflets	Coriaceous surface
7.	Leaf apex	Emarginate
8.	Leaf base	Cordate to cuneate
9.	Inflorescence	Axillary with 1-3 flower per axil
10.	Mid leaf	Reddish

3.1.2 MICROSCOPICAL CHARACTERISTICS OF THE LEAF SAMPLE

The results of the transverse sections (TS) of the leaf of *Landolphia duclis* are shown in Figures 3-4 below. The TS showed the presence of the outermost covering tissue - the single layered epidermis, and leaf Petiole. There was presence of closely packed palisade mesophyll cells with numerous chloroplasts (the main photosynthetic organ) and scattered spongy mesophyll cells that are loosely fitted to leave air spaces.

The Adaxial surfaces showing epidermal cells, stomata and subsidiary cells were represented in Figures 1-2 below.

Powder microscopy of leaf showing fragmented and sclariform fibre elements, prism-shaped calcium oxalate, spherically-shaped sclerids and lignified tissues were represented in Figures 5-8 below.

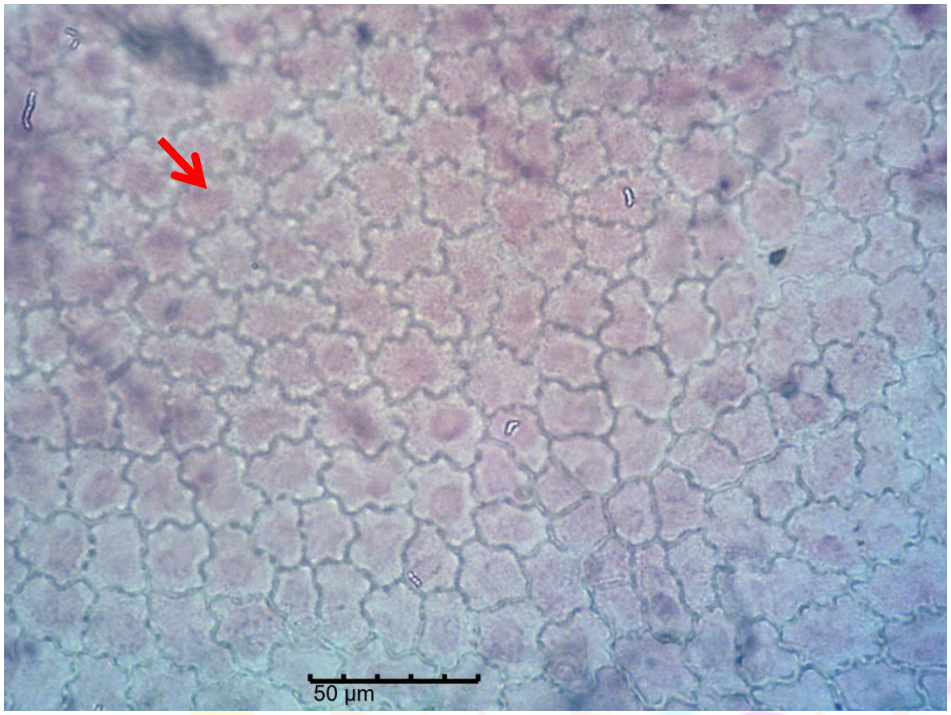

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Figure 1: Adaxial surface of the leaf of *L. dulcis* showing epidermal cells (red arrow)

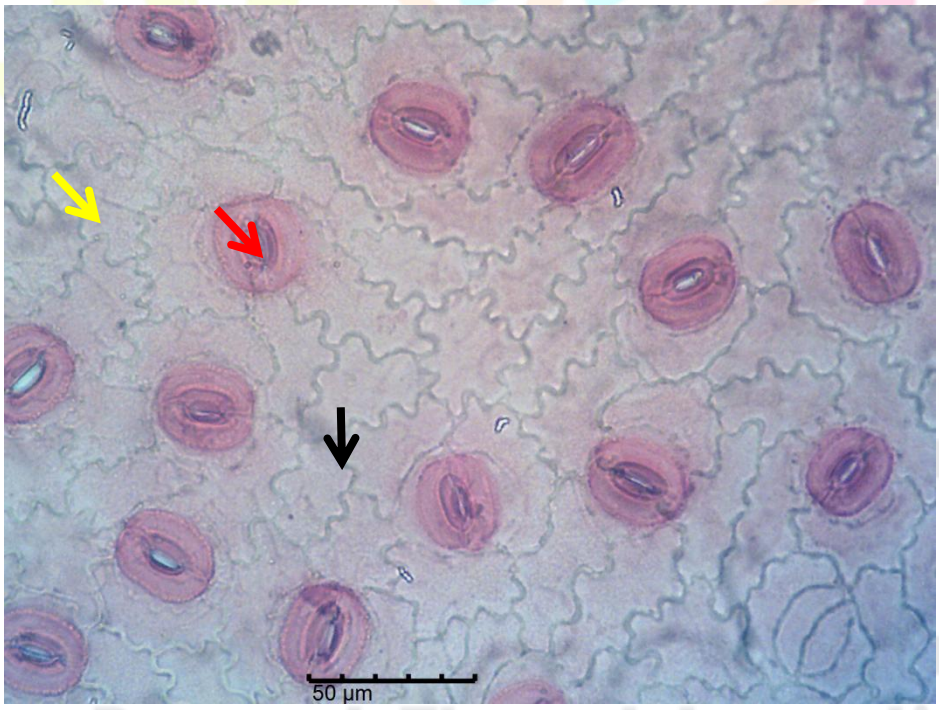

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Figure 2: Adaxial surface of the leaf of *L. dulcis* showing epidermal cells (red arrow), stomata (yellow arrow) and subsidiary cell (black arrow)

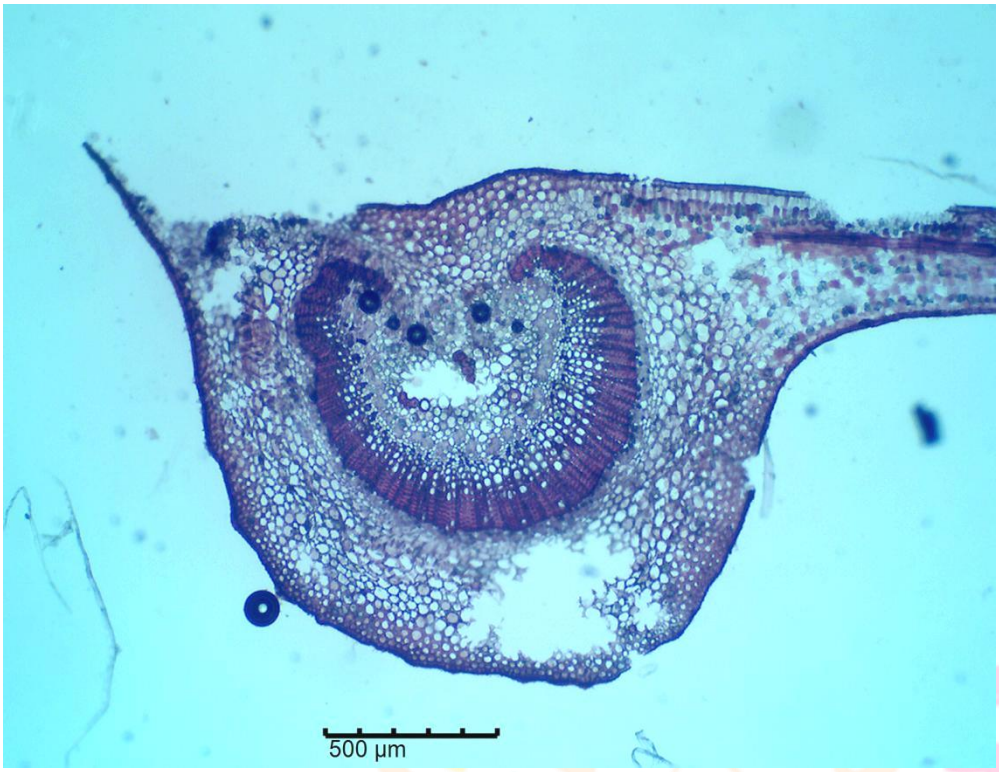

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Figure 3: Transverse section of the leaf of *L. dulcis* showing single-layered epidermal tissues, palisade and spongy mesophylls, U-shaped vascular bundle etc.

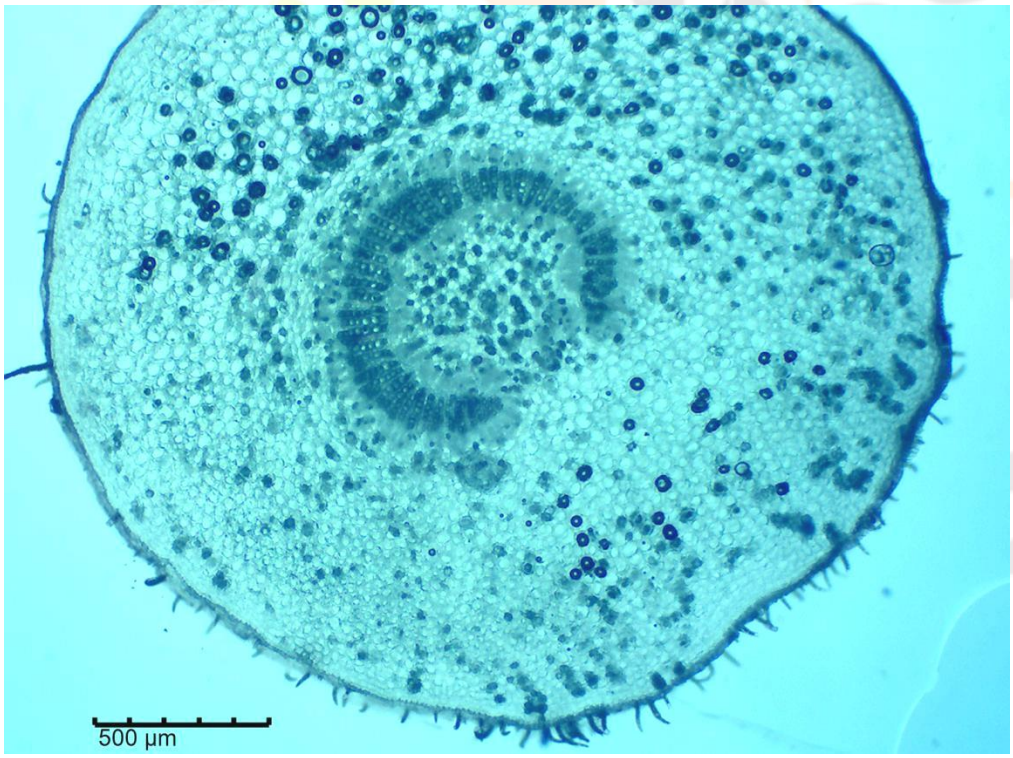

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Figure 4: Transverse section of the leaf petiole of *L. dulcis*

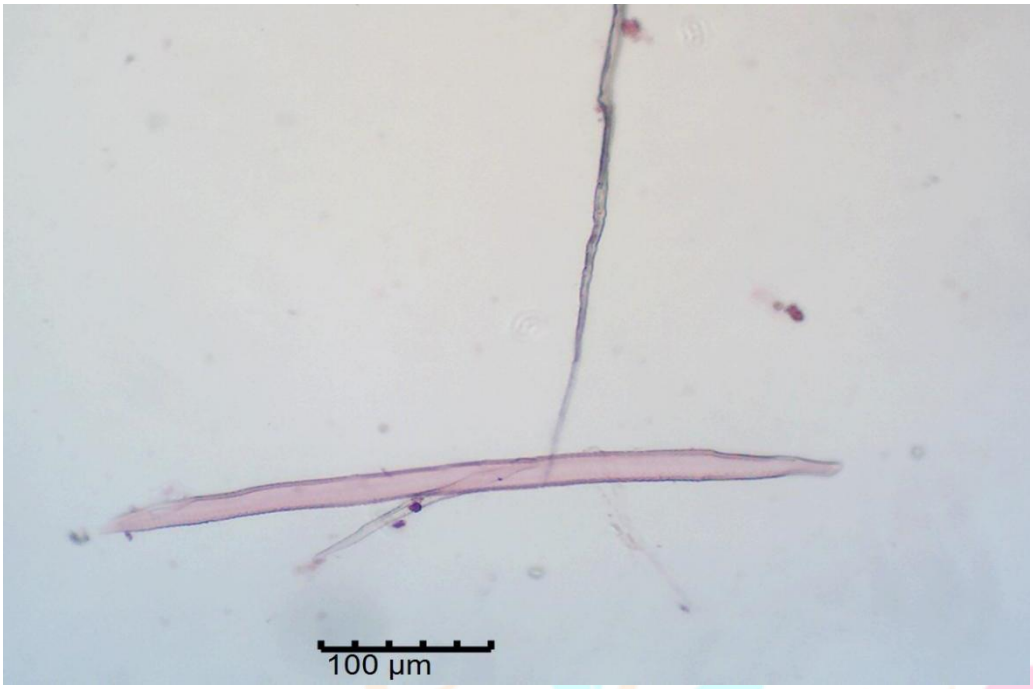

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Figure 5: Powder microscopy of the leaf showing a fragmented fibre element

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Figure 6: Powder microscopy of the leaf showing scalariform vessel element and lignified tissues

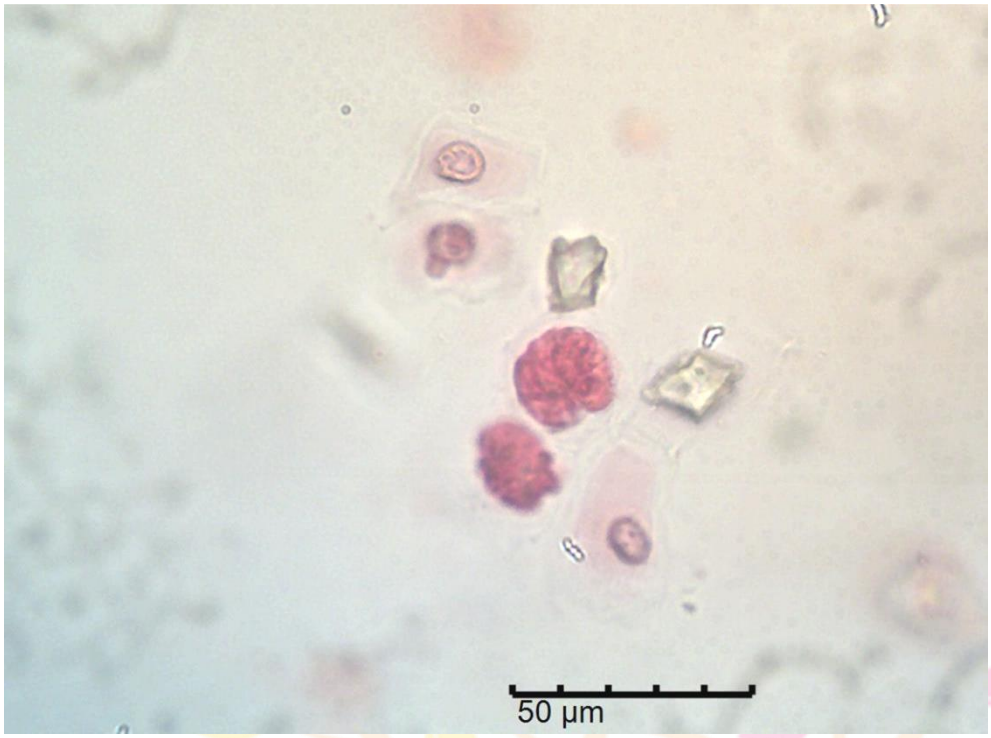

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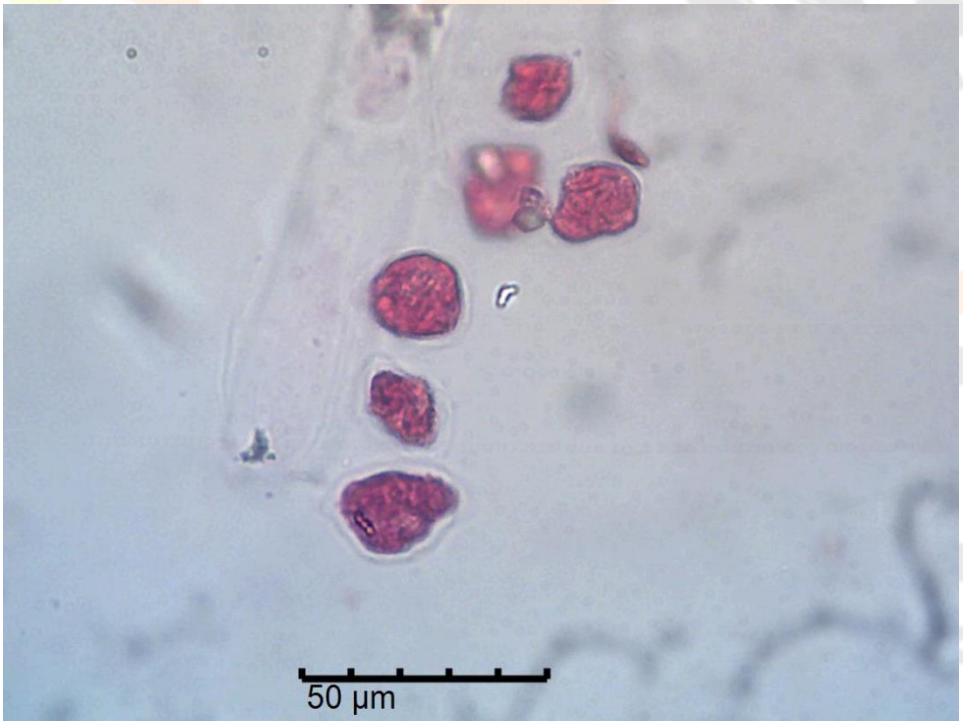
Figure 7: Powder microscopy of the leaf showing a prism-shaped calcium oxalate and lignified tissues



X400

Figure 8: Powder microscopy of the leaf showing spherically-shaped sclereids (lignified)

Table 2: Summary of microscopic study of *Landolphia dulcis*

Epidermal cell	Epidermal cells are irregular in shaped with waxy anticlinal cell walls on both the upper and lower surfaces
Stomata type	The leaf is hypostomatic (stomata occur only on the lower/abaxial surface) with paracytic type of stomata (two subsidiary cells lie parallel to the guard cells).
Trichome	Present - both unicellular and multicellular covering trichomes
Stomata number (p.f.v.)	15.25 ± 0.67
Stomata density (mm^{-2})	89.71 ± 3.70
Stomata length (µm)	30.46 ± 0.55
Stomata width (µm)	23.35 ± 0.25
Stomata size (μm^2)	711.04 ± 9.20
Stomata index (%)	18.75 ± 0.38
Vein islet number	10.25 ± 0.48
Vein islet termination number	7.75 ± 0.25
Palisade ratio	12.50 ± 0.65

3.1.3 CHEMOMICROSCOPY OF THE LEAF SAMPLE

Table 3: Result of powder chemomicroscopy of the leaf of *Landolphia dulcis*

Parameter	**Reagent(s)**	**Result**
Starch grains	Iodine solution	Present
Lignified tissues	Conc. HCl + Phloroglucinol	Present
Calcium oxalates	Iodine solution	Present; Prism-shaped

	Conc. Sulphuric acid	
Tannin	Ferric chloride	Present
Cellulose	Zinc chloride; Conc. Sulphuric acid	Present
Gum/Mucilage	Ruthenium red	Absent
Protein	Biuret reagent; Nihydrin	Present
Oil	Sudan III reagent	Present

EXTRACTIVE VALUES OF THE LEAF SAMPLE

Table 4: Result of the extractive values of the leaf of *Landolphia dulcis*

Reps/Solvent	**Alcohol (%)**	**Hexane (%)**	**ETOAC (%)**	**Chloroform (%)**	**Water (%)**
1	9.22	3.88	5.34	4.28	8.95
2	9.31	3.94	5.25	4.27	8.87
Value	9.27 ± 0.05	3.91 ± 0.03	5.30 ± 0.05	4.28 ± 0.01	8.91 ± 0.04

ASH VALUES AND MOISTURE CONTENT OF THE LEAF SAMPLE

Table 5: Result of the ash values and moisture content of the leaf of *L. dulcis*

Reps/Solvent	Total ash (%)	Water soluble ash (%)	Acid insoluble ash (%)	Moisture content (%)
1	7.42	3.35	3.81	9.98
2	7.38	3.37	3.81	10.04
Value	**7.40** ± 0.02	**3.36** ± 0.01	**3.81** ± 0.00	**10.01** ± 0.03

GC-MS Analysis

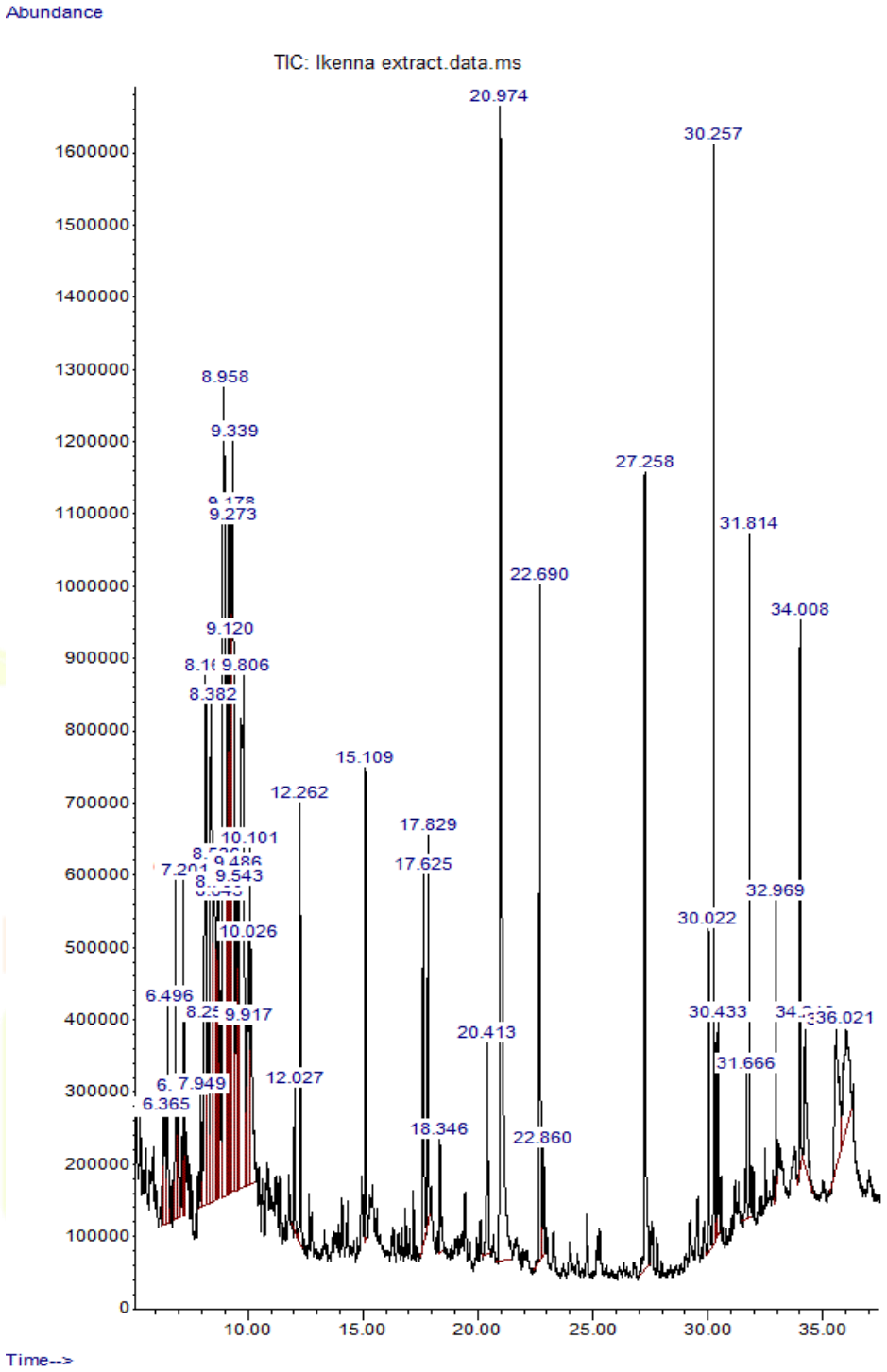


Fig. 2; GC-MS Chromatogram

GC-MS profiling of *Landolphia dulcis leaf* ethanol extract showed the presence of 45 compounds. The first compound to elute was .beta.-Myrcene which took 6.30 minutes, followed by Benzene, 1,2,4-trimethyl- (retention time 6.365) while the last compound, 5.alpha.-Cholest-8-en-3-one, 14-methyl, took 36.021minutes to elute. The most abundant compound judging by the percent area was Heptadecane, 2,6,10,14-tetramethyl

(9.43%), 2,4-Di-tert-butylphenol (9.22%), Octane, 3,5-dimethyl- (9.120%) , Undecane (8.00%), 5.alpha.-Cholest-8-en-3-one, 14-methyl (2.23%). The details of identified compounds are shown in Table 2

Table 2 : Compounds identified in GC-MS profile of methanol extract

S/NO	RT	% Conc	Quality	Compound
1	6.30	0.30	15	.beta.-Myrcene
2	6.365	0.63	64	Benzene, 1,2,4-trimethyl-
3	6.496	1.09	49	Oxirane, (chloromethyl)-
4	6.849	1.56		Benzene, 1,4-dichloro-
5	6.956	0.70	62	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
6	7.201	1.30	96	p-Cymene
7	7.949	0.40	41	Hexane, 2,2,5-trimethyl-
8	8.160	4.02	95	gamma.-Terpinene
9	8.258	1.19	58	Decane, 3,6-dimethyl-
10	8.382	3.59	64	Dodecane, 2,6,11-trimethyl-
11	8.536	3.24	83	Decane
12	8.645	1.09	59	Tridecane
13	8.700	1.57	64	Undecane, 3,7-dimethyl-
14	8.958	9.43	86	Heptadecane, 2,6,10,14-tetramethyl
15	9.120	9.120	72	Octane, 3,5-dimethyl-
16	9.178	3.39	78	Decane, 2,3,5,8-tetramethyl-
17	9.273	3.48	72	Heptadecane, 2,6,10,14-tetramethyl
18	9.339	5.19	86	Tetradecane
19	9.486	1.33	64	Undecane, 4,7-dimethyl-
20	9.543	1.35	72	Carbonic acid, nonyl vinyl ester
21	9.806	8.00	64	Undecane
22	9.917	0.50	59	Heptadecane, 2,6,10,14-tetramethyl
23	10.026	1.51	86	2,6-Dimethyldecane
24	10.101	2.64	53	Decane, 2,4-dimethyl-
25	12.027	0.51	81	5-Dodecene, (Z)-
26	12.262	1.80	94	Dodecane
27	15.109	1.82	95	Tridecane
28	17.625	1.61	90	Cetene
29	17.829	1.42	90	Tridecane
30	18.346	0.65	94	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4 Z,9S*)]-
31	20.413	1.06	90	Pentadecane
32	20.974	9.22	95	2,4-Di-tert-butylphenol
33	22.690	3.16	95	Z-8-Hexadecene
34	22.860	0.50	93	Hexadecane
35	27.258	3.94	95	1-Octadecene
36	30.022	1.13	90	1,2-Benzenedicarboxylic acid, buty l 2-ethylhexyl ester
37	30.257	3.01	95	1-Octadecene
38	30.433	0.62	49	6-(Trifluoromethoxy)-N-(trimethyls ilyl)-1,3-benzothiazol-2-amine
39	31.666	0.59	78	Ethyl Oleate
40	31.814	1.50	96	1-Docosene
41	32.969	0.60	97	1-Docosene
42	34.008	1.50	91	Bis(2-ethylhexyl) phthalate
43	34.216	1.11	32	Tetradecanoic acid, 2-hydroxy-, methyl ester
44	35.582	2.11	96	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-m-Camphorene
45	36.021	2.23	35	5.alpha.-Cholest-8-en-3-one, 14-methyl

Discussion and conclusion

The grounds for the earlier objection to the use of plants and plant-derived agents in medical treatment and management of diseases are being addressed through improvements in handling and processing of plant materials and introduction of new innovations in medicinal plants production. These include emphasis on toxicity studies, introduction and enforcement of SOPs in different areas, including standardization of products.

One of the criticisms level against traditional medical practitioners is that their potions re not standardized. Another complaint is that the quantity specified is not related to the age or weight of the patient. There is some truth, however, in the criticisms levelled against traditional medicine in its lack of precise dosage and standardization. It is not that traditional healers do not attempt to standardize their preparations at all, but such standardization are very imprecise. [13]. Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. Of these, the phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs. The fingerprint profiles serve as guideline to the phytochemical profile of the drug in ensuring the quality, while quantification of the marker compound/s would serve as an additional parameter in assessing the quality of the sample. [14] The moisture content of a drug will be responsible for decomposition of crude drugs either producing chemical change or microbial growth. So the moisture content of a drug should be determined and controlled. A moisture content of 9.50 ±0.1 (% w/w) by loss on drying method implies that the plant contains a lot of water or other volatile substances. And a lot of attention must be given to the dying process to discourage microbial infestation. The extractions of any crude drug with a particular solvent yield a solution containing different compounds the nature and type of which depends on the plant material, solvent and other processing activities. The compositions of extract will give an indication whether the plant material is virgin or exhausted. Therefore, the “extractive value” parameter apart from guiding in the choice of solvent for further work also helps in quality assurance of the plant material. The total ash usually consists mainly of carbonates, phosphates, silicates and silica. The total ash value is of importance and indicates to some extent the amount of care taken in the preparation of the drug.

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