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Pharmacognostic Standardization and Chemical Study of *Euphorbia nutans* Lag. Euphorbiaceae

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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

Aims: This study was designed to set macro/micro morphological standards, phytochemical and physicochemical parameters for the identification of *E. nutans*, a traditional remedy for the management of many disesases.

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Study Design: To establish pharmacognostic standards for proper identification of *E. nutans* and also study its phytochemicals using Gas Chromatography coupled to Mass Spectrometry (GC-MS).
Place and Duration: This work was undertaken at the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria for three month spanning from April through June, 2022.
Methodology: Examination of microscopic characters, venation, chemomicroscopy, micromeritic properties, fluorescence analysis and phytochemical profiling using Gas Chromatography-Mass Spectrometry (GC-MS) were carried out.
Results: Epidermal cell shapes were irregular with undulate-sinuous anticlinal walls. Stomatal distribution was amphistomatic with anisocytic and anomocytic stomata on both surfaces. Areolation was quadrangular, linear and biforked vein termination. The fluorescence characteristics showed the presence of different colours supporting the presence of various phytoconstituents for both leaf and stem. The flow properties for both leaf and stem were poor while GC-MS analysis of the dichloromethane extracts revealed lupeol (64.05%), 2-methylhexacosane (9.37%), stigmasterol (4.16%) and campesterol (1.29%) as major components in the leaf while campesterol (0.67%), stigmasterol (2.15 %), beta.-sitosterol (6.22%), lupeol (9.85%) and vitamin E (1.05%) for the stem extract.
Conclusion: The results of the study could be useful for correct identification, standardization and preparation of monograph.

Keywords: Euphorbia nutans; pharmacogostic; standardization; micromeritic; GC/MS analysis.

1. INTRODUCTION

Euphorbia is the third largest genus in the flowering plants after Fabaceae and Rubiaceae with about 2000 species distributed worldwide (Horn et al. 2012). It has been widely reported for its ethnomedicinal uses for the treatment of diseases ranging from respiratory infections, body and skin irritations, digestion complaints, inflammatory infections, body pains, microbial illness, snake/scorpion bite, endocrine and sensory disorders (Ernst et al. 2015). “Studies showed the purgative and emetic effects of *Euphorbia* species” (Chika et al. 2007, Kemboi et al. 2020). “They are also implicated in the treatment of skin diseases most such as warts, sores, carbuncles, boils, dermatitis, calluses, hair loss, irritation, psoriasis, pustules, sunburn and eczema” (Amtaghri et al. 2022). “The milky sap or latex of spurges is used to have a protective and defensive role in healing wounds” (Sandeep et al. 2009). “In the category of respiratory system disorders, *Euphorbia* was described to treat asthma and cough, but also included descriptions of treatment for bronchial complaints, breathlessness, pneumonia and use as and expectorant” (Olounlade et al. 2017).

“Plants in herbal medicine have become a basic interest for research as the major source of herbs for local people and the herbal drug industry is the wild source. Adulteration is often found in the raw materials when purchased from the market” (Adesina 2011). “It is also reported that herbal industry and local residents face the problems of adulteration and substitution at a raw material stage” (European Medicines Agency 2005). “Quality control of crude drugs and herbal formulation is of vital importance in justifying their acceptability in modern medicine” (Verma 2008). “One of the main obstacles to the acceptance of traditional medicine in developed countries is lack of documentation and stringent quality control. However, standardization of medicinal herbs is a function of proper identification, quality control and quality assurance thus building confidence the acceptability of crude drugs” (Thomas et al. 2008).

“Therefore, the evaluation of standards can be done by assessing the organoleptic (colour, odour, taste) macroscopic, microscopic and physicochemical parameters” (Burkill 2000). With the numerous uses of *Euphorbia* species, *Euphorbia nutans,* commonly known as nodding spurge*,* spotted sand mat*,* eye bane*,* spotted spurge, an important member of this genus, has not been explored of its taxonomic and chemical profiling hence this study. This study was designed to investigate the pharmacognostic/taxonomic parameters and also study the chemical constituents using GC-MS to aid in its identification for safe use.

Fig. 1. *Euphorbia nutans* in a natural environment

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plants Materials

Fresh samples of *Euphorbia nutans* were collected in August 2022 from a botanical garden and preserved in FAA (Formalin Acetic Acid). The plant was identified by Dr. Imoh I. Johnny, a taxonomist and voucher specimen (UUPH 31(e)) deposited in a herbarium. The collected leaves and stems were washed under running tap water, rinsed with distilled water, chopped into pieces, dried under shade at room temperature. The dried leaves and stems were powdered using electric blender, sift through 350 microns sieve size and stored in airtight bottles to avoid moisture and humidity prior to use

2.2 Microscopic Leaf Evaluation

2.2.1 Qualitative microscopic Study

“For anatomical studies, the standard median portion of the well expanded matured leaf was obtained. Epidermal peels of both adaxial and abaxial surfaces were made by placing the leaf on a clean glass slide with the surface to be studied facing down. The specimens were irrigated with water holding it downward from one end and then the epidermis above the desired surface was scrapped off carefully with sharp razor blade. The loose cells were then washed off with water and the epidermis was stained in 1 % aqueous solution of safranin-O for 2-3 minutes and washed again in water to remove excess stain and mounted in 10 % glycerol on a glass slide and covered with a glass cover slip before viewing with an Olympus CX21 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” (Burkill 2000).

2.2.2 Quantitative microscopic study

“Quantitative microscopic parameters such as leaf constant studies viz. stomatal length and width, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number, vein termination number, areole length and width were carried out using standard procedures” (Metcalfe 1979). All measurements were made using a calibrated ocular micrometer and thirty (30) microscopic fields chosen at random were

used and data presented as mean ± Standard Error of Mean (SEM). The stomatal index (S.I) was determined according to the formula: Stomatal Index (S.I) = S/E +S x 100, where S = number of stomata per unit area and E = number of epidermal cells in the same area (Killedar et al. 2014). The stomata index (S.I) was determined using the formula: Stomatal Index (SI) = S/E +S x 100 Where: S = number of stomata per unit area E = number of epidermal cells in the same area (Burkill 2000).

2.2.3 Evaluation of leaf and stem powders

"Chemomicroscopic studies of the coarse powders of both the leaf and stem were undertaken to study o microscopical characters as well as chemomicroscopic properties such as cellulose, mucilage, lignin, starch, protein, oils and calcium oxalate crystals" (Kokate et al. 2005, Evans 2009). "The fluorescent analysis of *E. nutans* dried leaf and stem powders was carried out using the standard methods" (Kumar et al. 2012, Khandelwal 2002). "The micromeritic characteristics of leaf and stem powder to study the bulk density, tap density, angle of repose, Hausner's ratio, Carr's index and pH were determined according to earlier reported methods" (Mbah 2012).

2.2.4 Chemical study with GC/MS analysis

"Thirty (30) grams of each of leaf and stem powder was marcerated in 100 mL of dichloromethane (analytical grade) for 48 hrs, filtered and concentrated using a rotary evaporator. The resultant lipophilic extracts were subjected to GC-MS analysis at Shimadzu Training Centre for Analytical Instruments (STC, Lagos, Nigeria) using standard experimental protocol" (Merlin et al. 2009).

3. RESULTS AND DISCUSSION

3.1 Qualitative and Quantitative Microscopic Studies

The results of the micro-morphological evaluation of leaf and stem of *E. nutans* are summarized in Fig. 2, Fig. 3 and Table 1 while the results of micromeritic, chemomicroscopic and fluorescence studies are captured in Tables 2, 3 and 4. Tables 5 and 6 captured the GC-MS phytochemical profiling of the dichloromethane fractions of both the leaf and stem of *E. nutans*.

Table 1. Qualitative and quantitative micro-morphological characters of *E. nutans*

Parameters	Abaxial	Adaxial
Stomata type	Anomocytic and Anisocytic stomata with T-pieces	Anomocytic and Anisocytic stomata with T-pieces
Anticlinal Wall Pattern	Sinous	Undulate
Stomata distribution	Amphistomatic	Amphistomatic
Stomata pore length	8.78(10.43±1.225)12.26	7.08(8.9±1.428)10.90
Stomata pore width	1.73(2.74±0.676)3.65	2.02(2.62±0.567)3.52
Stomata width	6.15(8.59±1.488)10.09	6.22(8.64±1.444)10.81
Stomata length	17.09(19.93±1.827)22.90	11.42(13.04±1.210)14.85
Stomata number (for area view)	40(42.6±2.011)46	59(65.6±4.993)72
Epidermal wall pattern	Irregular	Irregular
Epidermal layer number	167(222.2±34.656)276	241(279.7±22.39)300
Epidermal cell length (m)	31.82(39.16±6.748)53.17	32.05(38.84±5.50)47.90
Epidermal cell width (m)	23.67(27.59±2.915)32.64	13.02(17.51±3.559)22.28
Vein termination type	Linear and Biforked termination	Linear and Biforked termination
Vein termination number	3(3.8±1.229)7	9(12.3±1.636)14
Areole type	Quadrangular	Quadrandular
Width of areole	38.51(40.95±1.618)43.73	47.50(49.28±0.983)50.17
Length of areole	102.7(108.30±3.758)113.62	117.5(121.12±2.288)124.11
Length of Guard cell	10.51(12.71±1.330)14.29	13.05(13.71±0.192)14.51
Width of Guard cell	3.25(3.73±0.617)4.51	3.25(3.73±0.617)4.51
Stomatal Index	16.09%	21.40%

Values are represented as: Lowest (Mean± Standard Error of Mean) Highest of ten (10) replicates

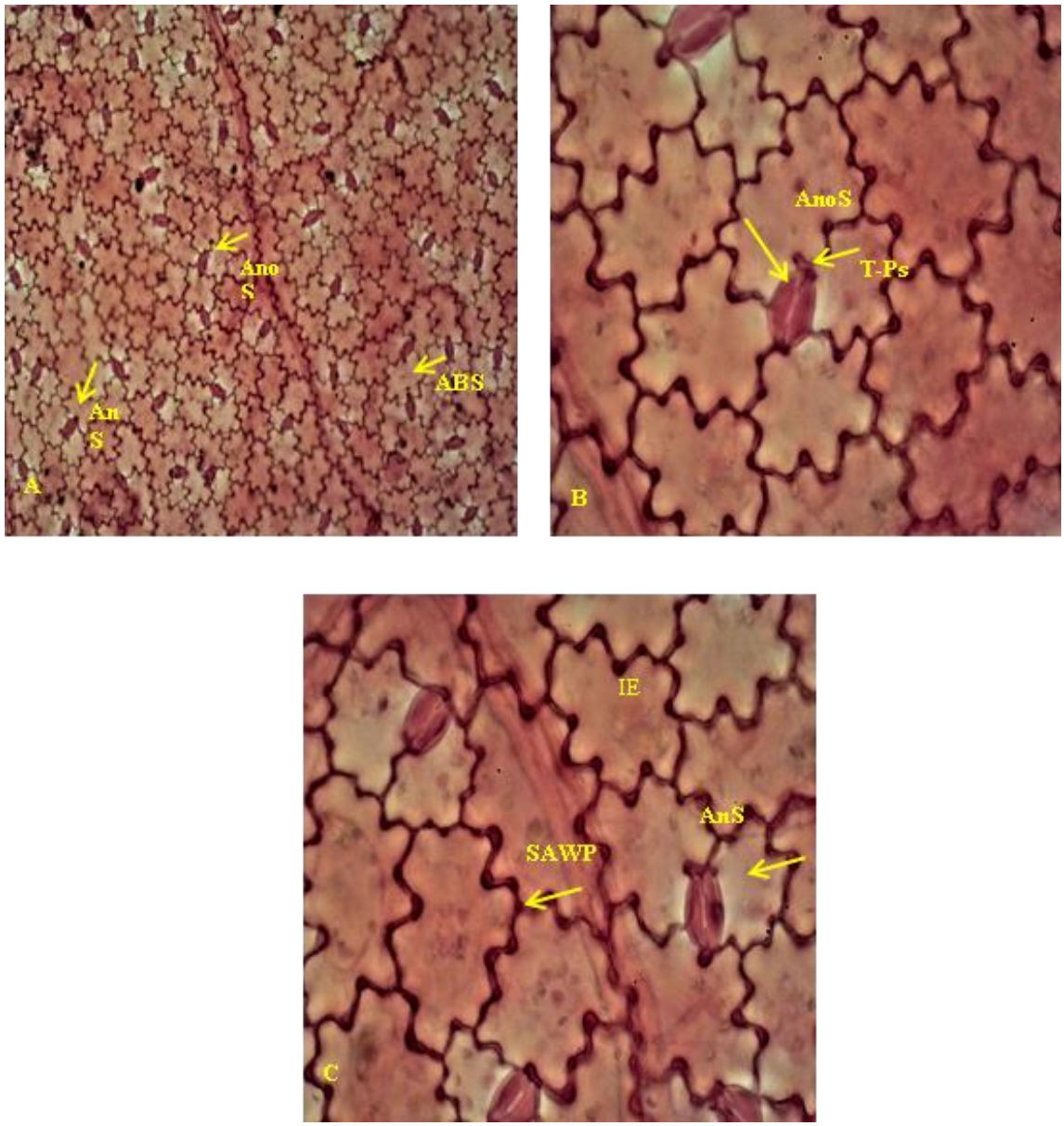

Fig. 2. (A): Abnormal stomata (AB), Anomocytic (AnoS) and Anisocytic (AnS) stomata ×1(B): Anomocytic stomata (AnoS) × Abaxial surface (C): Irregular epidermal cell (IE), Sinuous anticlinal wall pattern (SAWP) Abaxial surface × 40

Table 2. Micromeritic evaluation of powdered leaf and stem of *E. nutans*

Micromeritic parameters	Leaf powder	Stem powder
Bulk volume (mL)	36.6±0.62	49.33±0.57
Tapped volume (mL)	28±1.00	32.33±2.309
Bulk density (g/mL)	0.275±0.00	0.203±0.00
Tapped density (g/mL)	0.357±0.01	0.311±0.02
Flow rate (g/s)	0.499±0.07	0.063±0.00
Angle of repose (º)	33.77±2.57	37.18±0.78
Carr's index (%)	22.90±3.04	34.53±5.11
Hausner's ratio	1.298±0.05	1.533±0.12

Result presented as mean ± SEM of three (3) replicates

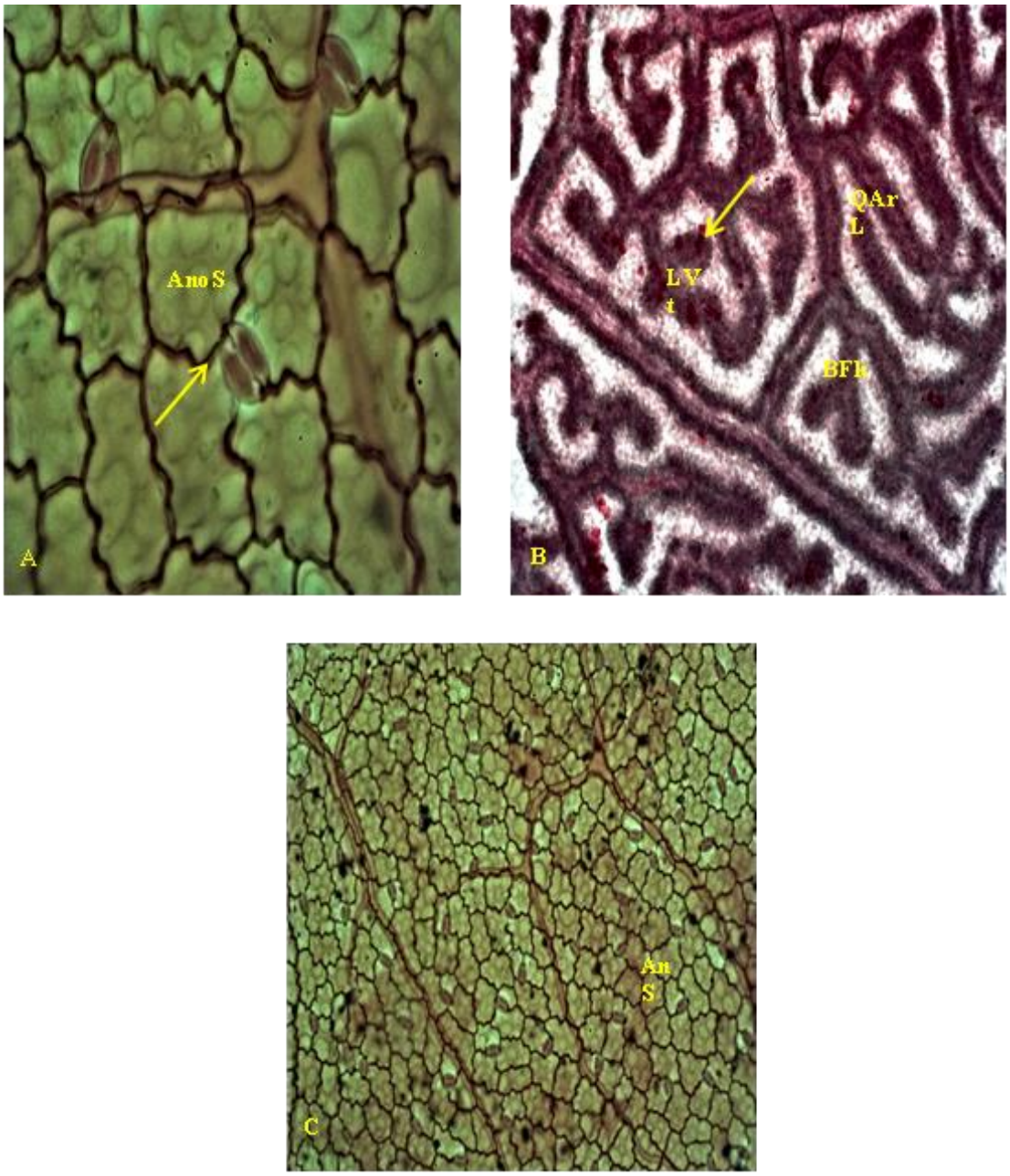

Fig. 3. (A): Anomocytic (AnoS) stomata adaxial × 40; Irregular epidermal cell (IR) ×40, (B): Linear vein termination (LVt), Bi-forked vein termination (BFK) ×10(VI) and Quadrangular areole (QArL) × 40

Table 3. Chemomicroscopic evaluation of the leaf and stem of *Euphorbia nutans*

Constituents	Leaf	Stem
Mucilage	+	+
Lignin	+	+
Starch	+	+
Cellulose	+	+
Oils	+	+
Proteins	-	-

+ = present and - =absent

Table 4. Fluorescence analysis of *Euphorbia nutans* leaf and stem powders

Extracts	Physical observation LEAF	Physical observation STEM	365 (nm) colour LEAF	365 (nm) colour STEM
Methanol	Pale green	Light brown	Brownish red	Greyish pink
DCM	Green	Light Green	Red	Pink
n. hexane	Yellowish green	Grey	Light red	Light pink
Ethylacetate	Light Green	Light brown	Red	Pink

Table 5. Phytochemical composition of dichloromethane leaf extract of *E. nutans* by GC-MS analysis

S/N	Retention Time	Compound Name	Molecular Formula	Molecular Weight	Area %
1	11.467	Bicyclo[4.4.0]dec-5-en-4-one-1-carboxylic acid	$C_{11}H1_4O_3$	194	0.04
2	11.767	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	180	0.04
3	12.970	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-	$C_{13}H_{20}O_2$	208	0.04
4	14.168	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$	222	0.32
5	14.244	2,3-Bis (1-methylallyl) pyrrolidine	$C12H_{21}N$	179	0.72
6	14.867	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.15
7	14.914	Phytol, acetate	$C_{22}H_{42}O_2$	338	0.58
8	15.111	3.14 5-Nonadecen-1-ol	$C_{19}H_{38}O$	282	0.18
9	15.272	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	298	0.16
10	15.923	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.55
11	16.376	4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester	$C_{13}H_{15}NO_3$	233	0.18
12	17.186	Phytol	$C_{20}H_{40}O$	296	1.64
13	17.499	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	3.33
14	17.693	Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.29
15	19.050	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	0.17
16	19.309	2-Methyl-7-nonadecene	$C_{20}H_40$	280	0.13
17	19.454	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	0.06
18	20.765	Decane, 1,9-bis[(trimethylsilyl)oxy]-	$C_{16}H_{38}O_2Si_2$	318	0.12
19	21.083	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	0.14
20	21.316	Campesterol	$C_{28}H_{48}O$	400	1.29
21	21.982	Stigmasterol	$C_{29}H_{48}O$	412	4.16
22	22.185	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	0.78
23	22.561	2-methylhexacosane	$C_{27}H_{56}$	380	0.34
24	23.018	beta.-Sitosterol	$C_{29}H_{50}O$	414	2.55
26	23.226	Tetracosyl trifluoroacetate	$C_{26}H_{49}F_3O_2$	450	0.35
27	23.277	. beta.-Alanine, n-pentafluoropropionyl-, hexadecyl ester	$C_{22}H_{38}F_5NO_3$	459	0.52
28	23.391	Squalene	$C_{30}H_{50}$	410	0.45
29	23.540	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	424	2.27
32	24.037	2-methylhexacosane	$C_{27}H_{56}$	380	9.37
33	24.654	Lupeol	$C_{30}H_{50}O$	426	64.05
34	24.762	Docosanedioic acid, dimethyl ester	$C_{24}H_{46}O_4$	398	0.40

Table 6. Phytochemical composition of dichloromethane stem extract of *E. nutans* by GC-MS analysis

S/N	Retention Time	Compound Name	Molecular Formular	Molecular Weight	Area %
1	8.885	2-Tridecenal, (E)-	$C_{13}H_{24}O$	196	0.23
2	10.561	5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol	$C_{15}H_{26}O_2$	238	0.21
3	10.903	1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	$C_{15}H_{24}$	204	0.05
4	11.373	2-Cyclopentene-1-butanal, .gamma.,.gamma.,2,3-tetramethyl-	$C_{13}H_{22}O$	194	0.30
5	11.671	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	0.17
6	11.767	2'-Acetonaphthone, 1',2'.alpha.,3',4',4'a,5',6',7',8',8'a.alpha.-decahydro-5'.beta.-hydroxy-4'a.beta.,8'.beta.-dimethyl-, (.+-.)-	$C_{14}H_{24}O_2$	224	0.22
7	12.257	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.18
8	12.551	Caryophyllene oxide	$C_{15}H_{24}O$	220	0.40
9	13.660	Octadecanal	$C_{18}H_{36}O$	268	0.21
10	13.958	1-Heptadec-1-ynyl-cyclopentanol	$C_{22}H_{40}O$	320	0.43
11	14.130	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.68
12	14.258	Bicyclo[2.2.1]heptan-2-one, 5-hydroxy-4,7,7-trimethyl-	$C_{10}H_{16}O_2$	168	0.21
13	14.865	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.63
14	14.910	Phytol, acetate	$C_{22}H_{42}O_2$	338	1.04
15	14.992	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.39
16	15.108	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.23
17	15.158	Cyclohexadecane	$C_{16}H_{32}$	224	0.38
18	15.268	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.30
19	15.527	3-Cyclopentylpropionic acid, 6-ethyl-3-octyl ester	$C_{18}H_{34}O_2$	282	0.23
20	15.648	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.66
21	15.753	2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin	$C_{18}H_{30}O_5$	326	0.19
22	15.975	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.93
23	16.083	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.24
24	16.358	4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester	$C_{13}H_{15}NO_3$	233	3.34
25	16.480	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.24
26	16.768	Kaur-16-ene	$C_{20}H_{32}$	272	0.24

S/N	Retention Time	Compound Name	Molecular Formular	Molecular Weight	Area %
27	16.881	n-Nonadecanol-1	$C_{19}H_{40}O$	284	1.09
28	17.049	2-Nonadecanone	$C_{19}H_{38}O$	282	0.46
29	17.183	Phytol	$C_{20}H_{40}O$	296	0.92
30	17.424	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	4.20
31	17.483	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	2.04
32	17.592	1,16-Hexadecanediol	$C_{16}H_{34}O_2$	258	0.66
33	17.706	Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-, [1R-(1.alpha.,3.beta.,4.alpha.,6.alpha.)]-	$C_{10}H_{18}O$	154	1.76
34	17.773	2-Nonadecanone	$C_{19}H_{38}O$	282	0.50
35	18.074	Tricosyl acetate	$C_{25}H_{50}O_2$	382	0.34
36	18.133	Heneicosane	$C_{21}H_{44}$	296	1.02
37	18.397	Vitamin E	$C_{29}H_{50}O_2$	430	1.05
38	18.475	Bicyclo[12.4.0]octadec-1(14)-ene, 16,17-diethyl-, (Z)-	$C_{22}H_{40}$	304	0.46
39	18.899	n-Nonadecanol-1	$C_{19}H_{40}O$	284	0.35
40	19.091	2-Nonadecanone	$C_{19}H_{38}O$	282	1.98
41	19.451	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	0.81
42	19.977	1,16-Hexadecanediol	$C_{16}H_{34}O_2$	258	0.37
43	20.050	Cyclopentadecanone	$C_{15}H_{28}O$	224	0.42
44	20.100	Nonadecane	$C_{19}H_40$	268	0.19
45	20.780	Hexanoic acid, heptadecyl ester	$C_{23}H_{46}O_2$	354	1.10
46	20.955	2-Pentacosanone	$C_{25}H_{50}O$	366	2.14
47	21.093	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	1.97
48	21.298	Campesterol	$C_{28}H_{48}O$	400	0.67
49	21.568	2-Pentacosanone	$C_{25}H50O$	366	0.29
50	21.704	Z-14-Octadecen-1-ol acetate	$C_{20}H_{38}O_2$	310	0.49
51	21.796	2-methylhexacosane	$C_{27}H_{56}$	380	0.19
52	21.926	Stigmasterol	$C_{29}H_{48}O$	412	2.15
53	22.417	Hexanoic acid, octadecyl ester	$C_{24}H_{48}O_2$	368	1.41
54	22.565	2-Pentacosanone	$C_{25}H_{50}O$	366	2.20
55	22.700	Heneicosane, 11-decyl-	$C_{31}H_{64}$	436	2.58
56	22.758	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	0.98
57	22.931	.beta.-Sitosterol	$C_{29}H_{50}O$	414	6.22
58	23.033	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	1.02

S/N	Retention Time	Compound Name	Molecular Formular	Molecular Weight	Area %
60	23.283	1,3-Dioxolane, 2-heptyl-4-octadecyloxymethy	$C_{29}H_{58}O_3$	454	3.31
62	23.377	Lanosterol	$C_{30}H_{50}O$	426	2.47
63	23.525	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	424	3.42
64	23.679	9,19-Cyclolanost-23-ene-3,25-diol, (3.beta.,23E)-	$C_{30}H_{50}O_2$	442	1.37
65	23.749	Heptadecafluorononanoic acid, undecyl ester	$C_{20}H_{23}F_{17}O_2$	618	0.92
66	23.808	Ergosterol	$C_{28}H_{44}O$	396	0.53
67	23.986	Lupeol	$C_{30}H_{50}O$	426	9.85
68	24.056	Tetratetracontane	$C_{44}H_{90}$	618	5.86
69	24.328	Cholest-4-ene	$C_{27}H_{46}$	370	0.42
70	24.456	Cholane-24-thioic acid, 3,12-bis(acetyloxy)-, S-ethyl ester, (3.beta.,5.beta.,12.alpha.)-	$C_{30}H_{48}O_5S$	520	4.37
71	24.569	7.alpha.-Methylthiotestosterone acetate	$C_{22}H_{32}O_3S$	376	0.23
72	24.783	Nonadecanoic acid, 2,2,2- trifluoroethyl ester	$C_{21}H_{39}F_3O_2$	380	1.31
73	24.845	Heneicosane	$C_{21}H_{44}$	296	0.43

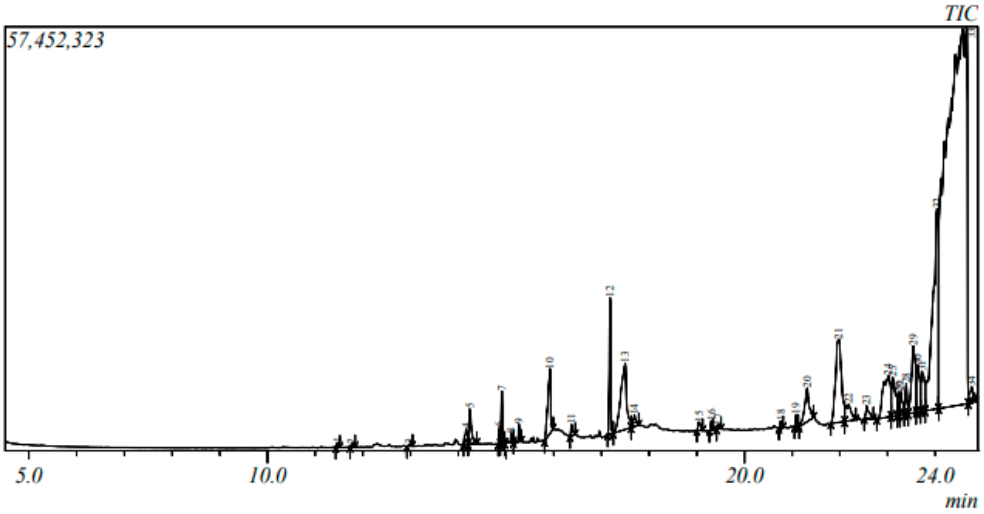

Fig. 4. GC/MS spectra of dichloromethane fraction of *E. nutans* leaf

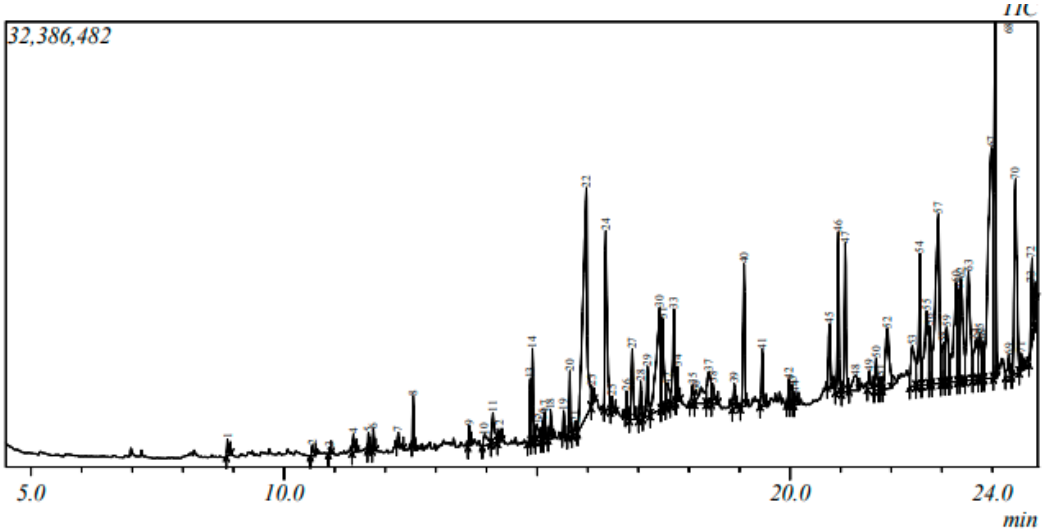

Fig. 5. GC/MS spectra of dichloromethane fraction of *E. nutans* stem

In the results obtained from microscopy of *E. nutans,* the plant was found to be amphistomatic, no trichomes were found on both abaxial and adaxial surfaces. The epidermal wall pattern was found to be irregular for both surfaces. The stomatal index of the abaxial surface was 16.09% and that of the adaxial surface was 21.40% as shown in Table 1. Also, the results of leaf microscopy in Table 1 revealed that *E. nutans* has more stomata on the adaxial surface than on the abaxial surface. The microscocpy of the leaf also revealed the stomata types as anisocytic and anomocytic on both the abaxial and adaxial surfaces and with t-pieces on the stomata (Figs. 2 and 3, respectively). It also showed an undulate anticlinal wall pattern for the adaxial surface and a sinuous anticlinal wall pattern for the abaxial surface with knots on the sinuous cell wall and the areole were quadrangular on both surfaces. Every plant possesses characteristic tissue features which can be identified by microscopy of leaf and stem powder analyses and used for identification and detection of adulteration. The results of microscopy evaluation of *E. nutans* leaf and stem furnished diagnostic features for judging the authenticity, quality, purity and differentiate the drug from its closely related species and also detect adulterant (European Pharmacopoeia 2007). Anatomic characters was used as a taxonomic tool for the identification of *Cola millenii* (Johnny et al. 2022, Adedeji 2008) hence the applicability of this study.

For the flow rate, the angle of repose in Table 2 for the leaf and stem were 33.77^0 and 37.18^0, respectively which showed a poor flow. The value of Hausner's ratio as seen in Table 2 for the leaf and stem powders were 1.298 and 1.533, respectively showing a poor flow. Hausner's ratio values of less than 1.25 indicates good flow while those greater than 1.25 indicates poor flow. The micromeritics properties help to characterize and standardize the pre-formulation properties of herbal drug powder in order to determine its suitability for formulation into solid dosage form (Adedeji et al. 2012, Li et al. 2004).

Chemomicroscopic analysis in Table 3 revealed the presence of mucilage, cellulose, lignin, oil and starch in both stem and leaf powders of the *E. nutans* and absence of protein in both leaf and stem of the plant. Most of the cell wall materials such as cellulose, lignin, etc. perform the functions of protection, strengthening, insulation and reinforcing vascular plants without which they topple over (Liu et al. 2018).

Flouorescence analysis of the leaf and stem on *E. nutans* as seen in Table 4. Different colors were observed when viewed in visible light and under UV light of wavelength 365nm. These colors were distinctive and reproducible revealing the solvent properties to the phytoconstituents. The various colours in *Chrysanthemum indicum* flowers were reported using florescence analysis (Wu et al. 2010).

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 which may serve as chemotaxomomic markers in plant identification (Mbah et al. 2012). The GC-MS analysis showed the presence of 34 phytochemical constituents (Table 5 and Fig. 4) for the leaf and 73 phytochemical constituents (Table 6 and Fig. 5) for the stem. Lupeol (64.05%), 2-methylhexacosane (9.37%), stigmasterol (4.16%) and campesterol (1.29%) were recorded as major components in the leaf while campesterol (0.67%), stigmasterol (2.15 %), beta.-sitosterol (6.22%), lupeol (9.85%) and vitamin E (1.05%) were recorded in the stem extract. Dichloromethane, a moderately non-polar aprotic solvent, is known to effectively dissolved and extract low boiling point components from the ethanol extracts thereby enabling the identification by GC/MS method (Dosso et al. 2020). These phytochemical constituents may function as chemotaxonomic markers, an important taxonomic tool in the identification of *E. nutans*. The phytochemical, lupeol is reported to act as anti-inflammatory, cancer preventive, hepatoprotective, and antiprotozoal agent (Devi et al. 2015, Ravi 2017). N-Hexadecanoic acid and hexadecanoic acid, both fatty acids are reported as antioxidant and anti-inflammatory agents (Ravi 2017, Mazumder et al. 2020) likewise phytol, an antioxidant and chemopreventive agent (Okpala et al. 2022, Gallo et al. 2009).

4. CONCLUSION

The pharmacognostic standards capturing qualitative and quantitative microscopic characters, micrometric properties of powders, chemomicroscopy and florescence characteristics coupled with the GC-MS chemical analysis of *E. nutans* can adequately provide data for the identification of *E. nutans* thus helping in its identity.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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