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Pharmacognostic studies of leaf of *Craterosiphon* scandens Engl. & Gilg. (THYMELAEACEAE)

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ABSTRACT

Background: Craterosiphon scandens Engl. and Gilg. (Thymelaeaceae), commonly known as african winged bean, bierie, or Itaka in various African region has been traditionally used for various medicinal purposes such as stomachache, malaria and fever. This study is aimed at identifying, describing and documenting the pharmacognostic, and diagnostic characters to establish standardization and quality control parameters for the leaves of *C. scandens*.

Methods: Standard procedures were used for microscopy, micrometry, chemomicroscopy, moisture content, ash values, extractive values, fluorescence, and physicochemical properties.

Results: The results of the leaf microscopy revealed that the epidermal cell wall was iregular for both abaxial and adaxial surface, with hypostomatic stomata, and anticlinal wall pattern as straight-undulate. For the micromeritic evaluation of the powdered leaf, the Hausner ratio was 1.546 and Carr's index was 36, indicating poor flow, the angle of repose was 42.520 indicating a passable flow. Chemo microscopy study revealed the presence of mucilage, cellulose, lignin, starch and calcium oxalate crystals (druse) while protein was absent. The moisture content of the leaf was 9.5% w/w, the total ash value was 8.6% w/w, the acid-insoluble ash value was 1.7% w/w, the water-soluble ash value was 1.8% w/w. The water-soluble extractive values, ethanol- soluble values and methanol-soluble values for the leaf of C. scandens were 16.67% w/w, 12.33 % w/w and 13.67% w/w respectively, all these falls within the limit when compared to the standard.

Conclusion: These findings support the identification and authentication of *C. scandens*, establishing standards for quality, purity, safety, and efficacy in Herbal medicine.

Keywords: Chemomicroscopy Craterosiphon scandens, Hypostomatic, Micromeritics, Thymeleaeceae,

1. INTRODUCTION

Craterosiphon scandens is native to Nigeria, Upper Guinea, Cameroons, Upper Guinea Buena. A climbing shrub, perfectly glabrous. Leaves opposite, subopposite or alternate, in 2 rows, short petiole, distant, oblong-oval, entire, narrowed at the base, long-acuminate and obtuse at the apex, sub coriaceous, 2 3/4–3 1/2 in. long, 1 1/4–1 1/2 in. broad, with spreading, parallel, distinct nerves. Flowers "greenish-yellow," in fascicles of 3–6 in the leaf-axils, subsessile, with minute bracteoles at the base. Calyx-tube glabrous, 11/4–1 1/2 in. long; lobes oblong, obtuse, minutely puberulous on the margin, otherwise glabrous, apparently erect during flowering, 1/3 in. long. Stamens inserted immediately below the middle of the tube. Ovary glabrous; hypogynous disc short, deeply lobed, bearing a few short erect hairs. Fruit not seen. [1]. Classification of *C. scandens* according to Angiosperm Phylogeny Group System (APG, 2016) [1].

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Kingdom Plantae Clade Tracheophytes Clade Angiosperms Clade **Eudicots** Rosids Clade Malvales Order Family Thymelaeaceae Genus Craterosiphon

Species: *C. scandens* Engl. and Gilg. Common name: Africa winged bean



Figure.1: Craterosiphon scandens Engl & Gigl. (Source: Field data (2025) Medicinal farm at Orba Local Government Area of Enugu State, Nigeria.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Materials

The leaves of the plant Craterosiphon scandens

2.1.2 Chemicals and reagents

The chemicals and reagents used include; distilled water, glycerol, sodium hydroxide, 10% Concentrated Hydrochloric acid, ferric chloride, concentrated sulphuric acid, dichloromethane, ethyl acetate, methanol, ethanol, n-hexane, dragendorff's reagent, ferric chloride, phloroglucinol, ruthenium red, millon's reagent, N/50 iodine, sodium hypochloride.

2.1.3 Equipment and Apparatus

Materials used include: Beakers, electronic weighing balance, test tubes, filter paper, oven, water bath, pen, pencil, funnel, glass stirrer, measuring cylinders, conical flashk, sieves, spatula, marker, masking tape, foil paper, thongs, evaporating dish, silica gel, knife, mortar and pestle, desiccator, furnace, ash less filter paper, Olympus CX21 electronic microscope, microscope slides, cover-slips, foolscap sheets, meter rule, Am scope MD 500.

2.2 Methods

2.2.1 Collection and Identification of Plant Material

Fresh leaves of *C. scandens* was collected from Orba Local Government Area of Enugu State, Nigeria. It was identified and authenticated by Mr. Alfred O. Ozioko, a taxonomist at the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Nigeria. The Plant was collected and deposited at the Herbarium in the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo. A voucher number UUPH No. A76 was assigned to it.



2.2.2 Microscopic Evaluation of Leaf

The standard median portion of the well-expanded matured leaf was obtained. Microscopical examinations of the transverse section were made, the Epidermis of both adaxial and abaxial surfaces were also made by placing the leaf on a glass slide, scraping gently with a sharp razor blade, irrigated with water until loose cells from the epidermis were washed away and the desired epidermis was reached. The epidermal peels were further cleared with sodium hypochlorite rinsed gently with water and stained with an 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 [2].

2.2.2.1 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 was presented as mean \pm Standard Error of Mean (SEM).

2.2.3 Stomatal Index Determination

The stomatal index (S.I) was determined according to Metcalfe and Chalk [3, 4, 5] The sample (quantitative microscopy) was placed under the microscope and the stomatal index was determined using the formula;

$$S.I = \underline{E + S} \times 100$$

Where S = Number of stomata per unit area, E = Number of epidermal cells in the same area

2.2.4 Evaluation of Powders

2.2.4.1 Micromeritic Analysis

The flow property was determined using standard methods [6]. Which constitutes; Bulk Density and Tapped Density. The weight of 10 g of dried powdered leaf was weighed into a 100 ml measuring cylinder and the volume occupied was noted as the bulk volume (Vb). The cylinder was gently tapped repeatedly to obtain a constant volume noted as the tapped volume (Vt). Bulk density was calculated using the formula below;

$$B\rho = M/Vb$$
; $Tv = M/Tv$

Where $B\rho = Bulk$ density, M=Mass of powder, Bv = Bulk volume of powder, $T\rho = Tapped$ density Tv = Tapped volume

2.2.4.2 Hausner's Ratio and Carr's index

Hausner's ratio a function of inter particle friction was calculated using the formula.

Hausner's ratio = Tp/Bp While $Carr's index = Tp - Bp/Tp \times 100$

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

2.2.5 Chemo microscopic Analysis of Leaf Powder

Powdered leaf was examined for its chemo microscopic properties namely mucilage, lignin, starch, oils, calcium carbonate and calcium oxalate crystals using standard procedures [7].

2.2.5.1 Fluorescence Analysis of Leaf Powders

The fluorescent analysis of dried leaf powder was carried out using the standard method [8].

2.2.5.2 Physico-chemical Evaluation of Leaf Powders

The physicochemical parameters such as moisture content, ash values (total ash, acid-insoluble ash and watersoluble ash values), soluble extractive values such as ethanol, methanol and water-soluble extractive values were performed according to the official method prescribed by the WHO guidelines on quality control methods for medicinal plant materials [3,4,8].



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2.3 Statistical Analysis

Data obtained were expressed in Mean \pm SEM using (Statistical Package for Social Sciences) SPSS 17.0 and the terminology used in describing epidermal features is that of Metcalfe and Chalk (1979) [3].

3 RESULTS

Table 3.1 Quantitative micro-morphological characters for the adaxial and abaxial surface of leaves of *C. scandens*.

Iregular Hypostomatic Straight-Undulate	Iregular Hypostomatic
• 1	Hypostomatic
Straight-Undulate	
Straight Chadiate	
	$18.73(19.07\pm0.75)21.53$
	14.47(14.74±0.66)14.29
	$13.45(10.62\pm0.50)10.54$
	4.60(4.44±0.29)3.86
	95(89.3±1.61)86
564(520.1±11.07)565	392(348±17.69)290
32.07(33.19±2.07)28.17	40.55(33.27±1.81)32.35
15.49(14.68±0.64)17.10	$15.12(16.00\pm0.89)11.17$
3.63(3.33±0.17)3.46	$2.64(2.31\pm0.10)2.02$
	11.53(13.46±1.11)15.04
	3.30(4.77±0.36)5.24
	32.07(33.19±2.07)28.17 15.49(14.68±0.64)17.10

Results presented as Mean±SEM of Ten (10) Replicates

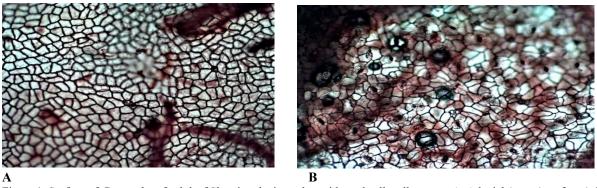


Figure 1: Surface of *C. scandens* fresh leaf Showing the irregular epidermal cell wall pattern. A. Adaxial (upper) surface (x10) B. Abaxial (Lower) surface (x10)

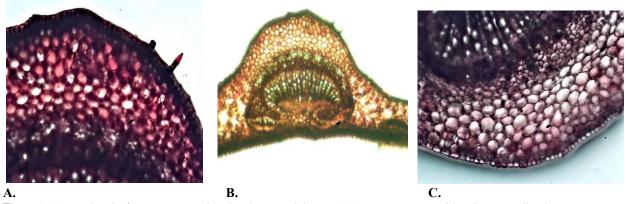


Figure 2: *C. scandens* leaf Transverse section showing A. Trichome (x10), B. UE; Upper epidermis, Co; Collenchyma, P; Parenchyma, Xy; Xylem, Ph; Phloem (x4) C. LE; Lower epidermis, Vb; Vascular bundle (x10).



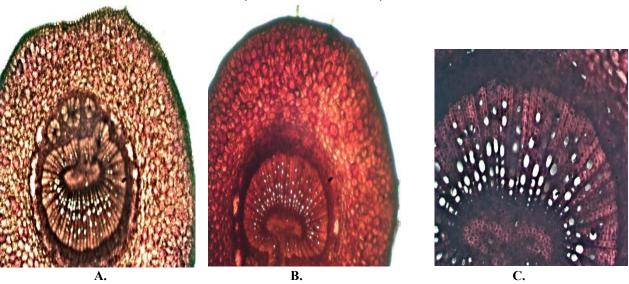


Figure 3 *C. scandens* Transverse section of petiole showing A. (x4) B. (x4) C. (x10) of UE; Upper epidermal cell,Vb;Vascular bundles, Xy; Xylem. LE; Lower epidermis, Co; Collenchyma, P; Parenchyma, Vb; Vascular bundle.

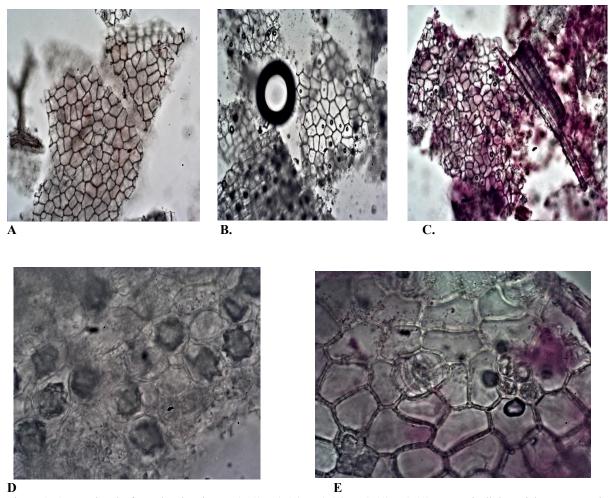


Figure 4: *C. scandens* leaf powder showing A. (x10) B.(x40) C. (x40) D, (x40) E (x40) UT; Unicellular Trichome, GT; Glandular Trichome. UAWP; Undulate Anticlinal wall pattern, Anis; Anisocytic stomata, Anos; Anomocytic stomata, IEC; Irregular epidermal Cell.

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Table 2: Micromeritic evaluation of powdered leaf of C. scandens

Micro-meritic Parameters	Leaf Powder
Bulk Volume (mL)	50.35±0.40
Tapped Volume (mL)	32.83 ± 0.20
Bulk Density (g/mL)	0.19 ± 0.00
Tapped Density (g/mL)	0.30 ± 0.00
Hausner Ratio	1.55±0.03
Carr's Index (%)	36.00 ± 1.22
Diameter of Heap (cm)	7.70 ± 0.14
Height of Heap (cm)	3.53 ± 0.08
Flow Time (sec)	38.18±4.11
Angle of Repose (0)	42.52

Mean±SEM (Standard Error of Mean) of Three (3) Replicate

Table 3.3 Results for Fluorescence analysis of C. scandens leaf powder

Extract Ordinary Light		UV - 365nm	
Water	Dark grey	Blue	
Methanol	Green	Red	
Ethanol	Green	Red	
Chloroform	Green	Red	
N-Hexane	Light grey	Reddish blue	
Ethylacetate	Grey	Red	

Table 4: Results of Water-soluble extractive value, Methanol soluble extractive value, Ethanol soluble extractive value, Moisture content, Total ash value, Water-soluble ash value, and Acid-insoluble ash value of *C. scandens* leaf.

Parameter	Result in weight(g)	Percentage (%w/w)
Moisture content	0.195±0.002	9.500±0.000
Total ash value	0.172±0.003	8.600 ± 0.000
Acid-insoluble ash value	0.033 ± 0.004	1.700 ± 0.000
Water-insoluble ash value	0.037 ± 0.000	1.800 ± 0.000
Water-soluble extractive value	0.167±0.004	16.670±0.000
Ethanol-soluble extractive value	0.123±0.004	12.330±0.000
Methanol-soluble extractive value	0.137±0.004	13.670±0.000

Values are represented as mean of five replicates (n=5) $\pm SEM$

Table 5 Results for Chemomicroscopic Evaluation of C. scandens Powdered Leaf

Parameters	Leaf	



Cellulose	+
Lignin	+
Starch	+
Calcium Oxalate	+ (druse)
Crystals	
Mucilage	+
Protein	-

⁺⁼ Present, -= Absent

Table 6 Percentage yield of Methanol extraction and partitioning of the leaf powder of C scandens.

Taxon	Solvent Used	Morphological	Quantity Used for	Quantitative	Percentage
		Part Used	Extraction (g)	Yield (g)	Yield (%)
C. scandens	N-Hexane	Leaf	601.96	21.11	3.51
	Dichloromethane	Leaf	601.96	13.23	2.20
	Ethyl Acetate	Leaf	601.96	5.26	0.87
	Partial Pure				
	Compound Salted Out			0.82	0.14
	Methanol	Leaf	601.96	62.34	10.36

Table 7: Identified compounds from GC-MS spectra of ethyl acetate fraction of C scandens leaf

Peak	R. Time (min)	Area %	Height %	Name of Compound
1	9.965	2.34	5.13	2-Acetylpiperidine
2	10.095	1.45	1.92	Phenol
3	11.248	1.05	2.93	Pantolactone
4	13.626	1.2	4.07	Benzofuran, 2,3-dihydro-
5	14.984	0.44	0.76	4-Hydroxy-3-methylacetophenone
6	16.356	9.05	5.77	L-Serine, ethyl ester
7	16.754	2.53	8.35	Piperidine, 1-acetyl-
8	17.11	10.14	24.7	Piperidine, 2,2,6,6-tetramethyl-
9	17.624	0.44	1.16	4-(7-Methoxy-3,3,7-trimethyl-oxepan-2-ylidene)
10	19.083	11.22	15.8	Ketone, vinyl-pyrrolidinyl-
11	19.906	48.45	16.31	1,5-Anhydro-d-mannitol
12	20.766	3.52	5.18	2-Dodecen-1-yl(-)succinic anhydride
13	21.263	2.33	1.96	1-(4-Hydroxy-2-methoxy-pyrrolidin-1-yl)-



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				ethanone
14	21.71	3.63	2.17	OctylbetaD-glucopyranoside
15	24.351	2.22	3.79	Phytol

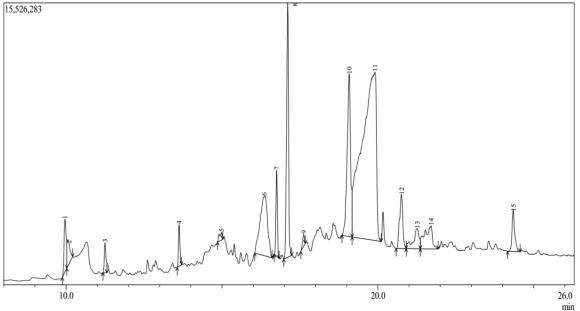


Figure 5: GC-MS spectra of ethyl acetate fraction of *C. scandens*

Figure 6: Structures of some identified chemical compounds from the GC-MS spectra of the ethyl acetate fraction of *C. scandens*



Table 8: Identified compounds from GC-MS spectra of aqueous fraction of *C. scandens* leaf

Peak	R. Time (min)	Area %	Height %	Name of Compound
1	9.958	0.81	2.23	2-Acetylpiperidine
2	10.642	5.11	4.16	Glycerin
3	12.615	1.04	2.72	L-Homoserine lactone, N,N-dimethyl-
4	15.281	0.71	1.74	Morpholine, 4-(2-methyl-1-propenyl)-
5	16.098	7.49	6.83	L-Serine, ethyl ester
6	16.746	1.49	4.6	Piperidine, 1-acetyl-
7	17.069	7.45	21.11	Piperidine, 2,2,6,6-tetramethyl-
8	17.525	1.2	1.8	β-D-Glucopyranose, 1,6-anhydro-
9	17.874	4.86	2.61	DL-Arabinitol
10	18.408	4.92	3.19	α-D-Galactopyranoside, methyl
11	18.963	12.7	15.72	Ketone, vinyl-pyrrolidinyl-
12	19.433	39.43	19.08	1,5-Anhydro-d-mannitol
12	10.725	2.15	2.72	Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-
13	19.735	2.15	2.73	I
14	20.158	1.14	1.31	Aspidofractinine-3-methanol, $(2\alpha,3\beta,5\alpha)$ -
15	20.726	3.87	5.2	2-Dodecen-1-yl(-)succinic anhydride
16	21.199	5.15	4.03	1-(4-Hydroxy-2-methoxy-pyrrolidin-1-yl)-ethanone
17	24.295	0.46	0.93	Phytol



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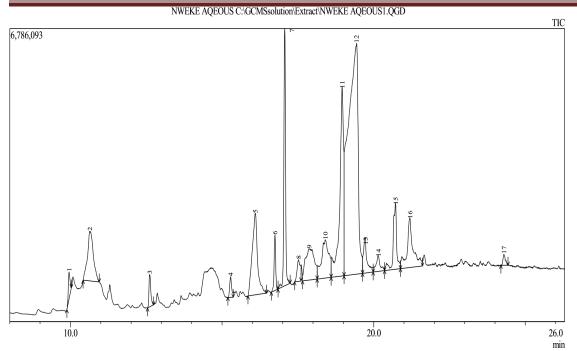


Figure 7: GC-MS spectra of aqueous fraction of C. scandens

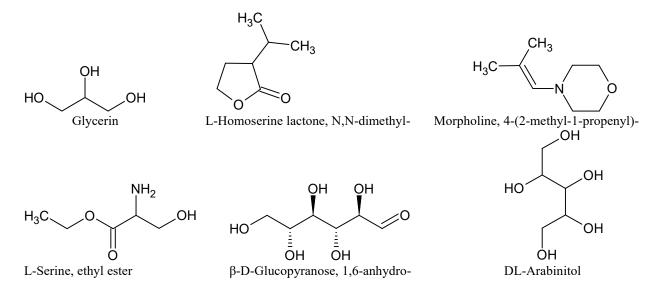


Figure 8: Structures of some identified chemical compounds from the GC-MS spectra of the aqueous fraction of C. scanden**s**

4. DISCUSSION

Craterosiphon scandens, a member of the Thymelaeceace family produces edible pods resembling green beans, rich in protein, vitamins, and minerals, serving as a nutritious food source for various African communities. It has therefore been used traditionally for various medicinal purposes such as stomachache, malaria and fever. These studies will offer this plant like any other medicinal plants that could be confused with other species due to their relative similarities and so provide some basis for its proper identification. The quality control of vegetable crude drugs is of paramount importance under current European Union (EU) regulations: herbal products can only be manufactured under license in uniformity with the rules and guidance for pharmaceutical manufacturers and



distributors. [9] According to WHO, all drugs synthetic or plant-based must meet safety and efficacy standards.[13] Hence, quality control of herbal drugs is critical for ensuring authenticity and detecting adulteration. The results obtained for the abaxial and adaxial surfaces were irregular epidermal cell wall shape, hypostomatic distribution of stomata and straight undulate anticlinal wall pattern for the adaxial and abaxial surface. Stomatal frequency (stomatal number) were seen on the abaxial surface (89.3 \pm 1.611), as shown in (Table 1). The micromeritic evaluation of the powdered leaf, the Hausner ratio was 1.546 indicating a poor flow when compared to the standard, Carr's index was 36, indicating another poor flow and finally the angle of repose was 42.520 which indicates a passable flow. The Hausner ratio and Carr's index are parameters that are used to determine the powder flow property and powder characteristics. Hausner ratio values less than 1.25 indicate good flow while those greater than 1.25 indicates poor flow. From the experiment, Hausner's ratio and Carr's index of the dried leaf was greater than 1.25 and 25 % respectively and these indicate that the powder has a poor flow property as shown in (Table 2). This could be as a result of some factors that affect a powder's flowability hence affecting the powder characteristics. The factors include: moisture content, temperature, particle size, particle shape (texture) and time of storage at rest. 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 interparticle friction or resistance to movement between particles[10]. Chemo microscopy study of the dried leaf revealed the presence of mucilage, cellulose, lignin, starch and calcium oxalate crystals (druse) while Protein was absent as shown in (Table 5). The moisture content of C. scandens leaf was 9.5% w/w as stated in (Table 4). The African pharmacopoeia limit of moisture contents for vegetable crude drugs range is between 8-14 % w/w with a few exceptions such as digitalis leaf with the value 6 % w/w, from the result of this research work, the range is found to be within limit. High moisture content is uneconomical, and in the presence of suitable temperature could lead to enzymatic activation and hydrolytic reactions as well as proliferation of microbial growth which may ultimately lead to degradation of active constituents. The total ash value for C. scandens leaf as obtained was 8.6% w/w respectively as shown in (Table 4). The European pharmacopoeia limit of total ash value for crude vegetable drugs range should not exceed 14 % w/w [11]. Therefore the total ash value of the leaf was found to be within acceptable limit. The total ash value is a method used to measure the total amount of residual substances that is not volatilized when the drug sample is ignited. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium, etc. Ash may be derived from the plant itself and it is usually called the "physiological ash" or may come from the extraneous matter, especially sand and soil that adhere to the surface of the drug and it is usually called the "non-physiological ash". Generally, the amount of ash contained in a crude vegetable must be low. It indicates to some extent the amount of care taken in the preparation of the drug [12]. The acid-insoluble ash value obtained from C. scandens leaf was 1.7% w/w as shown in (Table 4). The European pharmacopoeia limit of acid- insoluble ash value for crude vegetable drugs range should not exceed 2 % w/w [11]. The determination of the acid- insoluble ash is a method that is intended to measure the amount of silica, especially sand and siliceous material, present in the drug. The water-soluble ash value of C. scandens leaf was 1.8% w/w as shown in (Table 4). The African pharmacopoeia limit of ash value for crude vegetable drugs state that a lesser amount shows that there is less solubility of the ash in water while a higher value indicated a high solubility of the ash in water. The determination of the water- soluble ash value of a particular crude drug helps in the detection of the amount of the ash materials that are soluble in water. In addition, the water-soluble extractive values, ethanol-soluble values and methanol-soluble values for the leaf of C. scandens were 16.67% w/w, 12.33 % w/w and 13.67% w/w respectively as stated in Table 4. The determination of the extractive values helps to measure the number of constituents which are extractable by the solvents under the specified conditions. They also give an idea about the nature of the chemical constituent present in a crude drug. The GC-MS analysis of the ethyl acetate fraction of C. scandens revealed several bioactive compounds, including 2-Acetylpiperidine, Phenol, Benzofuran (2,3-dihydro-), Piperidine (1-acetyl-), Ketone (vinyl-pyrrolidinyl-), and Phytol (Table 7 and 8). These compounds contribute to the antioxidant activity of the extract through various mechanisms

5. CONCLUSION

The results obtained from the pharmacognostic studies have provided critical insights into the plant's botanical and phytochemical characteristics, ensuring proper identification and standardization for medicinal use of the plant *C. scandens*.



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DECLARATION

Acknowledgements

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Conflict of Interest

The authors have declared that no conflict of interest exists.

Contribution of the Authors

This work was carried out in collaboration with all other authors. UTN and RAU designed the study, performed the experimental procedures, and author IIJ prepared statistical analysis, and UTN wrote the first draft of the manuscript. Authors RAU and IIJ supervised lab experiments. Authors UTN, IIJ, and RAU organized data, managed the literature searches, and assisted in plant material preparation. All authors read and approved the final manuscript.

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