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Zacchaeus Kipkorir Rotich
1. Department of Public Health, Pharmacology, and Toxicology, College of Veterinary and Agricultural Sciences, University of Nairobi, P.O. Box 29053 - 00625, Nairobi, Kenya
2. Department of Chemistry and Biochemistry, School of Science and Aerospace Studies, Moi University, P.O. Box 3900- 30100, Eldoret, Kenya
3. Department of Pharmaceutics and Pharmaceutical Chemistry, School of Pharmacy, Kabarak University, Private bag- Nakuru, Kenya

James Mucunu Mbaria
Department of Public Health, Pharmacology, and Toxicology, College of Veterinary and Agricultural Sciences, University of Nairobi, P.O. Box 29053 - 00625, Nairobi, Kenya

Isaac Ole Mapanay
Department of Public Health, Pharmacology, and Toxicology, College of Veterinary and Agricultural Sciences, University of Nairobi, P.O. Box 29053 - 00625, Nairobi, Kenya

Correspondence:
Dr. Zacchaeus Kipkorir Rotich
1. Department of Public Health, Pharmacology, and Toxicology, College of Veterinary and Agricultural Sciences, University of Nairobi, P.O. Box 29053 - 00625, Nairobi, Kenya
2. Department of Chemistry and Biochemistry, School of Science and Aerospace Studies, Moi University, P.O. Box 3900- 30100, Eldoret, Kenya
3. Department of Pharmaceutics and Pharmaceutical Chemistry, School of Pharmacy, Kabarak University, Private bag- Nakuru, Kenya
Email: rotichkipkorir@gmail.com

Pharmacognostic, physicochemical and phytochemical profiles of *Euclea divinorum* (Ebenaceae)

Zacchaeus Kipkorir Rotich, James Mucunu Mbaria, Isaac Ole Mapanay

ABSTRACT

Background: *Euclea divinorum*, belonging to the family Ebeneceae, has extensive traditional medicinal use in Africa. However, it lacks sufficient published data on its pharmacognostic, physicochemical, and phytochemical properties. Aims and Objectives: Thus, this study aimed at comprehensively evaluating the pharmacognostic, physicochemical, and phytochemical properties of *E. divinorum* using established techniques. Materials and Methods: The evaluation included assessing organoleptic properties, macroscopy, and microscopy of leaves, stems, and roots. Various physicochemical parameters, such as loss on drying and extractive values via hot and cold maceration, were determined. Phytochemical screening was also conducted on root aqueous extracts. Results and Conclusion: Results revealed specific characteristics of *E. divinorum* leaves, including light green coloration, distinct odor, and bitterness. Macroscopic examination highlighted opposite phyllotaxy, simple leaf types with lamina length range of 71.09 - 93.89 mm and a width range of 6.05 - 17.59 mm, obtuse leaf apexes, cuneate leaf bases, entire leaf margins, and smooth leaf surfaces. Venation displayed a reticulate pattern, with the midrib prominently visible on the lower surface. Microscopic analysis of the lower leaf epidermis showed an average of stomatal density of 3.75±0.67, epidermal density of 35.75±2.16 and stomatal index of 10.47±1.11. Microscopic examination of the leaf lamina across the midrib revealed cortical parenchymal cells containing calcium oxalate crystals, lignified xylem, and non-lignified phloem. Stem cross-sections displayed single-layered cells comprising the epidermis, secondary phloem, secondary xylem, and pith. Similar structures were observed in root cross-sections. The plant's moisture content was found to be 44.8±1.962%, with water and alcohol-soluble extractive values of 7.27±0.17 and 1.13±0.05 g/100g, respectively, through cold maceration, and 14.77±0.28 and 11.43±0.39 g/100g, respectively through hot maceration. The total ash content was measured at 3.03± 0.103%. Phytochemical screening detected various compounds, with anthraquinone glycosides, coumarine glycosides, saponin glycosides, flavonoids, proteins, and tannins being abundant. Alkaloids, cardiac glycosides, and steroids were absent. This research contributes to standardizing *E. divinorum*, aiding in its identification, preventing adulteration, and ensuring therapeutic efficacy.

Keywords: Ebenaceae, *Euclea divinorum*, Ethnobotanical information, Nandi County.

INTRODUCTION

The botanical family *Ebenaceae*, commonly known as ebony, comprises a diverse array of flowering plants primarily located in tropical regions worldwide. This family is categorized into two primary genera: *Diospyros L.* and *Euclea L.*, collectively containing between 500 and 600 species [1]. The genus Diospyros encompasses approximately 500 species and is widely distributed across mainland tropical Africa, ranging from Senegal in the west to Eritrea, Ethiopia, and Kenya in the east, and extending southward to Namibia, northern South Africa, and Swaziland [2]. Diospyros species are commonly employed in traditional medicine within tropical regions to address various health issues. Leaves, bark, fruits, hardwood, and roots are utilized in forms such as tonics, powders, and poultices to treat conditions like asthma, dermatitis, hypertension, atherosclerosis, lumbago, bleeding, insomnia, anthelmintic, purgative, antidiuretic, lowering blood pressure, antipyretic, and tightening tissues [2].

The Euclea (Ebenaceae) genus has 16 identified species of flowering trees and shrubs that are mostly found in Africa, including the Comoro Island, Arabia, Socotra, and South East Asia [1]. These plants are extensively spread in tropical and subtropical regions of the world. As per an extensive examination of the literature, the plants from this genus is employed in folk medicine to address numerous ailments such as diabetes, diarrhea, toothaches, malaria, leprosy, cancer, as well as infections related to HIV/AIDS [3]. Some of the Euclea species of ethnomedicinal importance include *E. crispa* [4], *E. divinorum* [5], *E. latideus* [6], *E. natalensis* [7], *E. racemosa* [8] and *E. schimperi* [9]. The ethnomedicinal applications of other Euclea species have been documented in scientific literature [3]. Euclea divinorum, among the various species of Euclea, holds significant medicinal value in African traditional medicine systems.

Its diverse parts, including the bark, leaves, and roots, have been utilized for centuries across Africa to address a wide range of health issues. These include the treatment of HIV/AIDS-related diseases in Zambia [10], managing urine retention in Ethiopia [11], combating malaria, leprosy, gonorrhea, syphilis, and tapeworm in Ethiopia [12], addressing abdominal upsets, skin problems, kidney disorders, and respiratory issues [13], and inducing labor in Kenya [14]. Furthermore, it is employed to alleviate convulsions in Zimbabwe [15], manage schistosomiasis in South Africa [16], and act as a laxative for constipation [3]. This species (Figure 1) is an evergreen shrub or small tree that has male and female flowers located on separate plants, and can grow to heights of up to 9 meters, with a rounded canopy supported by one or more stems. Young stems display small rust-colored granules and have a smooth, pale gray surface. Its bark, characterized by longitudinal cracks, ranges in color from grey-brown to black [17]. Owing to the widespread ethnomedicinal applications of this plant, the main objective of this research was to evaluate the pharmacognostic, physicochemical and phytochemical profiles of *E. divinorum*.

MATERIAL AND METHODS

Chemicals, reagents and solvents

All chemicals, solvents and reagents used were of analytical grade.

Plant material collection and identification

Assisted by a recognized herbalist, fresh leaves and roots of *E. divinorum* were collected from Nandi escarpment in Nandi County, Kenya (Figure 2, coordinates: 0˚00'37.0"S 34˚59'03.8"E). The plant specimens were subsequently conveyed to the East Africa Herbaria situated within the National Museums of Kenya for the purpose of identification and authentication. Following its examination, the plant was verified to be Euclea divinorum (Family: Ebenaceae) and was designated with the voucher specimen number: NMK/BOT/CTX/2/ID/14//2023.

Plant material processing and aqueous extraction

After the collection and identification of plant samples, the roots were air-dried for two weeks at the laboratories of the Department of Chemistry and Biochemistry, School of Science and Aerospace Studies, Main Campus, Moi University, Kenya. Following this, the dried roots were ground into a powder using an electric plant mill and stored in labeled air-tight plastic containers on laboratory shelves for future aqueous extraction. The maceration technique [18], was employed, immersing the samples in distilled water at a ratio of 1g to 4 ml for 72 hours in tightly sealed conical flasks. Post-extraction, filtration with 0.45 µm filter paper was performed, followed by Lyophilization using a freeze dryer, using Harvest Right Medium Home PRO Freeze Dryer [19, 20] equipped with 1350 psi of vacuum pressure and maintained at 22.22°C for complete desiccation. The resultant dried extracts were stored in a refrigerator at 4°C for subsequent experimentation.

Evaluation of Pharmacognostic Profiles of *E. divinorum*

Pharmacognostic properties namely organoleptic properties, macroscopic, qualitative and quantitative microscopy were determined following standard methods.

Determination of Organoleptic Attributes of E. divinorum

The sensory assessments of *E. divinorum* were conducted in accordance with the WHO Quality Control guidelines for herbal medicine evaluation [21]. The various organoleptic attributes assessed encompassed the color, size, odor and taste parameters.

Determination of Macroscopic Attributes of E. divinorum

Macroscopic assessments of *E. divinorum* were conducted in accordance with the WHO Quality Control guidelines for herbal medicine evaluation [22]. The various macroscopic attributes assessed encompassed shape, markings, base, texture, veins, and apex characteristics.

Determination of Microscopic Attributes of E. divinorum

Microscopic studies were done following the protocols by Musharaf and co-workers [23] with minor modifications. Sections of leaves, stems, and root specimens were prepared using a sharp blade and then cleared with a chloral hydrate solution. They were stained using a phloroglucinol-hydrochloric acid solution and mounted in glycerin. Microscopic analysis was conducted using a Penta Head Microscope/Multi-view Head Microscope (EUROPE) at ×100 magnification, and photomicrographs were captured using a Top View digital camera.

The *E. divinorum* leaf underwent qualitative microscopic examination to determine the presence or absence of epidermal cells and the type and distribution of stomata. Additionally, qualitative microscopic analysis was performed on transverse and longitudinal sections of the leaf, stem, and root. Moreover, quantitative microscopic analysis of the leaves included assessing the stomatal number and stomatal index, calculated as the average number per square millimeter of leaf epidermis.

Evaluation of Physico-chemical Parameters of *E. divinorum*

Standard methods were used to evaluate for various physico-chemicals parameters of the plant, namely loss on drying, extractive values by hot and cold maceration, and total ash values [24].

Determination of Loss on Drying

The moisture content of *E. divinorum* was determined following the gravimetric method as loss on drying, in accordance with standard procedures [25]. A preheated tarred thin porcelain crucible was weighed, and its weight with the lid recorded as W_1 grams. The dried powdered sample of *E. divinorum* was weighed to obtain 100 grams (W_2 grams) and placed into the crucible. The sample was then dried in an oven at 105 °C until two consecutive weights did not differ by more than 0.5 mg. After cooling in a desiccator, the mass was determined as W3 grams. The moisture content was calculated as the percentage loss on drying (LOD) using the formula: % moisture = $(W_2-W_3/W_2-W_1) \times 100\%$ where W_2-W_3 represents the weight of moisture and W_2-W_1 represents the weight of the sample.

Determination of extractive values

The extractable matter from *E. divinorum* was analyzed for alcohol-soluble and water-soluble extractives using both cold and hot maceration methods, following established protocols [25]. For the determination of alcohol-soluble extractive value by cold maceration, 4.0 grams of coarsely powdered sample were soaked in 100 mL of 90% ethanol for 24 hours. The resulting extract was filtered, and 25 mL of the filtrate was evaporated to dryness to obtain the dried matter. The percentage w/w alcohol-soluble extractive value was then calculated using the formula: Alcohol-soluble extractive value (w/w%) = 100 (x), where x represents the weight (in grams) of the dried matter obtained from the 25 mL of extract. Similarly, the alcohol-soluble extractive value by hot maceration was determined following the same procedure, with refluxing of the sample for one hour before filtration and drying.

For the determination of water-soluble extractive values, the same procedure was repeated using chloroform water instead of ethanol. The percentage w/w water-soluble extractive value by both cold and hot maceration methods was calculated using the same formula as for the alcohol-soluble extractive values.

Total ash content

The total ash value of *E. divinorum* was determined following established procedures [14]. A thin, flat porcelain dish was weighed and ignited, after which 2.0 grams of *E. divinorum* powder were added to it. The dish was then heated using a burner placed on a triangle made of pipe-clay and a ritort support. After cooling in a desiccator, the ash was weighed. The total ash value was calculated using the formula:

Total ash value of the sample= (100(z-x)/y%

where x represents the weight of the empty dish, y is the weight of the drug taken, and z denotes the weight of the dish plus ash after complete incineration.

Preliminary phytochemical screening of *E. divinorum*

Aqueous extracts were screened for alkaloids, glycosides (anthraquinone, cardiac, coumarin cynogenetic and saponin), steroids, tannins, carbohydrates, proteins, and flavanoids using standard phytochemical methods [27, 22].

Screening for alkaloids - Dragendorff's test

To detect alkaloids in the aqueous root extract of *E. divinorum*, the root powder was mixed with hydrochloric acid (HCl), filtered, and then a few drops of Dragendorff's reagent were added to 2 to 3 mL of the filtrate. The formation of an orange-brown precipitate indicated the presence of alkaloids.

Screening for anthraquinone glycosides - Bornträger's test

The plant extract was first mixed with chloroform and filtered. Subsequently, the filtrate was shaken with 10% ammonia solution. The presence of anthraquinones was determined by observing the formation of a pink, red, violet, or purple color in the ammoniacal layer.

Screening for cardiac glycosides - Keller-Killiani tests

The plant extract (2ml) was mixed with glacial acetic acid containing a trace amount of ferric chloride solution, followed by the addition of 1 drop of 5% concentrated sulfuric acid. The appearance of a brown ring at the interface between the two layers, which subsequently changes to a violet or bluish-green color, indicates the presence of cardiac glycosides.

Screening for coumarin glycosides – Feric chloride test

To determine the presence of coumarins, the dried plant extract powder was placed into a test tube and moistened. The test tube was then covered with filter paper soaked in diluted sodium hydroxide and placed in a water bath. After exposure to ultraviolet light, the filter paper displayed a yellowish-green fluorescence, confirming the presence of coumarins.

Screening for saponin glycosides - honeycomb froth

The honey comp test, conducted to detect saponins, began by measuring 1 gram of the sample and placing it into a conical flask, followed by the addition of 10 mL of distilled water and boiling for 5 minutes. After filtration, 2.5 mL of the filtrate was measured and mixed with 10 mL of distilled water, vigorously shaken for approximately 30 seconds. The formation of honeycomb froth confirmed the presence of saponins.

Screening for flavonoids - Shinoda test

To screen for flavonoids in the plant extract, the dry powder was combined with 5 mL of 95% ethanol/t-butyl alcohol, along with a few drops of concentrated HCl and 0.5 grams of magnesium turnings. The presence of flavonoids was indicated by the appearance of colors ranging from orange, pink, to red and purple.

Screening for proteins - Biuret's test

The test solution, comprising 3 mL, was combined with 4% NaOH and a small amount of 1% CuSO4 solution. The appearance of a violet or pink color indicates the presence of proteins.

Screening for sugars - Barfoed's Test

The plant extract was combined with Barfoed's reagent, consisting of copper acetate dissolved in acetic acid, and heated in a boiling water bath for 1-2 minutes before cooling. The emergence of a brick-red precipitate within 3 to 4 minutes confirmed the presence of monosaccharides.

Screening for steroids - Salkowski test

The extract (2 mL) was combined with chloroform (2 mL) and concentrated sulfuric acid (2 mL), and thoroughly shaken. A positive indication of steroid presence was given by a red color observed in the chloroform layer and a greenish-yellow fluorescence in the acid layer.

Screening for tannins

To screen for tannins in the plant extract, 1 gram of the extract was mixed with 10 mL of distilled water and filtered. Then, 1 milliliter of a 5% ferric chloride solution was added to the filtrate. The presence of tannins was confirmed by the formation of a blue-black, green, or blue-green precipitate.

RESULTS

Pharmacognostic Characteristics of *E. divinorum*

Organoleptic Properties

The sensory characteristics of *E. divinorum* leaves are listed in Table 1. The lamina length of *E. divinorum* light green leaves was measured to be between 71.09 - 93.89 mm and between 6.05 - 17.59 mm in width. During testing, they were discovered to be mucilaginous and to have a slight odor.

Macroscopic Properties

Figure 3 depicts the morphological arrangement of *E. divinorum* leaves, with the findings from the macroscopic analysis presented in Table 2. The observation revealed opposite phyllotaxy, with alternate leaf types characterized as simple. The leaf apex displayed obtuse angles, while the base appeared cuneate. Moreover, the leaf margin was noted to be smooth, and the surface texture exhibited a smooth texture. Venation followed a reticulate pattern, with the midrib distinctly visible on the lower surface.

Microscopic evaluations

Figure 4 displays the photomicrograph from the microscopic examination of the lower leaf epidermis of *E. divinorum*, used to derive leaf constants in Table 3 through quantitative microscopy. Further microscopic examinations on the Transverse Sections (T.S.) of the leaf lamina, stem, and root yielded photomicrographs (x100) shown in Figures 5, 6, and 7 respectively. Figure 8 illustrates photomicrographs depicting the Longitudinal Section (L.S.) of the *E. divinorum* root.

The examination of the lower leaf epidermis of *E. divinorum* revealed an average stomatal density of 3.75±0.67 per square millimeter, 35.75±2.16 epidermal cells per square millimeter and an average stomatal index of 10.47±1.11. The Transverse Section (T.S.) of the leaf lamina across the midrib (Figure 5) revealed cortical parenchymal cells containing calcium oxalate crystals, lignified xylem, and non-

lignified phloem. Similarly, both the T.S. and Longitudinal Section (L.S.) microscopy of the stem (Figures 6 and 8) showed single-layered cells forming the epidermis, secondary phloem, secondary xylem, and pith. Corresponding structures were also observed in the T.S. microscopy of the roots (Figure 7).

Figure 1: Photograph of *E. divinorum* plant (Pictured by Z. K. Rotich)

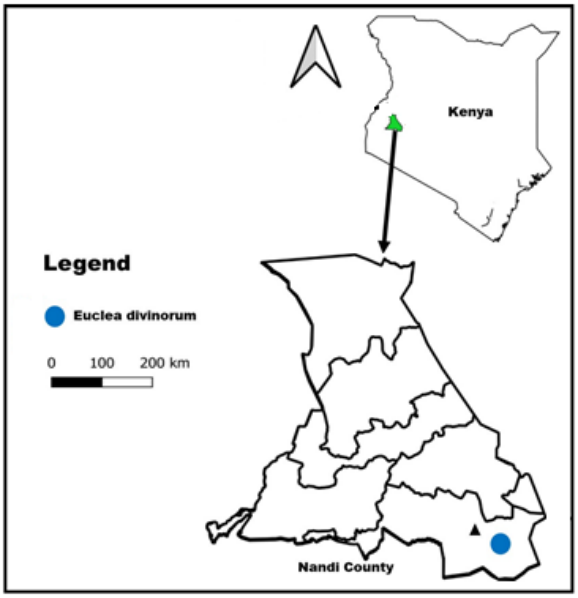

Figure 2: Geographical site map

Figure 3: Morphological structure of *E. divinorum* leaves (Z. K. Rotich)

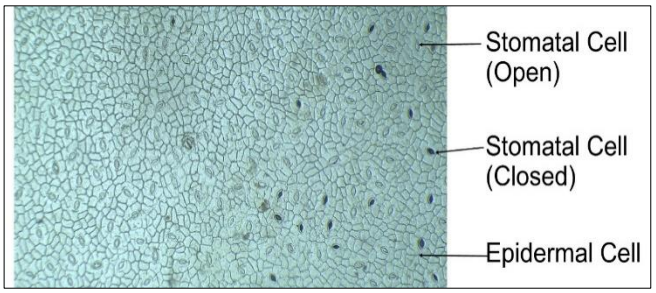

Figure 4: Photomicrograph of leaf of *E. divinorum* showing abaxial (lower) surface (Z. K. Rotich)

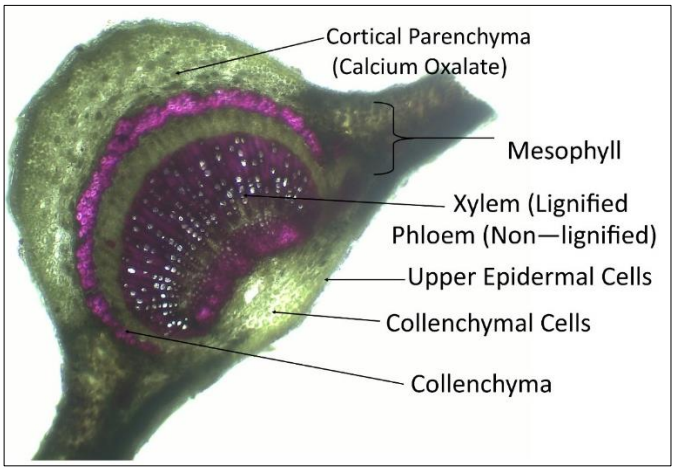

Figure 5: Photomicrograph showing the T. S. of *E. divinorum* leaf lamina across the midrib 100x (Z. K. Rotich)

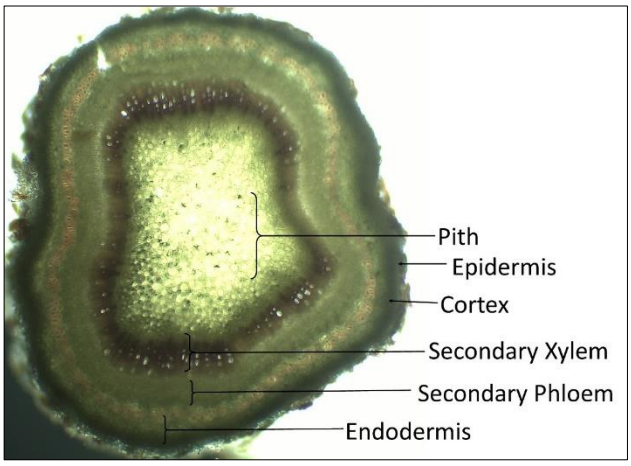

Figure 6: Photomicrograph showing the T. S. of *E. divinorum* stem 100x (Z. K. Rotich

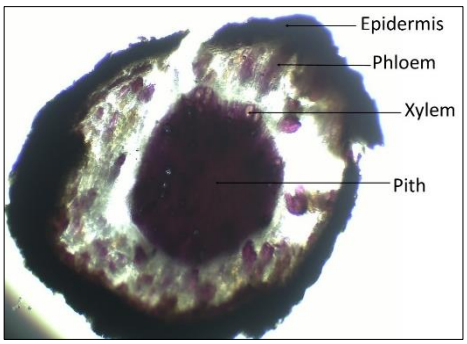

Figure 7: Photomicrograph showing the T. S. of *E. divinorum* root 100x (Z. K. Rotich)

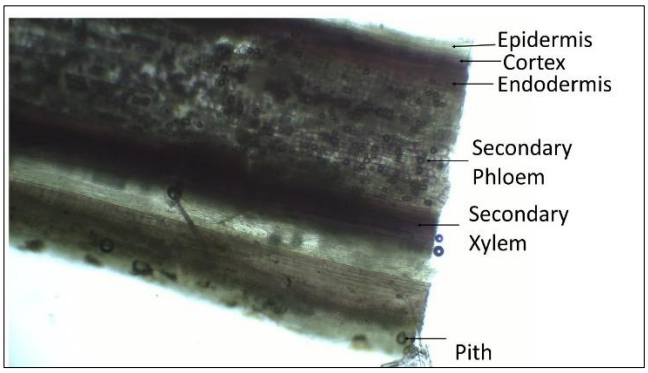

Figure 8: Photomicrograph showing the L. S. of *E. divinorum* root 100x (Z. K. Rotich)

Physico-chemical Parameters of *E. divinorum*

Moisture content

Table 4 illustrates the findings regarding moisture content, measured as loss on drying (LOD). The moisture content recorded was 44.8±1.96%.

Extractive values

Table 5 displays the results of extractive values obtained through hot and cold maceration methods. For cold maceration, the water-soluble extractive value was 12.9%, and the alcohol-soluble extractive value was 0.4%. In contrast, hot maceration yielded higher values, with water-soluble and alcohol-soluble extractive values of 20.4% and 15.5%, respectively.

Total ash content

Table 6 presents the results of calculating the total ash value, which was found to be 3.05%.

Qualitative Phytochemical screening of *E. divinorum*

The qualitative phytochemical screening of E. divinorum yielded various compounds, including anthraquinone glycosides, coumarine glycosides, saponin glycosides, flavonoids, proteins, and tannins, which were abundant. Sugars were moderately present, while alkaloids, cardiac glycosides, and steroids were absent, as shown in Table 7.

Table 1: Organoleptic Properties of *E. divinorum* Leaf

Organoleptic property	Leaf characteristics
Color	Light green
Lamina size	Length: 71.09 - 93.89 mm; Width: 6.05 - 17.59 mm
Odour	Non-distinct
Taste	Bitter and mucilaginous

Table 2: Macroscopic Properties of *E. divinorum* Leaf

Organoleptic property	Leaf characteristics
Phyllotaxy	Opposite with some alternate
Leaf type	Simple leaf
Leaf apex	Obtuse
Leaf base	Cuneate
Margin	Entire
Surface texture	Smooth
Venation	Reticulate
Midrib	Provident on the lower surface

Table 3: Quantitative leaf microscopy of *E. divinorum*

No of stomata (S)/ sq. mm	No of epidermal cells (E+S)/ sq. mm	Stomatal Index = 100S/(E+S)
3	33	9.1
4	34	11.8
4	38	10.5
Average = 3.75±0.67	Average = 35.75±2.16	Average =10.47±1.11

Table 4: Loss on drying (moisture content) of *E. divinorum* root

Weight of sample (S) ($W_2 - W_1$) g	Weight of moisture ($W_2 - W_3$) g	% moisture = ($W_2 - W_3$)/ ($W_2 - W_1$)x100
S_1= 100	42.5	42.5
S_2= 100	44.7	44.7
S_3= 100	47.3	47.3
Average = 100	Average = 44.8±1.962	Average =44.8±1.962

Table 5: Extractive values of *E. divinorum* root

Experiment	Replicates	Water-soluble extractive value (%)	Alcohol-soluble extractive value (%)
Cold maceration	1	7.2	1.2
	2	7.5	1.1
	3	7.1	1.1
	Average	7.27±0.17	1.13±0.05
Hot maceration	1	14.8	11.8
	2	15.1	10.9
	3	14.4	11.6
	Average	14.77±0.28	11.43±0.39

Table 6: Total ash content of *E. divinorum* root

Initial mass of sample (g)	Final mass (ash) (g)	Total ash content (%)
2.0	0.061	3.05
2.0	0.058	2.90
2.0	0.063	3.15
Average	0.06± 0.002	3.03± 0.103

Table 7: Preliminary Phytochemical Screening *of E. divinorum*

Phytochemical class	Specific test	Result
Alkaloids	Dragendoffr's test	-
Anthraquinone glycosides	Borntrager's test	+++
Cardiac glycosides	Baljet's test	-
Coumarine glycosides	Feric chloride test	+++
Saponin glycosides	Foam test	+++
Flavonoids	Alkaline test (general test)	+++

Proteins	Biurets test	+++
Sugars	Barfoeds test (monosaccharides)	++
Steroids	Salkwolski test	-
Tannins	Ferric chloride test	+++

DISCUSSION

The macroscopic examination of *E. divinorum* revealed that the leaf arrangement (phyllotaxy) is predominantly opposite, occasionally exhibiting a whorl or verticillate pattern. The leaves were observed to be simple, dorsiventral, and light green in color. Other macroscopic features are consistent with those reported for *Diospyros* and *Euclea* in the literature [1].

E. divinorum leaves were measured to have a lamina length ranging from length: 71.09 - 93.89 mm and width: 6.05 - 17.59 mm. This suggests smaller leaf dimensions compared to *E. divinorum*, documented to have lengths between 35 to 90 mm and widths of 10 to 25 mm [28].

Microscopic analysis of the lower epidermis leaf revealed anticlinal walls varying from straight to curved or undulating. Each stoma typically contains four to six guard cells, resembling the surrounding epidermal cells, a pattern known as anomocytic state or ranunculaceous type. Quantitative leaf microscopy indicated an average stomatal density of 3.75±0.67, epidermal cell density of 35.75±2.16, and a stomatal index of 10.47±1.11. There is paucity of published literature on the leaf constants for *E. divinorum* [1].

Total ash value serves as a critical parameter for assessing the purity and quality of herbal drugs or plant extracts, reflecting the mineral content which influences therapeutic properties, stability, and safety. While variations are anticipated due to geography and seasonality, total ash values for a given plant species typically conform to a specific range. Deviations may indicate adulteration, contamination, or improper processing. Despite a total ash value of 3.03± 0.103% found in *E. rdivinorum* roots in this study, no literature documentation on these ash values was available.

Qualitative phytochemical screening of *E. divinorum* aqueous root extract identified the presence of glycosides (anthraquinone, coumarins, and saponins), polyphenols (flavonoids), tannins, and proteins, while cardiac glycosides, steroids, and alkaloids were absent. These findings align with previous studies [29, 30]. Similarly, phytochemical analysis of methanolic extracts of *E. schimperi* leaves revealed the presence of polyphenols (flavonoids), saponins, glycosides, tannins, and terpenoids [30]. This study also yielded negative results for steroids and alkaloids, which were detected in the n-hexane, DCM, and ethanol extracts of various parts of *E. divinorum* and *E. schimperi* leaves [2]. Methanol leaf and stem extracts of *E. undulata* and *E. crispa* also showed the presence of alkaloids [31].

The presence of anthraquinone glycosides in *E. divinorum* aqueous root extract supports its traditional use as a laxative in folkloric medicine. The ethnobotanical use of *E. divinorrum* in managing mycotic skin conditions could be attributed to the presence of quinones [33, 32].

CONCLUSION

Guaranteeing the uniform quality of herbal medicines depends on verifying the origins of the ingredients, prompting a notable increase in standardization endeavors for medicinal herbs. Despite modern techniques being accessible, pharmacognostic examinations persist as the most reliable approach for identifying medicinal plants. Therefore, researchers have directed their efforts towards examining the microscopic and macroscopic features of roots to advance this aim. The ongoing research has produced pertinent identifying traits for *E. divinorum*, offering potential benefits for enhancing quality control measures specific to this medicinal plant.

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Conflict of interest

The authors declare that they have no conflict of interest.

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ORCID ID

Kipkorir Rotich: https://orcid.org/0000-0001-8499-1347

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