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Standardization of *Aloe emodin* Compound in *Aloe sinkatana* Plant Extract Using TLC and UV-visible Spectroscopic Methods

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A B S T R A C T

Objectives: Aloe-emodin is one the of dynamic compounds and has several pharmacological activities such as anti-inflammatory, antitumor, antiviral, anticancer, antifungal antidiabetic, and antipsoriatic. It is an important ingredient in several traditional herbal formulations. This study was done for qualitative and quantitative standardization of the Aloe-Emodin compound in Aloe sinkatana plant extract.

Methods: TLC (Thin layer chromatography) and UV-visible spectroscopic methods were developed for both qualitative and quantitative standardization of aloe emodine in Aloe sinkatana plant extract.

Results: The results indicated that TLC and UV-visible spectroscopic methods are simple, accurate, sensitive, precise, and reproducible.

Conclusion: In conclusion, we can say that the TLC and UV-visible spectroscopic methods can be effectively used for standardizing aloe-emodin in various pharmaceutical dosage forms.

Keywords: *Aloe-emodin*, Aloe sinkatana plant, Standardization, Thin layer chromatography, and UV-visible spectroscopic

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Introduction

Plant-based medicines, nutraceuticals, and cosmetics have grown in popularity in recent years. In non-allopathic systems, they can be prescribed as drugs or bought over-the-counter in pharmacies and health food stores as self-medicating [1,2]. Eighty percent of people worldwide still use herbs and other traditional medicines for their fundamental medical requirements, according to estimates from the World Health Organization (WHO) [4,5].

As individuals around the world turn back to natural cures, the usage of herbal medications has greatly expanded [6]. Herbal medicine items, which include capsules, tablets, powdered materials, herbal teas, extracts, and dry or fresh plants, are used by people to improve their health [7]. An increasing number of people are utilizing herbal treatments without a prescription because they are historically believed to be safe. However, some may cause health problems, others may interfere with other medications, and some are ineffective. Standardization of herbal formulations is essential for assessing

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the quality of drugs based on the concentration of their active components [8].

The quality assessment of herbal preparation is a crucial prerequisite for manufacturers and other entities that deal with medicinal and ayurvedic goods. The public's growing use of botanicals—drugs and other products derived from plants—is motivating attempts to assess the agents' alleged health advantages and set criteria for their production and quality. It is evident that the herbal industry must adhere to strict standards and that such regulations are necessary [9].

Herbal medicine technology is the method of making pharmaceuticals from botanical resources. It includes quality control and standardization as well as the proper fusion of modern scientific techniques and traditional wisdom. Numerous drug delivery methods for herbal remedies have been reported [10–11].

Both herbal and conventional pharmaceutical medicines can differ in their qualities and composition, and a growing number of regulatory bodies are requesting that herbal formulations be standardized in response to adverse reaction reports.

A procedure known as standardization guarantees a certain quantity, purity, and therapeutic impact of substances in every dosage [12]. The herbal remedy cannot be considered scientifically legitimate if the tested medication has not been documented and validated to ensure repeatability in the product's manufacturing. The medicinal effectiveness of a herbal preparation is determined by its phytochemical constituents.

The development of trustworthy analytical methods that can precisely profile the phytochemical content, including quantitative analyses of marker/bioactive compounds and other crucial components, is one of the main issues facing scientists. In light of the aforementioned, standardization is an essential initial step in developing a consistent biological activity, a uniform chemical profile, or simply a program for quality assurance in the manufacturing and production of herbal medications [13]. Pharmaceutical companies, public health, and the assurance of reproducible quality in herbal medicine depend on the authentication of herbal drugs and the separation of adulterants from authentic medicinal herbs [14].

Bearing in mind all this, the main task of this study is standardized of the Aloe-Emodin compound in *Aloe sinkatana* plant extract. *Aloe sinkatana* grows naturally in Eastern Sudan in the Red Sea Mountains, mainly in the Sinkat area, where it is popularly used traditionally by residents of the region to treat several diseases [15].

Aloe sinkatana leaves and leaf exudates are used to cure a number of conditions, including as diabetes, fever, inflammatory colon, skin conditions, constipation, and immune system issues.

Asim *et al.* (2020), conducted an investigation into the antioxidant activity of Aloe sinkatana using the DPPH free radical scavenging assay. The findings revealed that the extracts were highly effective in scavenging free radicals, supporting the idea that Aloe sinkatana could be a valuable source of natural antioxidants or nutraceuticals, with potential applications in alleviating oxidative stress and promoting health benefits [16].

Antibacterial and antifungal activity studies of the Aloe sinkatana plant were conducted by Ali et al. (2013) [17]. The results showed that the extracts of A. sinkatana leaves exhibited antimicrobial activities greater than the commercial standard drug (positive control), and these findings agree with Azza et al. (2022) [18].

A study conducted by Kamal et al. (2013) aimed to assess the impact of *Aloe sinkatana* on blood sugar levels and lipid profiles in patients with Type 2 diabetes. The study involved 110 randomly selected Type 2 diabetic patients from those regularly visiting Eldaba Chinese Hospital. Of these, ten patients served as the control group, while the remaining 100 were assigned to experimental groups, each consisting of ten individuals. Aloe sinkatana extract was administered to one of the experimental groups over a 30-day period, with all other factors kept consistent to facilitate statistical analysis. At the conclusion of the study, blood samples were taken, and various biochemical parameters were measured. The findings of the study suggest that Aloe sinkatana has anti-diabetic and hypolipidemic effects in Type 2 diabetic patients. It significantly reduced fasting blood glucose levels ($P < 0.05$) and led to a notable decrease in triglycerides (TG), total cholesterol (TC), and very low-density lipoproteins (VLDL) ($P < 0.04$). Furthermore, there was a significant increase in high-density lipoproteins (HDL) ($P = 0.03$) [19].

Ten active compounds from Aloe sinkatana extracts were separated by Gihan *et al*. (2012), and the chemical structures of these compounds were identified. These compounds are: Compound 2, 8-dihydroxy-6-(hydroxymethyl)-1-methoxyanthracene-9,10-dione, Aloe-emodin, feralolide, 1-hydroxy-5-methoxy-3-methyl-9,10-dihydroanthracene-9,10-dione, β-sitosterol, microdontin, homoaloins A, homoaloins B, aloins A, and aloins B [20]. The Aloe sinkatana plant contains a hydroxyl anthraquinone compound called aloe-emodin, which has a several biological activities. Despite the traditional use of Aloe sinkatana and its promising medicinal properties, there have been limited studies on the standardization of its active compound, Aloe-emodin, for consistent therapeutic outcomes. This work attempts to fill this gap by examining the Aloe-

Emodin compound's qualitative and quantitative standardization in Aloe sinkatana plant extract.

Aloe-emodin (1,8-dihydroxy-3-hydroxymethyl-anthraquinone) is a naturally occurring anthraquinone derivative found in many common medicinal herbs, including Aloe sinkatana, Polygonum multiflorum Thunb, Cassia occidental is, and Rheum palmatum L., which are used as traditional medicines in many countries [21-27].

Aloe-emodin has garnered significant attention in recent years due to its exceptional antitumor activity against a variety of tumor cells, including those from the lungs, stomach, liver, melanoma, breast, and colon [28-30]. Additionally, it has been shown that aloe-emodin has a broad range of pharmacological effects, including hepatoprotective, anti-inflammatory, antibacterial, antiparasitic, neuroprotective, and antivirus properties [31-36]. This has led to its widespread usage in the treatment of a number of conditions, including growth problems, influenza virus, inflammation, sepsis, Alzheimer's disease, glaucoma, malaria, liver fibrosis, psoriasis, Type 2 diabetes, and various malignancies. But especially when taken in large quantities and over an extended period of time, aloe-emodin may potentially cause phototoxicity, hepatotoxicity, and renal toxicity. The present understanding of aloe-emodin's pharmacology, toxicology, and pharmacokinetics over the last few decades is reviewed in order to identify any therapeutic potentialities and gaps that may be filled by further study.

Materials and Methods

Materials

The standard compound

Aloe-Emodin standard compound was received as a gift from Prof. Masaki, (Kobe University, Japan)

Plant Material Collection and Authentication

The *Aloe sinkatana* plants were collected from the Sinkat area, Red Sea state, Sudan, they were authenticated by the Department of Chemistry, Medicinal and Aromatic Plants & Traditional Medicine Research Institute, National Center of Research, Sudan.

Methods

Extraction & Preparation of *Aloe sinkatana* gel

Fresh water was used to wash mature, healthy, and fresh Aloe sinkatana leaves that measured between 75 and 90 cm in length.

Cut the inside gel into pieces by scraping it off. Aloe leaves were processed using the age-old hand filleting method [37]. Sharp blades were used in this manner to cut off the lower leaf base, the tapering tip at the apex of the leaf, and the short spines along the leaf edges. The top rind was then cut off after the blade was inserted into the gel layer beneath the green rind, avoiding the vascular bundles. The firm, colorless gel was sliced into pieces after the leaves' epidermis was peeled off [37]. Then 250 gm of gel was loaded into a 1000ml flask and 500 ml solvent (Ethanol) was added. Ultrasound-assisted extraction was performed at a frequency of 35 kHz with a maximum input power of 240 W, for 60 mins, at 60°C. The solution was then filtered, and the solvent was extracted using a rotary evaporator at a lower pressure until it was totally dry [15].

Quantitative determination of yield of extract

The total yield of extract was calculated by weighing the dish empty and recording its weight; the weight of the dish and its contents after evaporation in a rotary evaporator was also recorded; and the yield of the extract was calculated using the formula given by Okigbo (2009) [38].

Yield % = weight of dried extract (g) x 100 / weight of dried plant (g)

Detection of the presence of aloe-emodin compound in *A. sinkatana* plant

Thin layer chromatography technique was used to detect the Aloe-emodin contents in *A. sinkatana* extract using the aloe-emodin standard compound

Thin-layer Chromatographic studies (TLC)

Aloe sinkatana extract was subjected to thin-layer chromatography using a one-way ascending approach on a pre-coated 20 x 10 cm plate. A pencil was used to mark the plates at a distance of approximately 1 cm from the bottom. After being individually dissolved in methanol, the plant sample and the aloe emodin reference component were evenly applied to the plate using capillary tubes and left to dry.

The plate was developed in a chromatographic tank using dichloromethane: ethylacetate (8:2 V/V) as a solvent system for 1 h at room temperature. The plate was dried and sprayed with 10% KOH solution [39].

The retention factor (Rf) was calculated using the following equation;

Rf = Distance moved by the solute /compound Distance moved by the solvent

Preparation of the spray reagents

The potassium hydroxide solution was prepared by adding 10 gm of KOH to 100 ml methanol.

Standardization of Extract Using UV-Spectrophotometer

UV-visible was recorded on Shimadzu UV-1700 Spectrophotometer in the chemistry Laboratory of the Faculty of Pharmacy, University of Khartoum.

Determination of λ max of Aloe Emodin standard compound

After allowing blank correction for methanol in the aforementioned region, the standard solution (100µg/ml) was scanned in the UVVIS range (200-800 nm) for maximum absorbance [40].

Preparation of Standard Solution of Aloe-emodin

A standard solution of 100µg/mL (stock solution) was obtained by accurately weighing 0.01g of aloe-emodin, transferring it to a 100 ml volumetric flask, and dissolving it with methanol using an ultrasonic shaker. In order to measure the absorbance of different dilutions, a UV spectrophotometer was used to make stock solutions with different concentrations (2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, 10 µg/mL, and 12 µg/mL). The absorbance was plotted against the concentration to create the calibration curve. [41]

Determination of Aloe Emodin content in *A.s* extract

Using an ultrasonic shaker, 0.5 grams of dry extracts were dissolved in 100 milliliters of 0.5% methanol. A UV spectrophotometer was used to measure the absorbance of the resulting solution at 430 nm.

Results

Yield percentage of extracts

Table 1: The yield percentage of the extract

solvent of Extract	Initial weight(g) of plant powder (gm)	Final weight(g) of plant extract (gm)	Yield (%) W / W
Ethanol	1000 gm	8gm	0.8%

The yield percentage was calculated for the ethanolic extracts of *Aloe sinkatana* gel and was found to be 0.8% (As shown in Table no.1)

Results of detection of the presence of aloe-emodin compound in *A. sinkatana* plant using TLC plate

Spraying the TLC plate with a potassium hydroxide reagent shows two pink color spots, one of them for the plant sample and another one for the standard compound (aloe-emodin) with the same R_f value= 0.47.

The previous results confirm the presence of aloe-emodin in the *Aloe sinkatana* plant and agree with the result of Gihan, 2013 [39].

Results of Standardization of Extract Using UV-Spectrophotometer

Result of determination of λ max of Aloe Emodin standard compound

The maximum absorbance of the Aloe Emodin standard compound was found to be at 430nm., as shown in Fig no.1.

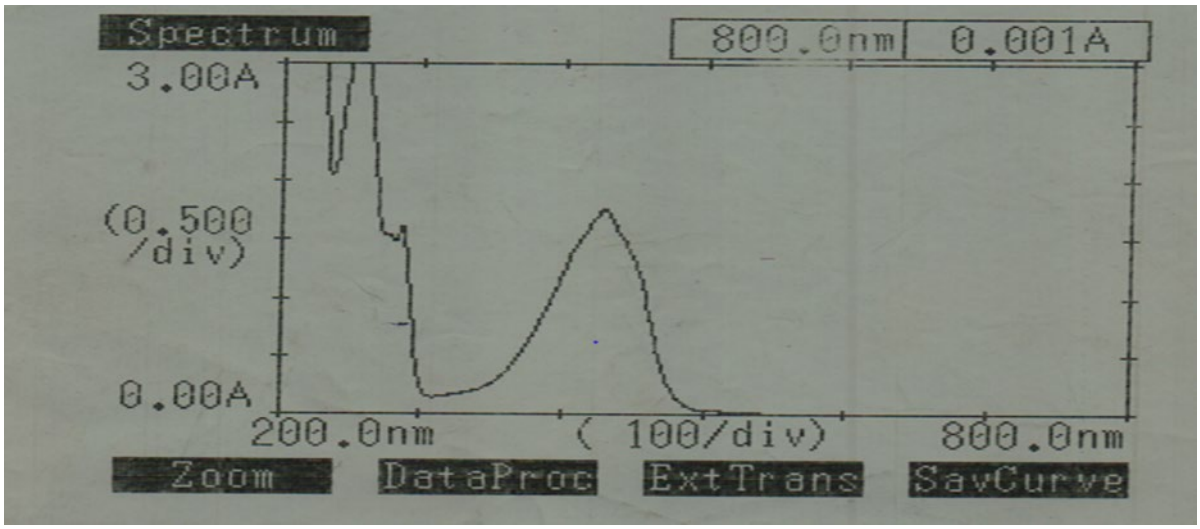

Fig No.1. The wavelength of maximum absorption Aloe Emodin standard compound in methanol (430nm)

Results of Calibration Curve

The absorbance for the concentration of Aloe Emodine standard compound is shown in Table No.2, and the calibration curve is illustrated in Figure 1.

Table 2: The absorbance data of serial concentration of Aloe Emodin

NO.	Concentration (µg /ml)	Absorbance at 430nm in Methanol
1	0	0
2	2	0.05
3	4	0.148
4	6	0.235
5	8	0.349
6	10	0.435
7	12	0.545

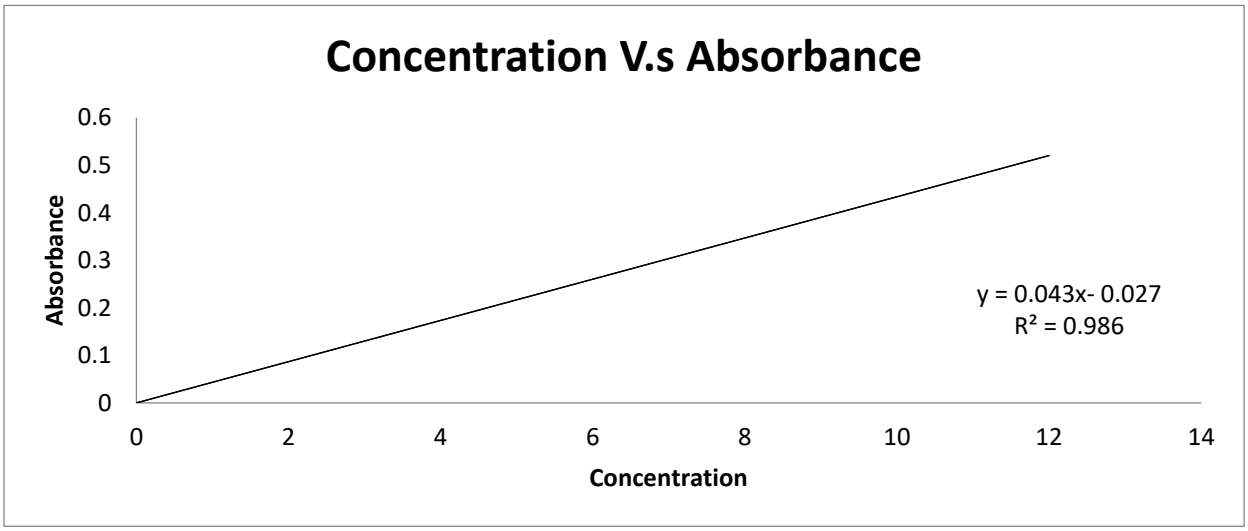

Figure 1: Calibration curve of absorption of various concentrations of Aloe Emodine standard compound at 430nm

Determination of Aloe Emodin content in *Aloe sinkatana* extract

The absorbance of *Aloe sinkatana* solution at wavelength 430 nm was 0.148

The aloe-emodin concentration in *Aloe sinkatana* extract was estimated from the following equation of the calibration curve.

The final concentration of Aloe-emodin in the plant extract was found to be equal to 4 µg /5mg = 0.08%

Y= 0.046× **X** - 0.027……Eq.1.

Y: The Absorbance of the plant extract.

X: The Concentration of the plant extract (µg /mL).

Calculation

Step 1

Y= 0.043× **X** - 0.027

0.148= 0.043 x - 0.027

X= 0.004mg /mL (4µg /mL)

Step 2

The concentration of plant extract solution was 0.5%

5mg /mL..... concentration of plant extract

That means the final concentration of Aloe-emodin in the plant extract is equal to 4 µg /5mg = 0.08%

Discussion

Plants typically contain little amounts of biologically active substances. An extraction method is one that can produce extracts with the least amount of alteration to the necessary functional characteristics of the extract [42]. Numerous investigations have documented differences in the biological activity of extracts made using various extraction methods. As a result, the appropriate extraction technique and solvent must be chosen based on the desired qualities, matrix analyte interaction, analyte chemical properties, sample matrix properties, and efficiency [43,44]

The technique known as ultrasound assisted solvent extraction employs solvents and high-frequency, high-intensity sound waves to extract specific chemicals from a variety of matrices. The propagation and interaction of sound waves as they break down plant cell walls alter the physical and chemical properties of the materials exposed to ultrasound. This facilitates the release of extractable compounds and improves the mass transport of solvent from the continuous phase into plant cells.

In the instance of microwave assisted solvent extraction, specific chemicals are extracted from plant matrices using both solvents and microwave energy.

In this study the yield percent of aloe sinkatana extract was 0.8%

Aloe emodin's UV spectra were measured between 200 and 800 nm when compared to a blank solution of methanol. Figure 1 displays a band of absorption with λ max at 430 nm in the standard solution. The UV spectra make it clear that the greatest wavelength at which aloe emodin could absorb UV light was 430 nm.

The linear regression equation with a regression coefficient is guided by the calibration curve for emodin that was derived from different concentrations. Methanol concentrations between 2 and 12 µg/ml (r2 = 0.986) follow the standard deviation (SD=0.001) of Beer's law. The higher precision of the suggested approach is indicated by the lower standard error values (0.07).

The aloe-emodin content in Aloe sinkatana extract was found to be 0.08%, and it has been identified as one of the active constituents of Aloe sinkatana and used as a marker compound.

Conclusion

The study findings demonstrated that UV-VIS spectroscopy exhibits strong accuracy, cost-effectiveness, and efficiency, making it a valuable tool for aloe-emodin estimation in various dosage forms. However, when compared to methods such as HPLC and LC-MS, UV-VIS may face limitations in sensitivity and selectivity, especially when dealing with complex mixtures or trace-level analytes. While HPLC provides superior resolution and quantitative precision, and LC-MS offers unparalleled specificity and structural elucidation, UV-VIS remains advantageous due to its simplicity, lower operational costs, and speed. Future research could focus on integrating UV-VIS with complementary techniques or enhancing its capabilities through advanced chemometric approaches to address its current limitations.

Statements and Declarations

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Competing interests

The authors declare there is no Competing interests

Authors' contributions

Investigation, data collection Writing original draft, writing review, & editing: Azza Dawoud; Editing and data analysis: Mohammed abdalbagi

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