Proximate Analysis, Phytochemical Screening, and Structural Analysis of Aloin Isolated from the Gel of *Aloe elegans* Todaro

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ABSTRACT

Plants contain several bioactive compounds distributed in all their organs, such as leaves, flowers, bark, seeds, and roots, among others. The gel found in *Aloe* species primarily consists of water, which makes up approximately 98% of its composition. The gel also contains several classes of both primary and secondary metabolites, including polysaccharides, glycoproteins, phenolic anthraquinones, flavonoids, enzymes, minerals, amino acids, sterols, saponins, and vitamins. The distribution and concentration of these metabolites vary among the different *Aloe* species. *Aloe elegans* Todaro is one of the *Aloe* species that are commonly found in Ethiopia. The plant grows in areas with rocky or limestone landscapes, mainly in regions covered by evergreen bushland or wooded grasslands. This preliminary study aimed to investigate the proximate composition and perform phytochemical screening on the gels of this rarely distributed plant, along with isolating an anthraquinone molecule for the first time. The results showed that the gel had a moisture content of 98.12 ± 0.06%, ash content of 0.16 ± 0.02%, fat content of 0.03 ± 0.02%, protein content of 0.11 ± 0.01%, carbohydrate content of 0.60 ± 0.03%, and energy content of 13.18 kcal. Furthermore, a phytochemical screening analysis revealed the presence of tannins, alkaloids, anthraquinones, and terpenoids, indicating the distribution of several classes of secondary metabolites. Aloin, an anthraquinone compound, was isolated from the crude gel extract using column chromatography assisted by thin-layer chromatography analysis. Structural identification of this compound was achieved by analyzing ^{1}H-NMR, ^{13}C-NMR, DEPT, and IR data, as well as by comparing the results with literature and chemical profiling databases. To summarize, the results of this study highlight the phytochemical richness and nutritional potential of *A. elegans*, underscoring its value as a source of pharmacologically relevant compounds and laying a strong foundation for further research. Therefore, to fully understand the biomedical and nutraceutical potential of the plant, future investigations should focus on comprehensive metabolite profiling, bioactivity-guided fractionation, and evaluation of its therapeutic properties.

Keywords: *Aloe elegans* Todaro, Aloe gel, Aloin, Phytochemical screening, Proximate analysis.

1. INTRODUCTION

Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization (WHO) estimates that up to 80% of the world's population still relies on traditional medicines, mainly those derived from medicinal plants (Ba et al., 2015).

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This is because of the different phytochemicals they possess in their specific parts, such as leaves, flowers, bark, seeds, fruits, and roots, among others. The therapeutic effects of plants usually arise from the combined role of these secondary metabolites, which establish plants as the basis for modern medical knowledge (Gomes et al., 2018; Joseph and Raj, 2010). Specifically, screening natural products has proven to be a highly effective strategy in the field of drug discovery, as it allows researchers to identify novel lead compounds that could be used directly or as a basis for drug development (Ahmed and Hussain, 2013). Overall, there has been an increasing focus on the investigation, advancement, and application of plant-derived natural products as therapeutic agents (Joseph et al., 2013).

The genus *Aloe,* belonging to the Aloaceae family, consists of succulent species known for their medicinal values and their use in different parts of the world. Most *Aloe* species can grow to a height of 80−100 cm and take about 4−6 years to reach full maturity. If the growing conditions are favorable, *Aloe* species can survive for up to 50 years (Sahu et al., 2013). The leaves of *Aloe* species are heterogeneous and usually divided into three major parts: the outer green epidermis, the outer pulp region below the epidermis, and the inner leaf pulp, consisting of *Aloe* gel containing parenchyma cells. Studies have shown that the different leaf portions of *Aloe* species possess distinct classes of bioactive compounds, which exhibit wide-ranging biological properties (Coke, 2015). The gel found in *Aloe* species consists of approximately 98% water. Despite this, the gel is also rich in a wide range of primary and secondary metabolites like polysaccharides, glycoproteins, phenolic anthraquinones, flavonoids, enzymes, minerals, essential and non-essential amino acids, sterols, saponins, and vitamins (Gebremariam et al., 2023; Ray and Aswatha, 2013; Gu et al., 2012; Sampedro et al., 2004). Some *Aloe* species have also been documented for their use in traditional medicine to treat various ailments, which may be attributed to the presence of bioactive metabolites (Gebremedhin et al., 2024; Salehi et al., 2018).

The distribution and contents of both primary and secondary metabolites differ among the various *Aloe* species. For instance, the gel of *A. vera*, one of the most extensively studied *Aloe* species, was found to exhibit strong anti-inflammatory effects due to the presence of anthraquinones and chromones. Moreover, intake of oral *A. vera* gel has been reported to be effective in decreasing the severity of pain and wound size, while the combination of *A. vera* gel with *Lactobacillus rhamnosus* has been shown to reduce the risk of cardiovascular diseases (Radha and Laxmipriya, 2015; Kang et al., 2014; Kumar et al., 2013). Studies have also shown the potential of *A. vera* gel to enhance the absorption of drugs with low bioavailability (Carien et al., 2013). Apart from these, several proximate analyses have demonstrated the role of *Aloe*

species in the development of functional and nutraceutical food products and as ingredients in beverage industries. The gels of some *Aloe* species, such as *A. maculata* and *A. zebrina*, have also been used as sources of food during famine and emergency cases (Yadeta, 2022).

Figure 1. Aloe elegans Todaro (Photo credit: Researchers, February 18, 2018).

Ethiopia is one of the countries where various *Aloe* species are widely found. It is estimated that a total of 46 *Aloe* species are found in the flora of Ethiopia and are distributed in all floristic regions of the country. *Aloe elegans* Todaro (Fig 1), known by the name "*era*" (Tigrinya), is one of the *Aloe* species that are commonly found in Ethiopia. The plant grows in areas with rocky or limestone landscapes, mainly in regions such as the Tigray, Wollo, Gojjam, and Shewa floristic regions. These areas are characterized by evergreen bushland or wooded grasslands at elevations ranging between 1500 m and 2400 m. The specific epithet 'elegans' refers to the overall elegant nature of the plant. This refers, particularly, to the attractive and conspicuous bright colors of the flowers. Despite the plant's wide distribution, its phytochemical composition and proximate contents have not been investigated. In general, much of the research on Ethiopian *Aloe* species focuses mainly on the botanical descriptions (Demissew and Nordal, 2010; Dessalegn, 2006), *in vitro* micropropagation (Abraha et al., 2014; Berhe et al., 2023; Hailu et al., 2020; Niguse et al., 2020; Welehaweria and Sbhatu, 2023), and biocidal properties (Asamenew et al., 2011; Brhane et al., 2018; Minale et al., 2014; Tewabe and Assefa, 2018). Only a few studies have investigated the chemical constituents of the different tissues of *Aloe* species (Mudin et al., 2018; Teka et al., 2016; Girma et al., 2015; Megeressa et al., 2015). Studies that explore the various aspects of less-studied *Aloe* species are essential to enhance their potential applications in the food, pharmaceutical, and cosmetics industries (Gebremedhin et al., 2024; Sbhatu et al., 2020a, 2020b). Therefore, this preliminary study aims to isolate and characterize Aloin, an anthraquinone molecule. The results of this research will lay a foundation for initiating further metabolomics investigations and exploring the biological activities of the plant.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

All the chemicals and reagents used in this study were analytical grade. Hydrochloric acid and sodium hydroxide were purchased from Hi-Media (Hi-Media, Mumbai, India), while chloroform and methanol were obtained from Carlo Erba (Carlo Erba, Normandie, France). Sulfuric acid was obtained from Unichem (Roha, Maharashtra, India). The remaining chemicals and reagents, including boric acid, sodium carbonate, potassium sulfate, copper sulfate, potassium iodide, ferric chloride, petroleum ether, ethyl acetate, ammonia, iodine, *n*-hexane, D-glucose, ammonium sulfate, and silica gel, were obtained from Loba Chemie (Loba Chemie, Mumbai, India).

2.2. Instruments and Apparatus

The major instruments and the apparatus used during sample preparation, extraction, and purification in the study include thin-layer chromatography (TLC) plates (Merck, Darmstadt, Germany), a laboratory-made preparative thin-layer chromatography (PTLC), column chromatography (CC) (Merck, Darmstadt, Germany), a UV lamp (Shimadzu, Model 1800, Kyoto, Japan), and a rotary evaporator (Buchi Rotavapour, Flawil, Switzerland). The NMR analysis was conducted with a 400 MHz Bruker instrument, while the IR spectrum was recorded using a PerkinElmer FTIR instrument (PerkinElmer, Waltham, MA, USA).

2.3. Plant Material Collection and Sample Preparation

A. elegans leaves were collected from the vicinity of Mesebo Mountain, located in Mekelle City (Tigray Region, Ethiopia). Plant collection was conducted in February 2018, and the plant identification was conducted at the Department of Biology, Addis Ababa University, Ethiopia. Permission for the collection of plant materials by Ethiopian researchers for research is granted by Article 15, Clause 1, of the Access to Genetic Resources and Community Knowledge, and Community Rights Proclamation of Ethiopia (No. 482/2006). During sample collection, the leaves were selected from those with fully expanded, healthy, and mature leaves. Specifically, leaf selection was achieved by counting the leaves from top (7th leaf) to bottom (9th leaf) and then cutting with a knife. The collected leaves were packed in plastic bags and transported to the Organic Chemistry Laboratory (Mekelle University, Ethiopia). The leaves were subsequently washed with distilled water to remove any dirt. Then, the gel was separated from the other leaf sections by cutting the edges and skin with a knife. The separated gel was cut into small pieces and kept in a shaded area to facilitate the drying process (Kang et al., 2014; Kumar et al., 2013). The dried gel was subsequently powdered using an electric grinder and stored in a refrigerator pending extraction and analysis.

2.4. Proximate Analysis

The proximate parameters, including moisture content, ash content, total fat content, and crude protein content, were determined using the official methods of the Association of Official Analytical Chemists (AOAC), as described below (AOAC, 2000). Furthermore, crude carbohydrate content was determined using the phenol-sulfuric acid method as reported in a previous study (Sadasiyam and Manickam, 2008). During each of these assays, measurements were made in triplicate (n = 3). The data obtained was then computed in Excel and the results were expressed as the mean along with the corresponding standard deviation (mean ± SD).

2.4.1. Determination of Moisture Content

Initially, 2.0 g of powdered gel was placed in a porcelain crucible. The crucible with the sample was heated in an oven for 6 h at 105 °C. Then, the crucible was cooled in a desiccator and weighed again until a constant weight was measured. The procedure was repeated three times, and the moisture content (in percent) was calculated using the formula in Equation 1.

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 10 \quad (1)$$

Where, W_1= weight of the sample before drying; W_2 = weight of the sample after drying.

2.4.2. Determination of Ash Content

During ash content analysis, 2.0 g of powdered gel sample was placed in a porcelain crucible, followed by placement in a furnace for 4 h at about 600 °C. Then, the sample containing the crucible was cooled in a desiccator and weighed. To ensure completion of the process, the crucible was again heated in the furnace for 30 min, cooled, and weighed. This activity was repeated until the same value of weight was obtained, and the ash was almost white. Then, the ash content (in percent) was determined using the formula indicated in Equation 2.

$$\text{Ash (\%)} = \frac{W_2}{W_1} \times 100 \quad (2)$$

Where, W_1 is the weight of the sample before ash; W_2 is the weight after the formation of ash.

2.4.3. Determination of Fat Content

For total fat content, 10 g of powdered gel sample was mixed with 200 mL of petroleum ether and subjected to reflux in a Soxhlet extractor for 5 h at 40 °C. After the extraction, the flask was removed from the apparatus and kept in a water bath, followed by placement in an oven. Then, the flask was cooled and weighed, and the crude fat content (in percent) was calculated using the following formula (Equation 3).

$$\text{Fat (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (3)$$

Where, W_1 is the weight of the sample before extraction; W_2 is the weight after extraction.

2.4.4. Determination of Total Protein Content

Protein content was determined using the Kjeldahl method. Specifically, 0.1 g of powdered gel sample was digested by heating it until the sample became clearer with 15 mL of concentrated H_2SO_4 in the presence of a digestion mixture including K_2SO_4 and $CuSO_4$ in an 8:1 ratio. Then, 10 mL of 40% NaOH was added to the mixture, and the released ammonia was collected in 4% H_3BO_3 solution and titrated against 0.1 N HCl. Finally, the total protein was calculated by multiplying the amount of nitrogen (%) released with the appropriate factor (6.25) using the formula shown in Equation 4 (Sadasiyam and Manickam, 2008).

$$\%N = \frac{(1.4 \times (\text{mL HCl} - \text{mL NaOH} \times \text{Conc.of HCl})}{\text{Weight of Sample}} \times 100 \quad (4)$$

2.4.5. Determination of Total Carbohydrate Content

During the carbohydrate analysis, 0.1 g of powdered gel sample was hydrolyzed with 5 mL of 2.5 N HCl. The acid-digested sample was cooled to room temperature and neutralized by adding Na_2CO_3. The final volume was made up to 100 mL with distilled water and centrifuged at 5000 rpm for 15 min. The supernatant was then collected, and 0.5 mL and 1 mL aliquots were taken for analysis of total carbohydrates. D-glucose at the concentration of 100 µg mL^{-1} was used to prepare working concentrations, and a standard curve was plotted. The absorbance was read at 490 nm, and the carbohydrate content was calculated using the following formula (Equation 5).

$$\text{Amount of Carbohydrate in 100 mg Sample} = \frac{\text{mg of Glucose}}{\text{Volume of Sample}} \times 100 \quad (5)$$

2.4.6. Energy Determination

The total energy value (in kcal) was calculated from the calorie values of the proximate parameters assessed, including fat, protein, and carbohydrate contents, using the formula shown in Equation 6 (Asuk et al., 2015).

$$\text{Energy Value} = (37 \times \text{Fat}) + (17 \times \text{Carbohydrate}) + (17 \times \text{Protein}) \quad (6)$$

2.5. Extract Preparation for Phytochemical Screening and Compound Isolation

Crude extract preparation for phytochemical screening and compound isolation was conducted according to a previously reported protocol with some modifications (Tala et al., 2018). Briefly, 350.0 g of powdered gel sample was mixed with 700.0 mL of methanol and subjected to extraction through maceration for 72 h (Fig 2). The mixture was then filtered, and the extract was concentrated using a rotary evaporator. The prepared crude methanol extract (35 g) was used for phytochemical screening and compound isolation as briefed below.

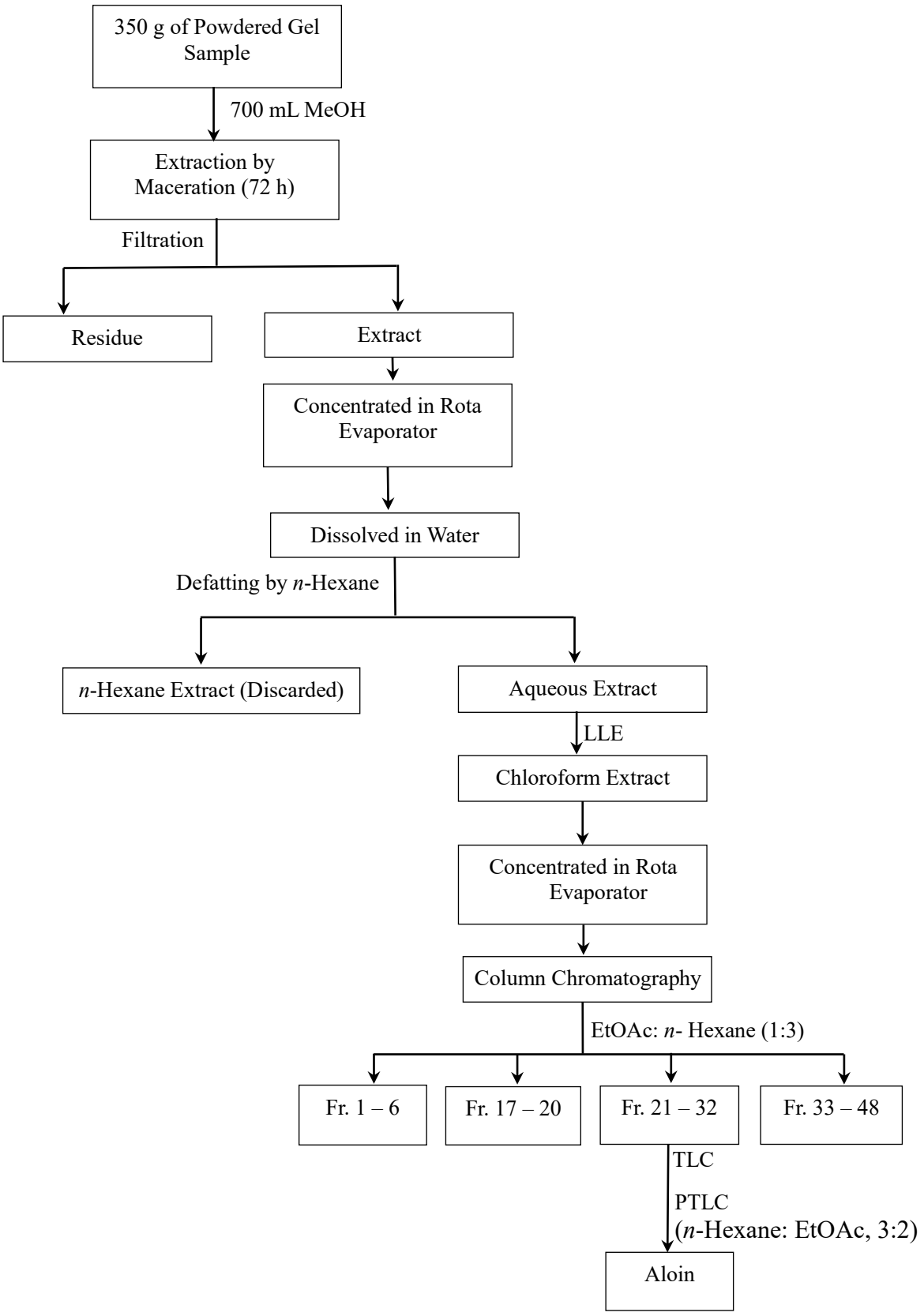

Figure 2. Scheme of extraction and isolation of Aloin from the gels of A. elegans (EtOAc − ethyl acetate, LLE – Liquid-liquid extraction, MeOH – methanol, PTLC − Preparative thin layer chromatography, TLC − Thin layer chromatography).

2.6. Phytochemical Screening

In order to determine the presence and/or absence of major phytochemicals, including tannins, saponins, alkaloids, anthraquinones, flavonoids, and terpenoids, in the gels of *A. elegans,* phytochemical screening was conducted. During each assay, standard methods were followed, and, in most cases, changes in color or precipitate formation were used as indicators of positive results.

For the tannin test, 0.15 g of methanol extract was mixed with water and heated in a water bath. The mixture was filtered, and 0.15 g solid $FeCl_3$ was added to the filtrate. The formation of a dark green color was inspected for the presence of tannin (Tala et al., 2018). During the saponin test, 0.2 g of the methanol extract was shaken with 10 mL of distilled water in a test tube and heated in a water bath for 15 min. The formation of froth was used to confirm the presence of saponins (Al Faham et al., 2013). The presence of alkaloids was tested using Wagner's reagent. Specifically, 0.15 g of methanol extract was stirred with 2 mL of diluted HCl and filtered. Then, six drops of Wagner's reagent were added, and the formation of a reddish-brown color was inspected to confirm the alkaloid's presence (Banso and Adeyemo, 2006). The presence of anthraquinones was tested using the ammonia assay.

Initially, 0.5 g of methanol extract was boiled with concentrated HCl for 5 min in a water bath and filtered, followed by the addition of $CHCl_3$. Three drops of ammonia were then added to the mixture and heated in a water bath. The formation of a rose-pink color was checked to authenticate the presence of anthraquinones (Khanam et al., 2015). During the flavonoid test, two drops of sodium hydroxide were added to a small amount of methanol extracts to give an intense yellow color. Then, dilute HCl was added dropwise, and the disappearance of the yellow color was checked to confirm the presence of flavonoids (Iqbal et al., 2015). A test for terpenoids was also achieved using the chloroform-sulfuric acid assay. Initially, 0.1 g of methanol extract was shaken with 2 mL of $CHCl_3$ in a test tube, followed by the addition of 2 mL of concentrated H_2SO_4 along the side of the test tube. Then, a reddish-brown coloration at the interface was inspected to confirm the presence of terpenoids (Alabri et al., 2014).

2.7. Compound Isolation and Structural Analysis

During compound isolation, column chromatography, liquid−liquid extraction, and TLC analysis were used. The overall process is illustrated in Figure 3. To provide a brief overview, the concentrated methanol extract (30 g) was initially dissolved in 150 mL of distilled water and subjected to sequential liquid-liquid extraction with *n*-hexane and chloroform. Briefly, the aqueous solution of the sample was poured into a separatory funnel and defatted three times using 60 mL of *n*-hexane. Following that, the remaining aqueous solution was extracted using

45 mL of chloroform. The chloroform extract was tested by TLC with a solvent system composed of ethyl acetate and *n*-hexane in a 1:3 ratio.

Following the TLC profile, the chloroform extract was dried using a rotary evaporator and applied to a silica gel-packed column chromatography (mesh size of 100–200). The same solvent system used in the TLC development was used to collect a total of 48 fractions of 40 mL each. The collected fractions were grouped into four subgroups based on their R_f values. Accordingly, fractions 1–16 were in subgroup 1, fractions 17–20 were in subgroup 2, fractions 21–32 were in subgroup 3, and fractions 33–48 were in subgroup 4. The extract in each of the subgroups was dried and inspected by using TLC with various solvent systems, including ethyl acetate, chloroform, dichloromethane, *n*-hexane, and their combinations, and visualized by UV lamp at 254 nm and 366 nm for the detection of anthraquinones (Megeressa et al., 2015). Multiple spots were observed from visualization for all fractions. Based on the results, compounds detected in fractions 21–32 (subgroup 3) were purified by PTLC using *n*-hexane:ethyl acetate (3:2) as a solvent system. Five clear spots were observed in the fractions 21–32. These fractions were selected due to the fact that spots in this subgroup were visible separately and clearly. Among the five spots, the spot that was yellow and whose R_f related to Aloin was selected. The purity was again confirmed with various solvents using TLC, which resulted in a single spot. Then, the pure sample that was isolated from fractions in subgroup 3 was kept for structural analysis using NMR and IR instruments. NMR analysis, including ^{1}H, ^{13}C, and DEPT 135, was conducted using DMSO as a solvent, while IR was recorded in the range of 4000–400 cm^{-1} using KBr pellets.

3. RESULTS AND DISCUSSION

3.1. Proximate Analysis

The proximate analysis was carried out for the determination of moisture, ash, total fat, total protein, and total carbohydrate contents, as described before. Moisture content was found to be the highest (98.12%) when compared to other parameters (Table 1). This could be ascribed to the fact that the gel of several *Aloe* species contains a high amount of water, as reported by other studies (Takaidza et al., 2018). Likewise, the ash content was found to account for 0.16%. Among the nutritional components, carbohydrate content dominated, followed by protein content and fat content, contributing to a total energy of 13.18 kcal.

In support of our findings, previous studies highlighted that aloe gel is rich in sugars, which could contribute to its high level of carbohydrates (Ibegbulem and Chikezie, 2013). A review by Salehi et al. (2018) highlighted the variation in proximate composition and other

metabolites reported in previous studies among different *Aloe* species, which may be attributed to differences in the plant tissue analyzed, genotype, and environmental factors. For example, the gel of *A. barbadensis* (*A. vera*) was reported to contain 0.01% fat, 0.12% protein, and 0.66% carbohydrates, findings that were comparable to our results (Lucini et al., 2015). In a separate study, it was found that *A. schweinfurthii* gel contained 3.18% protein, 0.67% fat, and 0.93% carbohydrates (Karina et al., 2013). Despite their low nutritional content, some *Aloe* species are widely utilized in the food industry owing to their amino acid compositions and carbohydrate levels (Oyesomi et al., 2021; Salehi et al., 2018).

Therefore, *A. elegans* could also have the potential to be used as additives in the development of food products. Moreover, comprehensive studies on fatty acid and amino acid compositions would provide valuable insights and are, therefore, strongly recommended.

Table 1. Proximate analysis results from the gel of A. elegans Todaro.

SN	***Proximate parameters***	***Results (%)***
1	Moisture	98.12 ±0.06
2	Ash	0.16 ±0.02
3	Fat	0.03± 0.02
4	Protein	0.11 ± 0.01
5	Carbohydrate	0.60 ± 0.03
6	Energy (kcal)	13.18

3.2. Phytochemical Screening

Phytochemical screening analysis provides first-hand information regarding the range of metabolites found in plants that promote health and prevent diseases (Singha et al., 2021). In this study, the presence and/or absence of some of the major plant secondary metabolites, including tannins, saponins, anthraquinones, alkaloids, flavonoids, and terpenoids, were assessed. Table 2 summarizes the phytochemical screening results. Accordingly, the gel of *A. elegans* was verified to contain tannins, alkaloids, anthraquinones, and terpenoids. Nevertheless, saponins and flavonoids were not detected in the gel sample. Several previous studies have also shown different patterns of secondary metabolite distribution among different species of *Aloe* plant (Mudin et al., 2018; Ibegbulem and Chikezie, 2013; Tiwari et al., 2011). Such discrepancies could arise due to several factors, including the genetic variance among *Aloe* species, the difference in growing conditions or environment, and the variations in sample handling techniques during analysis, among others (Singha et al., 2021). Furthermore, Mudin et al. (2018) have detected saponins, tannins, anthraquinones, and terpenes in the roots of *A. elegans*, while flavonoids and alkaloids were not detected. These findings, along with our results, demonstrate the variations of metabolite distributions across *A. elegans* tissues.

Generally, the detection of various metabolites like tannins, alkaloids, anthraquinones, and terpenoids suggests that it is crucial to prioritize further studies on these particular components present in the gels of *A. elegans*.

Table 2. Phytochemical screening results of the methanol extract of the gel of A. elegans Todaro.

SN	***Secondary metabolite***	***Tests***	***Results***
1	Tannin	Ferric chloride test	+
2	Saponin	Froth test	−
3	Alkaloid	Wagner's test	+
4	Anthraquinones	Borntrager's test	+
5	Flavonoids	Alkaline test	−
6	Terpenoids	Salkowski test	+

Note: (+): detected, (−): not detected.

3.3. Structural Determination of Aloin

Aloin is one of the most widely distributed anthraquinones in different *Aloe* species. Recent reviews by Xiao et al. (2022) and Zimbone et al. (2024) have detailed the wide-ranging health benefits and bioactivities of Aloin, including its anticancer, anti-inflammatory, antioxidant, and antimicrobial properties, among others. In this study, the isolation of Aloin from the methanol extract of *A. elegans* gel was achieved using a series of chromatographic procedures as highlighted before. *Aloin* was previously isolated and/or reported from different *Aloe* species. To the best of our knowledge, this study is the first to isolate the molecule from *A. elegans*, signifying the health benefits of the plant (Zimbone et al., 2024; Xiao et al., 2022).

The structure of Aloin was determined using data obtained from IR, ^{1}H-NMR, ^{13}C-NMR, and DEPT 135 analysis. Corresponding spectra are included as supplementary materials. The IR spectrum showed us major peaks, which indicated the presence of typical functional groups commonly found in anthraquinones. Specifically, the absorption at 3403 cm^{-1} indicated the presence of the −OH group. Likewise, the absorption band at 2920 cm^{-1} was a characteristic feature of C−H_{str}. Furthermore, C−H bending was observed at 752 cm^{-1}, while the presence of C=O_{str} and C=C_{str} was observed at 1601 cm^{-1} and 1458 cm^{-1}, respectively (Brhane et al., 2018). The characteristic C−O_{ben} was also confirmed by the absorption bands observed at 1287 cm^{-1} and 1170 cm^{-1}. In support of our observations, previous studies have also reported comparable IR absorption data for Aloin (Brhane et al., 2018).

NMR analysis was also conducted to further support the structural identification of Aloin (Table 3). The ^{1}H-NMR data exhibited characteristic chemical shift values that were consistent with the IR data. In particular, chemical shift values ranging from δ 1.22 to 3.93 were due to –CH and CH_2 groups of the glycosyl substituent (Oliveira et al., 2017). The singlet

peaks observed at chemical shift values of δ 6.82, 7.10, and 4.97 were due to methine (C−H) protons at positions C_{-2}, C_{-4}, and C_{-10}, respectively. Likewise, the chemical shift values δ 8.14(m), 6.80(d), and 5.47(s) were attributed to the CH protons at positions C_{-6} and C_{-7}, and CH_2 protons at position C_{-15}, respectively. The multiplicity observed at C_{-6} could be attributed to the effect of CH protons at the neighboring C_{-7} and C_{-5} positions, while the doublet at C_{-7} could be due to the influence of the only neighboring CH proton at C_{-6}.

Likewise, the chemical shift values δ 11.04 and 10.08 were attributed to the O−H protons at positions C_{-1} and C_{-8}, respectively. In general, the ^{1}H-NMR data that were observed aligned with previous research that characterized the structure of Aloin and its derivatives isolated from other *Aloe* species (Mudin et al., 2018; Tewabe and Assefa, 2018; Oliveira et al., 2017; Teka et al., 2016; Minale et al., 2014). In addition, the ^{13}C-NMR spectra showed a total of 21 carbon peaks, which are commonly linked to anthraquinone structures (Table 3).

Table 3. ^{1}H−NMR, ^{13}C−NMR, and DEPT−135 spectral data of Aloin compared to literature data.

C-Position	***^{1}H−NMR (δ, ppm)***	***^{13}C-NMR (δ, ppm)***	***DEPT−135 (δ, ppm)***	***Oliveira et al., 2017***	
				^{1}H−NMR	***^{13}C−NMR***
C_{-1}	11.04 (O−H)	162.1	No peak	11.97	162.0
C_{-2}	6.82 (s, C−H)	112.8	112.8 (CH)	6.88	113.0
C_{-3}	−	152.6	No peak	−	150.1
C_{-4}	7.10 (s, C−H)	116.7	116.7	7.04	117.7
C_{-5}	7.44 (d, C−H)	118.2	118.2 (CH)	7.06	118.6
C_{-6}	8.14 (m, C−H)	136.3	136.3 (CH)	7.5	135.7
C_{-7}	6.80 (d, C−H)	116.2	116.2 (CH)	6.87	115.4
C_{-8}	10.08 (O−H)	161.3	No peak	11.96	161.5
C_{-9}	−	194.5	No peak	−	1941
C_{-10}	4.97 (d, C−H)	44.3	44.3	4.58	44.5
C_{-11}	−	117.5	No peak	−	117.8
C_{-12}	−	142.6	No peak	−	141.8
C_{-13}	−	146.3	No peak	−	145.2
C_{-14}	−	116.3	No peak	−	117.2
C_{-15}	5.47 (s, CH_2)	62.5	−62.5 (CH_2)	4.67	63.12
$C_{-1'}$	4.57 (d, C−H)	85.6	85.6 (CH)	3.41	85.2
$C_{-2'}$	3.93 (s, C−H)	71.8	71.8 (CH)	3.02	70.6
$C_{-3'}$	3.99 (1H, s, C−H)	78.5	78.5 (CH)	3.28	78.6
$C_{-4'}$	1.22 (1H, O−H)	70.7	70.7 (CH)	2.91	70.5
$C_{-5'}$	2.51 (1H, C−H)	81.3	81.3 (CH)	2.93	80.3
$C_{-6'}$	3.92 (2H, CH_2)	61.8	−61.8 (CH_2)	3.36	61.8

Specifically, the presence of a carbonyl carbon (C=O) was easily verified due to the chemical shift value at σ 194.5 (C_{-9}). The high chemical shift value for this carbon could be attributed to the shielding effect of the OH groups located at the neighboring carbons, C_1 and C_{-8}. Similarly, chemical shift values related to the methine carbons were observed at δ 112.8

(C_{-2}), 116.7 (C_{-4}), 118.2 (C_{-5}), 136.3 (C_{-6}), and 116.2 (C_{-7}). On the other hand, the chemical shift values observed in the range of δ 61.8−85.6 were attributed to the glycosyl carbons (Teka et al., 2016). The nature and degree of substituents of the Aloin carbons were further confirmed by the DEPT spectra, which indicated the presence of eight quaternary carbons (C), two secondary carbons (CH_2), and six tertiary carbons (CH) (Table 3). Overall, the data obtained from IR, ^{1}H-NMR, ^{13}C-NMR, and DEPT analyses, as well as from previous literature, confirmed the isolation of Aloin from the gels of *A. elegans* for the first time (Fig 3).

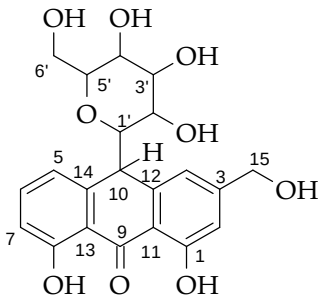

Figure 3. The chemical structure of Aloin.

4. CONCLUSION

This study examined the proximate composition and phytochemical screenings of *A. elegans* Todaro, a rarely distributed species of *Aloe*. The levels of total fat, total protein, and total carbohydrates in *A. elegans* gel were relatively low. In contrast, the presence of various classes of secondary metabolites, including tannins, alkaloids, anthraquinones, and terpenoids, was confirmed. Although the macronutrient content of *A. elegans* gel is low, the abundance of these bioactive secondary metabolites signifies potential functional health benefits. Furthermore, this study successfully isolated aloin, an anthraquinone molecule known for its anticancer, anti-inflammatory, antioxidant, and antimicrobial properties, from *A. elegans* gel for the first time, reinforcing its potential for pharmacological applications. The detection and isolation of this molecule further highlight the potential of *A. elegans* for metabolomics and natural product research. Accordingly, future studies should focus on quantifying the bioactive compounds, assessing their pharmacological effects, and evaluating the safety and efficacy of *A. elegans* extracts for potential therapeutic applications.

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6. CONFLICT OF INTEREST

There is no conflict of interest.

Authors' contributions

Adamu Tizazu Yadeta: Conceived and designed the study, performed the experiments, analyzed and interpreted the data, wrote the draft manuscript

Kebede Taye Desta: Conceived and designed the study, supervised the experiments, interpreted the analyzed data, and reviewed and enhanced the manuscript.

Desta Berhe Sbhatu: Conceived and designed the study, administered the projects, acquired funding, interpreted the analyzed data, and reviewed and enhanced the manuscript.

Data availability statement

Data will be made available on request.

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