International Journal of Pharmacology

Int. J. Pharmacol. **2025**; 21(7): 44072
https://doi.org/10.31083/IJP44072

Research Article

Comparative Phytochemical Analysis and Ethnopharmacological Potential of Nine *Aloe* Species Grown in the Western Highlands of Saudi Arabia

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Academic Editor: Mehmet Ozaslan
Submitted: 20 June 2025 Revised: 29 September 2025 Accepted: 10 October 2025 Published: 11 November 2025

Abstract

Background: Medicinal plants are abundant in bioactive phytochemicals, which act as shields against harm and disease, and enhance the color, flavor, and aroma of these plants. Therefore, this study aimed to examine and compare the ethnopharmacological potential and phytochemical components of nine *Aloe* plant species cultivated in the western highlands of Saudi Arabia. **Methods**: Nine *Aloe* plant species were collected from different locations (Taif, Al-Baha, Abha, and Jazan). High-performance liquid chromatography (HPLC) was performed on the nine *Aloe* species, demonstrating the production of six flavonoid compounds with different retention times. **Results and Conclusion**: *A. parvicoma* exhibited the highest concentration of apigenin (45.36 mg/g), *A. hijazensis* presented the highest for rutin (29.46 mg/g), *A. sabaea* had the highest of kaempferol (40.12 mg/g), and *A. armatissima* presented the highest for naringin (60.14 mg/g). Additionally, HPLC was used to separate the six phenolic compounds. *A. armatissima* had the highest concentrations of ellagic acid, quercetin, and gallic acid (27.99, 39.50, and 40.12 mg/g, respectively), while *A. hijazensis* had the highest for resorcinol (35.17 mg/g), *A. fleurentiniorum* had the highest for syringic acid (7.13 mg/g), and *A. brunneodentata* had the highest for ferulic acid (28.47 mg/g). Four alkaloid compounds were identified, with the highest concentration of coniine (6.58 mg/g) recorded in *A. fleurentiniorum*, while conhydrine and conmaculatin (5.60 and 4.99 mg/g) were recorded in *A. abhaica*, and 2-methylpiperidine (3.66 mg/g) was recorded in *A. sabaea*. The nine *Aloe* species exhibited significant divergence, as indicated by long Euclidean distances. The considerable concentration of identified compounds denotes the potential use of the *Aloe* plant species for different pharmacological purposes.

Keywords: HPLC; flavonoid compounds; pharmacological potential; phenolic compounds; *Aloe*

1. Introduction

The Kingdom of Saudi Arabia is the largest country in the Arabian Peninsula, in western Asia. Thus, Saudi Arabia is mostly composed of sand desert, with Rub Al-Khali representing the largest sand desert worldwide [1]. Nonetheless, most of the *Aloe* species found in Saudi Arabia are located in the diverse ecosystems supported by the southern and western mountain ranges in the country, some of which have peaks that reach nearly 10,000 feet (3000 meters) above sea level. These regions also have more rainfall and more hospitable climates [2]. Among the most well-known succulent plants in the world, *Aloe* plants can thrive in various habitats and assume different growth forms [3].

Although some *Aloe* species are harmful, most are thought to provide therapeutic and/or aesthetic benefits [4]. For centuries, *Aloe* plants have been utilized medicinally [5] to treat ailments [6]. *Aloe* plants have been used as a traditional cure for several illnesses, including diabetes, heart disease, skin conditions, ulcers, and digestive issues; thus, the use of *Aloe* plants has transitioned into the pharmaceutical sector [1]. Additionally, *Aloe* plants are utilized in health and cosmetic items, such as sunscreen, shampoos, lotions, and disinfectants [7]. According to this perspective, bioactive compounds extracted from medicinal plants have been modified to enhance the effectiveness of these compounds in the production of semi-synthetic drugs [6]. Since *Aloe* plants store water and essential chemical components in their tissues, these plants can tolerate hot and dry weather, making *Aloe* plants a unique source of phytochemicals [8]. *Aloe* plants are rich in naturally occurring phytochemicals, including proteins, amino acids, vitamins, hormones, polyphenols, alkaloids, and organic acids [9]. Additionally, *Aloe* species produced several phenolic compounds, and many more are likely to be discovered in the future [9].

Plants have long been recognized as a great source of bioactive substances with special pharmacological qualities that support human life and wellness [10]. Medicinal plants are rich in bioactive phytochemicals, which protect plants from disease and damage, and contribute to the color, aroma, and flavor of the plant [11]. Compounds dissolved in solution can be isolated, identified, and quantified using high-performance liquid chromatography (HPLC) [5]. This technique is used as a potential tool in biological and chemical research and is also employed in industry to extract

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and assay complex substance combinations [12]. In addition to developing new drugs or drug formulations, HPLC is utilized in biotechnology, agrochemicals, pharmaceuticals, forensic science, and environmental analysis. Moreover, HPLC is used to quantify compounds in biological samples, purify compounds at the process scale, and improve and characterize biocatalysts [12].

Ethnobotany, a branch of botany and pharmacology, involves examining, documenting, introducing, and publishing information about the traditional use of medicinal plants by local and ethnic groups in traditional, rural, and nomadic areas worldwide [13]. At the end of the 19th century, researchers began extracting, purifying, and identifying bioactive chemicals from plants to examine the pharmacological potential of these compounds as medicinal drugs. The ethnopharmacological qualities of *Aloe* species, including wound healing, antitumoral, anti-inflammatory, antibacterial, antimalarial, and anticancer capabilities, have been validated in numerous *in vitro* and *in vivo* investigations [14]. These traits were mostly attributed to a range of chemicals present in the phytochemical profile of *Aloe* extracts rather than to a specific class of compounds [15]. The current study hypothesis is that the estimated *Aloe* species constitute various chemical compounds with high potential for different medicinal purposes. Consequently, this study aimed to assess the phytochemical constituents of nine *Aloe* species and separate the chemical compounds using HPLC. The output of this study will enhance the understanding of how these plants are utilized for various pharmacological purposes.

2. Materials and Methods

2.1 Plant Collection

Nine *Aloe* species were gathered from various locations (Jazan, Abha, Al-Baha, and Taif) in the Kingdom of Saudi Arabia, and the phytochemical constituents of these plants were assessed (Fig. 1). These species were collected during the spring season of 2022. The included species were as follows: *Aloe parvicoma* Lavranos & Collen., *Aloe x abhaica* Lavranos & Collen., *Aloe brunneodentata* Lavranos & Collen., *Aloe armatissima* Lavranos & Collen., *Aloe vera* var. *officinalis* (Forssk.) Baker, *Aloe sabaea* Schwe-

Fig. 1. A map of the location of the study area with the included sample sites. 18° 56′ 34.57″ N and 43° 33′ 54.21″ E. Source: Google Earth, 1 January 2021.

inf., *Aloe castellorum* Wood, *Aloe fleurentiniorum* Lavranos Newton, and *Aloe hijazensis* Lavranos & Collen. The collected species were identified and named according to Chaudhary [16] and Collentette [17].

2.2 Phytochemical Study

2.2.1 Phytocomponents

The total flavonoid compounds (TFCs) were determined according to the methods described by Solich *et al.* [18] and Tofighi *et al.* [19]. The $TFC_{herbal\ material}$ results were calculated, as quercetin, using the equation: $TFC_{herbal\ material} = (TFC_{tested\ solution} \times 1.25 \times 50) / (w - ld)$, where $TFC_{test\ solution}$ is the total concentration of flavonoids in the test solution (mg mL^{-1}), 1.25 corresponds to the dilution factor, 50 is the volume of the stock solution (mL), w is the mass of herbal material (g), and ld is the loss on drying of herbal material. The standard for flavonoids' determination was quercetin. The results are expressed as the amount of flavonoid mg/g of herbal material (corrected for moisture content). Additionally, the spectrophotometric method was used to determine the amount of phenolics in the plant ethanol extract [19,20], while the bromocresol green (BCG; Aldrich chemicals) dye was used to estimate the total alkaloids [21]. The concentration of phenolics is expressed as gallic acid (mg GA/mL), and the total alkaloid content is described as the atropine equivalent (AE)/g crude extract.

2.2.2 Estimation of Phytochemical Compounds using HPLC

The HPLC technique was used to estimate the flavonoid and phenolic compounds of the assessed *Aloe* species. Plant flavonoids and phenolic acids were frequently separated, identified, and quantified using HPLC–mass spectrometry (MS). The column (ZORBAX Eclipse C18 column) size was 4.6 × 150 mm, 3–5 µm, with an injection volume of 10 µL. The HPLC–MS system comprises a quaternary pump, a photodiode-array detector, an ultraviolet (UV)/Vis detector, and a single quadrupole MS detector with an electrospray ionization (ESI) ion source (Agilent 1100: Agilent Corp., Palo Alto, CA, USA). Flavonoids were separated over 70 minutes, detected at 280 nm, and identified by ESI-MS using a gradient solvent system consisting of a 0.1% formic acid solution at a flow rate of 1.0 mL min^{-1} [21]. Phenolic acids were separated in 60 minutes at a flow rate of 0.8 mL min^{-1} and detected at 325 nm 22 using a gradient mobile phase composed of water/acetonitrile/glacial acetic acid (980/20/5, v/v/v, pH 2.68) and acetonitrile/glacial acetic acid (1000/5, v/v). Alkaloids were further examined by HPLC using 0.2% diethylamine and 0.16% formic acid as solvent system A and 0.2% diethylamine and 0.16% formic acid in acetonitrile as solvent system B (0 min, 80:20 (A–B); 5 min, 80:20; 20 min, 60:40; 25 min, 0:100). The Grace Smart RP18 column (Grace Vydac, Hesperia, CA, USA) measuring 5 µm and 250 mm × 4.6 mm was employed with a flow rate of 1.0 mL min^{-1}. The peaks were identified at 226 nm.

2.3 Data Analysis

The matrix of the separated chemical compounds from the different involved species was analyzed using PAST (free software available on the web) (https://past.en.lo4d.com/windows) [22]. Similarity of the quantitative data was calculated using the Nei and Li/Dice similarity index [23], and similarity estimates were performed using the unweighted pair group method with arithmetic averages (UPGMA). The matrices of the mutual coefficients of similarity that were calculated using PAST were clustered (agglomerative clustering), and the resulting clusters are expressed as a dendrogram.

2.4 Statistical Analysis

One-way analysis of variance (ANOVA) was used to evaluate the differences in chemical traits across the various study species after the normality of the data had been checked using SPSS software (Version 22, SPSS Inc., Chicago, IL, USA) [24]. The occurrence of substantial differences resulted in a post-hoc test being performed according to Duncan's test.

3. Results and Discussion

3.1 Phytochemical Constituents

The use of herbal/natural pharmaceuticals as complementary/alternative therapies is becoming increasingly widespread worldwide, with many drugs derived directly from plants and others chemically modified [25]. Studies on the phytochemistry of the genus *Aloe* have revealed that *Aloe* species plants are abundant in various classes of compounds, including phenolic compounds, flavonoids, and alkaloids. These compounds have been demonstrated to have antiviral, anti-tumor, and antibacterial properties. Thus, the *Aloe* genus is being investigated further as a potential alternative medicinal source [26,27]. Moreover, these compounds can act as a defense mechanism against numerous insects, microorganisms, and herbivores [28]. The results of the current investigation indicated a significant variation in the phenolic compounds, flavonoids, and alkaloids among the shoots of nine *Aloe* species (Fig. 2). These results aligned with previous findings [29–31], which recorded varying concentrations of the same chemical constituents in different *Aloe* species.

Secondary metabolites derived from plants are known to comprise numerous structurally varied groups of polyphenols with possible pharmacological actions, such as anti-inflammatory, anticancer, antioxidant, and antipathogenic qualities [26]. *A. hijacensis* were found to have the highest value of phenolic compounds, but the lowest concentration of alkaloids (25.02 and 2.30 mg/g, respectively). On the contrary, *A. parvicoma* had the highest content of alkaloids and the lowest concentration of phe-

nolic compounds (13.08 and 4.21 mg/g, respectively). Di Scala *et al*. [32] recorded higher phenolic contents (0.37 mg/g), but Adesuyi *et al*. [33] recorded lower phenolic and higher alkaloid contents (2.32 and 24.7 mg/g, respectively) in the tissues of *A. vera* var. *officinalis* than those in the investigated *Aloe* plants. Furthermore, the flavonoid content (32.46 mg/g) recorded by Adesuyi *et al*. [33] was higher than the highest content (6.33 mg/g) recorded in the tissues of *A. castellorum*. Phenols are a significant group of substances and can serve as antioxidants or free radical scavengers [33]. However, alkaloids have potential uses in eliminating and reducing the proliferation of human cancer cell lines [34] and as a potential painkiller [35]. Furthermore, flavonoids are antioxidants that have been shown to possess a variety of biological qualities, including cytostatic, antibacterial, anti-inflammatory, antiaggregatory, analgesic, and anti-allergic [36].

High Performance Liquid Chromatography

Generally, a chromatographic technique, such as HPLC, requires reference material that serves as an external standard to determine the amount of an active ingredient or a marker compound present in a crude extract or in preparations made from the material [37]. Quercetin was used to determine flavonoids, while gallic acid was utilized for phenolic compounds. A total of 16 compounds were separated from flavonoids, phenolic acids, and alkaloids (6, 6, and 4, respectively). Similar to this study, Añibarro-Ortega *et al*. [38] also recorded approximately 17 phenolic compounds in the leaf extracts, and categorized these compounds as anthrones, chromones, flavonoids, and phenolic acids.

3.2 Flavonoids

Numerous bacterial strains are inhibited or killed by flavonoids, which also eliminate several harmful protozoans and key viral enzymes, including reverse transcriptase and protease [36]. Flavonoids also act as antioxidants and radical scavengers, exerting a protective effect in several diseases [39]. The HPLC data on the nine *Aloe* species in this study exhibited six flavonoid compounds with different retention times (RTs) (Table 1 and Fig. 3a–i). These compounds were apigenin, luteolin, naringin, rutin, kaempferol, and hesperetin (RTs = 18, 26, 33, 43, 51, and 59 min, respectively). Kaempferol plays a pivotal role in human nutrition and disease treatment [40]. Furthermore, kaempferol and its related chemicals have antibacterial, an-

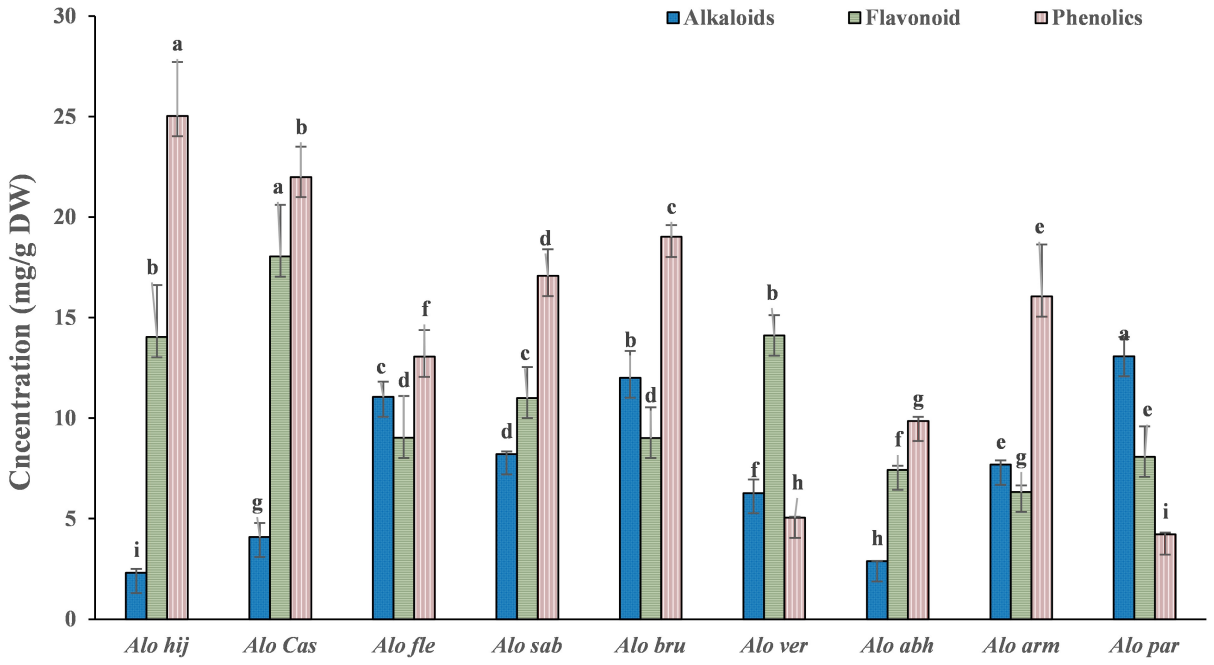

Fig. 2. Secondary metabolite contents in the shoots of the nine *Aloe* species. Data are presented as the mean. Vertical bars represent the standard deviation. Columns with the same letter are not significant ($p < 0.05$) according to Duncan's test.

Table 1. Identified flavonoid compounds produced from the HPLC analysis of nine *Aloe* species.

Peak	1	2	3	4	5	6
Compound	Apigenin	Luteolin	Naringin	Rutin	Kaempferol	Hesperetin
RT (min)	18	26	33	43	51	59

RT, retention time; HPLC, high-performance liquid chromatography.

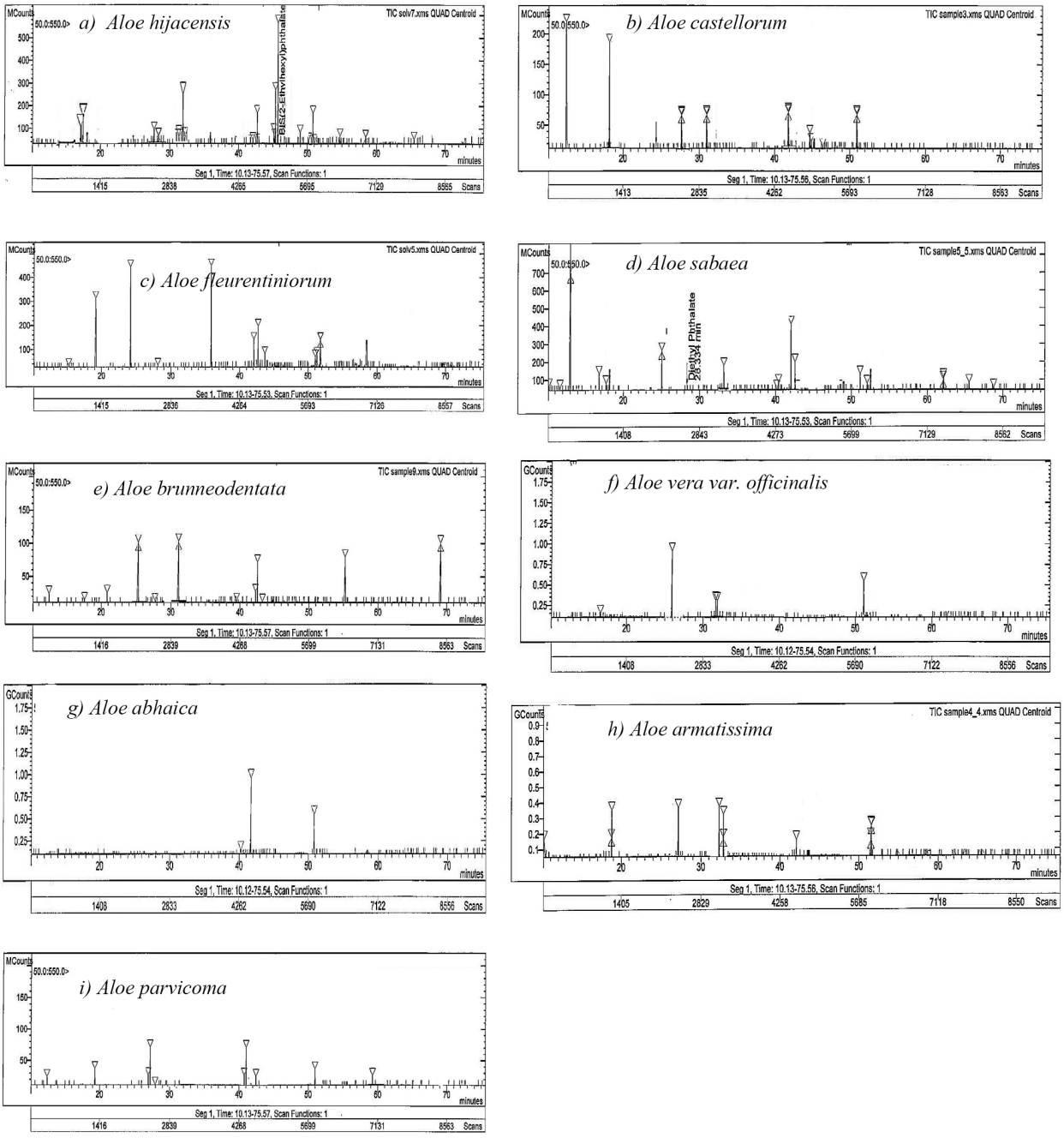

Fig. 3. HPLC profile of the flavonoid compounds in the leaf exudates of the nine *Aloe* species (a–i). The peaks correspond to the compounds presented in Table 1.

tifungal, and antiprotozoal properties in addition to the anticarcinogenic and anti-inflammatory effects [41]. Moreover, apigenin shows efficiency in preventing a wide range of ailments. Apigenin protects against diseases, such as cancer, heart disease, and neurological disorders, by lowering inflammation and oxidative stress, and by promoting cellular health [42]. Luteolin, with potential antioxidant activity, prevents ROS-induced damage and reduces oxidative stress, which is mainly responsible for the pathogenesis of many diseases, and prevents cancer by modulating numerous pathways [43]. Rutin is a polyphenolic flavonoid widely found in garlic and other foods, and improves the health, function, and integrity of the liver and has several biological benefits, including antimicrobial, anticarcinogenic, antithrombotic, cardioprotective, and neuroprotective properties [44].

Moreover, there were marked variations in the concentrations of the separated flavonoid compounds among the nine *Aloe* species (Table 2). Notably, *A. parvicoma* had the highest concentration of apigenin (45.36 mg/g). Among all

Table 2. Concentrations (mg/g) of flavonoid compounds in the shoots of the nine *Aloe* species.

Species	Flavonoids concentration (mg/g)					
	Apigenin	Luteolin	Naringin	Rutin	Kaempferol	Hesperetin
Aloe hijazensis	5.36^{d}	2.66^{f}	ND	29.46^{a}	ND	ND
Aloe castellorum	19.36^{c}	32.07^{a}	56.39^{a}	ND	20.14^{de}	ND
Aloe fleurentiniorum	7.59^{d}	12.33^{de}	ND	15.78^{c}	24.19^{c}	ND
Aloe sabaea	ND	28.61^{ab}	45.63^{b}	4.69^{e}	40.12^{a}	ND
Aloe brunneodentata	ND	20.25^{c}	14.36^{d}	8.56^{d}	ND	ND
Aloe vera var. *officinalis*	ND	28.65^{ab}	17.6^{c}	ND	17.69^{e}	ND
Aloe abhaica	ND	ND	ND	17.6^{c}	22.4^{cd}	ND
Aloe armatissima	36.77^{b}	9.61^{e}	60.14^{a}	22.45^{b}	35.06^{b}	ND
Aloe parvicoma	45.36^{a}	14.9^{d}	ND	27.69^{ab}	16.07^{e}	6.99^{a}

The maximum and minimum values are underlined. ND, not detected. The mean values with the same letters are not significant ($p < 0.05$) according to Duncan's test.

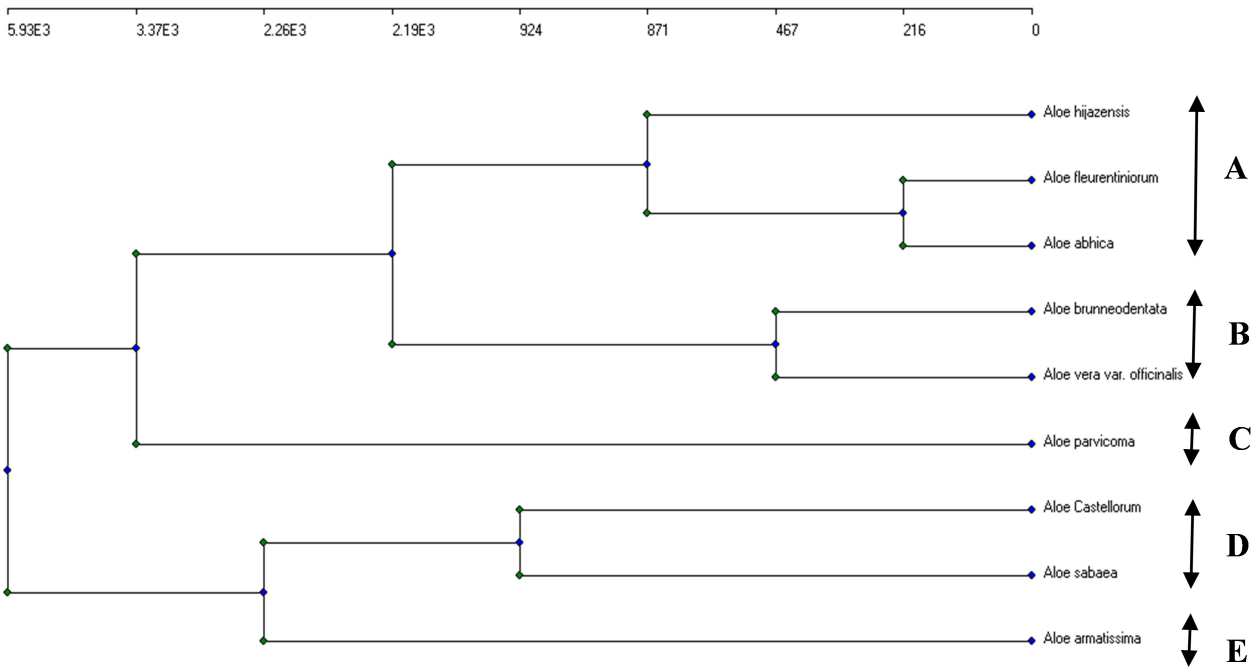

Fig. 4. The agglomerative clustering dendrogram of the concentrations of the six flavonoid compounds identified in the HPLC analysis of the nine *Aloe* species. (A) *A. hijazensis*, *Aloe fleurentiniorum*, and *Aloe abhaica*; (B) *A. brunneodentata* and *Aloe vera* var. *officinalis*; (C) *A. parvicoma*; (D) *A. sabaea* and *A. castellorum*; (E) *A. armatissima*. Arrows represent five segregated clusters.

species, it also had the lowest concentration of kaempferol (16.07 mg/g). Meanwhile, *A. hijazensis* had the highest concentration of rutin (29.46 mg/g), but the lowest apigenin and luteolin (5.36 and 2.66 mg/g, respectively). Comparatively, the highest concentration of kaempferol (40.12 mg/g) and the lowest of rutin (4.69 mg/g) were recorded in *A. sabaea*, while *A. armatissima* contributed the highest concentration of naringin (60.14 mg/g), whereas *A. brunneodentata* had the lowest (14.36 mg/g). Naringin is a common bioactive polyphenol found in citrus fruits, which have been consumed since ancient times and are beneficial to human health. Notably, naringin is an excellent alternative supplemental remedy that can alleviate the situations of cancer patients by suppressing the development of cancer in different body areas [45]. Furthermore, hesperetin (6.99 mg/g) was exclusively recorded in the tissues of *A. parvicoma*. Hesperetin is abundant in orange and grape juices and is consumed in daily diets. Hesperetin also has vitamin-like properties and can reduce fragility, leakiness, and capillary permeability (vitamin P). Additionally, hesperetin has exhibited strong anti-inflammatory, antioxidant, and neuroprotective properties in many neurodegenerative models [46].

The agglomerative clustering technique was used to group the nine *Aloe* species into five clusters based on the flavonoid chemicals found in the plant leaves (Fig. 4): (A)

A. hijazensis, *Aloe fleurentiniorum*, and *Aloe abhaica*; (B) *A. brunneodentata* and *Aloe vera* var. *officinalis*; (C) *A. parvicoma*; (D) *A. sabaea* and *A. castellorum*; (E) *A. armatissima*.

3.3 Phenolic Compounds

Plant phenolics are considered an essential part of the human diet and offer many health advantages, including strong antioxidant activity [47]. The HPLC data for the nine study *Aloe* species identified six phenolic compounds with different RTs (Table 3 and Fig. 5a–i). These compounds were identified as ellagic acid, ferulic acid, quercetin, resorcinol, gallic acid, and syringic acid (RT = 20, 36, 40, 54, 59, and 64 min., respectively).

Marked variations were observed in the concentrations of the separated phenolic substances among the nine *Aloe* species (Table 4). Plant phenolics are considered an essential part of the human diet and offer many health advantages, including strong antioxidant activity [48]. Thus, phenolics are an important compound that can be used as an anticarcinogenic [49], multiple-function protector against oxidative stress [50], and anti-inflammatory substance for treating chronic ulcerative colitis [51]. In addition, quercetin, a flavonoid present in fruits and vegetables, has a common role in preventing various pathogeneses, as this compound can inhibit inflammation, oxidative stress, and strengthen the natural antioxidant defense systems in the body [52]. Humans have been consuming gallic acid, a phenolic acid found in fruits and vegetables, for generations. Indeed, gallic acid is known to promote antimicrobial, antioxidant, anticancer, anti-inflammatory, and antiviral qualities, among other well-established health advantages [53].

The highest concentrations of ellagic acid, quercetin, and gallic acid were noted in *A. armatissima* (27.99, 39.50, and 40.12 mg/g, respectively), while *A. hijazensis* had the highest concentration of resorcinol (35.17 mg/g). In addition, *A. fleurentiniorum* presented the highest concentration of syringic acid (7.13 mg/g), and *A. brunneodentata* had the highest concentration of ferulic acid (28.47 mg/g). Fruits, vegetables, and drinks, such as coffee and beer, are all rich sources of ferulic acid, a compound that has shown promise as a treatment option for several illnesses, including viral and bacterial infections, inflammation, diabetes, neurological problems, and heart disease [54]. Topical pharmaceutical medicines that treat skin conditions and infections, such as seborrheic dermatitis, acne, psoriasis, eczema, calluses, corns, and warts, contain resorcinol as an antiseptic and disinfectant. Moreover, resorcinol has a keratolytic effect. Although, resorcinol has anti-thyroidal properties, it is not used for any official therapeutic indication [55]. Syringic acid is frequently found in vegetables and fruits and contains antioxidant, antibacterial, anti-inflammatory, antiendotoxic, neuro, and hepatoprotective properties [56]. Thus, syringic acid exhibits many therapeutic uses, including in preventing cancer, diabetes, and cerebral ischemia.

Conversely, the lowest concentration of ellagic acid (4.19 mg/g) was recorded in the aboveground tissues of *A. abhaica*, while the lowest concentrations of ferulic acid and resorcinol (4.17 and 3.12 mg/g) were recorded in *A. fleuren-*

Table 3. The phenolic compounds identified in the *Aloe* species by HPLC.

Peak	1	2	3	4	5	6
Compound	Ellagic acid	Ferulic acid	Quercetin	Resorcinol	Gallic acid	Syringic acid
RT (min)	20	36	40	54	59	64

RT, retention time.

Table 4. Concentrations (mg/g) of the phenolic compounds in the shoots of the nine *Aloe* species.

Species	Phenolic compounds (mg/g)					
	Ellagic acid	Ferulic acid	Quercetin	Resorcinol	Gallic acid	Syringic acid
Aloe hijazensis	ND	22.44^{b}	16.54^{d}	35.17^{a}	7.21^{g}	ND
Aloe castellorum	ND	15.69^{c}	1.47^{h}	19.14^{b}	10.14^{fg}	1.23^{bc}
Aloe fleurentiniorum	18.66^{b}	4.17^{g}	15.24^{de}	3.12^{g}	22.67^{cd}	7.13^{a}
Aloe sabaea	27.60^{a}	4.63^{g}	19.55^{cd}	4.12^{f}	28.99^{bc}	ND
Aloe brunneodentata	ND	28.47^{a}	9.25^{f}	7.14^{e}	19.88^{de}	ND
Aloe vera var. *officinalis*	5.33^{c}	9.56^{de}	8.65^{g}	ND	15.36^{e}	1.25^{bc}
Aloe abhaica	4.19^{d}	8.26^{ef}	ND	10.25^{cd}	20.16^{cd}	ND
Aloe armatissima	27.99^{a}	6.78^{f}	39.50^{a}	7.66^{de}	40.12^{a}	ND
Aloe parvicoma	15.36^{b}	10.22^{de}	24.19^{b}	ND	7.45^{g}	0.99^{c}

The maximum and minimum values are underlined. ND, not detected. The mean values with the same letters are not significant according to Duncan's test. $p < 0.05$.

tiniorum. Moreover, the lowest concentrations of quercetin, gallic acid, and syringic acid (1.47, 7.21, and 0.99 mg/g) were found in *A. castellorum*, *A. hijazensis*, and *A. parvicoma*, respectively.

Based on the phenolic compounds recorded in the nine *Aloe* species, seven clusters were segregated by applying the agglomerative clustering technique (Fig. 6): (A) *A. hijazensis*; (B) *A. castellorum*; (C) *Aloe vera* var.

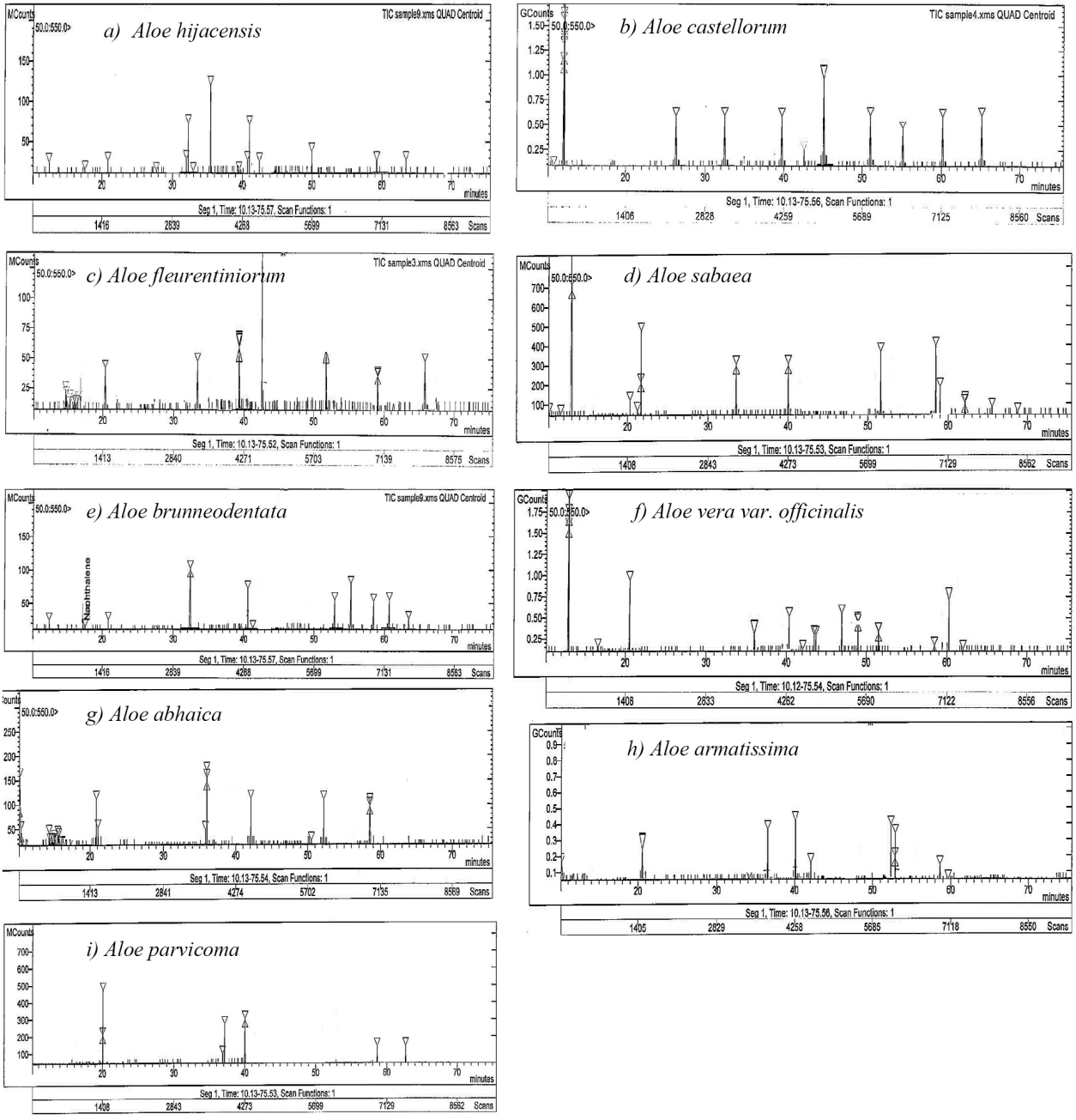

Fig. 5. HPLC profiles of the phenolic compounds in the leaf exudates from the nine *Aloe* species (a–i). The peaks correspond to the compounds presented in Table 4.

Table 5. The alkaloid compounds identified in the *Aloe* species by HPLC.

Peak	1	2	3	4
Compound	Coniine	2-methylpiperidine	Conhydrine	Conmaculatin
RT (min)	18	30	44	50

RT, retention time.

officinalis and *A. abhaica*; (D) *A. brunneodentata*; (E) *A.fleurentiniorum* and *A. sabaea*; (F) *A. parvicoma*; (G) comprised *A. armatissima*.

3.4 Alkaloids

The HPLC data on the nine studied *Aloe* species identified four alkaloid compounds with different RTs (Table 5 and Fig. 7a–i). These compounds are coniine (RT = 18 min), 2-methylpiperidine (RT = 30 min), conhydrine (RT = 44 min), and conmaculatin (RT = 50 min). This result aligns with that published by [57], who reported a few alkaloid compounds in *A. vera* var. *officinalis*. Various plant species, including *Aloe*, are known to contain the alkaloid coniine. However, despite the possibility that coniine is hazardous, this alkaloid is no longer used medicinally due to a limited window for treatment [58]. Additionally, piperidine causes musculoskeletal abnormalities in newborn animals and is acutely hazardous to adult cattle species. These teratogenic effects include several congenital contracture deformities and the introduction of a cleft palate in sheep, goats, pigs, and cattle. Furthermore, tobacco (*Nicotiana tabacum*), lupine (*Lupinus* spp.), and poison hemlock (*Conium maculatum*) are among the poisonous plants that contain teratogenic piperidine alkaloids [59]. Moreover, conhydrine is extracted from the leaves and seeds of the toxic plant *C. maculatum* L. (Apiaceae), whose extracts were used in Greece for executions [60]. Another volatile alkaloid, conmaculatin, is related to coniine and was recorded in the well-known poisonous weed *C. maculatum*. Indeed, in mice, conmaculatin exhibited potent central and peripheral antinociceptive action within a limited dose range (10–20 mg/kg) [61].

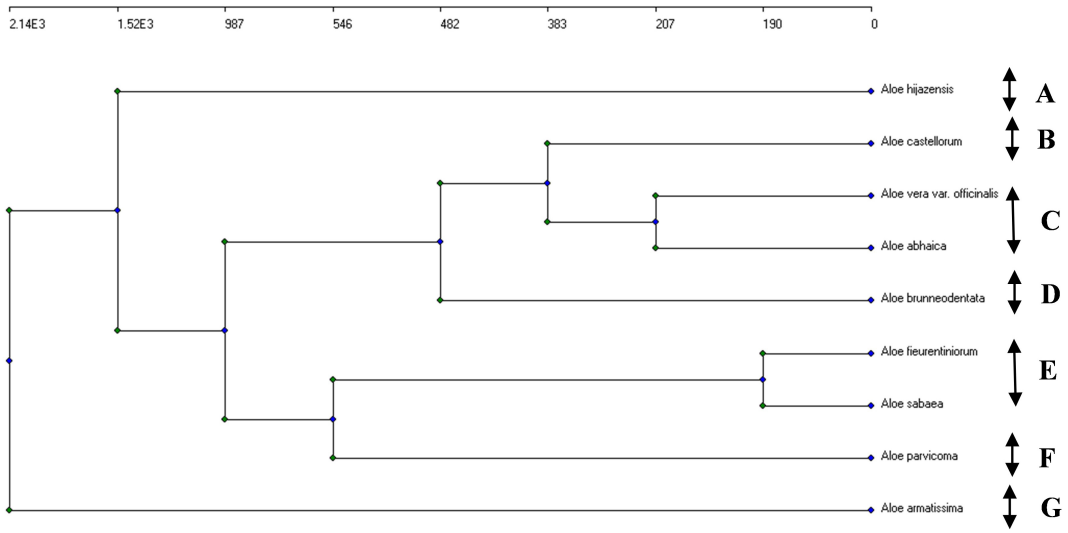

Fig. 6. An agglomerative clustering dendrogram of the concentrations of the six phenolic compounds identified in the HPLC analysis of the nine *Aloe* species. (A) *A. hijazensis*; (B) *A. castellorum*; (C) *Aloe vera* var. *officinalis* and *A. abhaica*; (D) *A. brunneodentata*; (E) *A.fleurentiniorum* and *A. sabaea*; (F) *A. parvicoma*; (G) comprised *A. armatissima*. Arrows represent five segregated clusters.

Table 6. Concentrations (mg/g) of the alkaloid compounds in the shoots of the nine *Aloe* species.

Species	Alkaloids concentration (mg/g)			
	Coniine	Conhydrine	Conmaculatin	2-methylpiperidine
Aloe hijazensis	1.25^{cd}	2.66^{c}	3.14^{c}	ND
Aloe castellorum	2.60^{c}	ND	4.01^{bc}	3.09^{ab}
Aloe fleurentiniorum	$\underline{6.58^{a}}$	1.05^{d}	$\underline{0.29^{e}}$	ND
Aloe sabaea	ND	4.21^{b}	ND	$\underline{3.66^{a}}$
Aloe brunneodentata	$\underline{0.99^{d}}$	$\underline{1.02^{d}}$	2.36^{d}	2.57^{b}
Aloe vera var. *officinalis*	ND	ND	4.90^{ab}	ND
Aloe abhica	ND	$\underline{5.60^{a}}$	$\underline{4.99^{a}}$	ND
Aloe armatissima	5.20^{b}	4.26^{b}	ND	ND
Aloe parvicoma	ND	3.55^{bc}	ND	$\underline{1.48^{c}}$

The maximum and minimum values are underlined. ND, not detected. The mean values with the same letters are not significant ($p < 0.05$) according to Duncan's test.

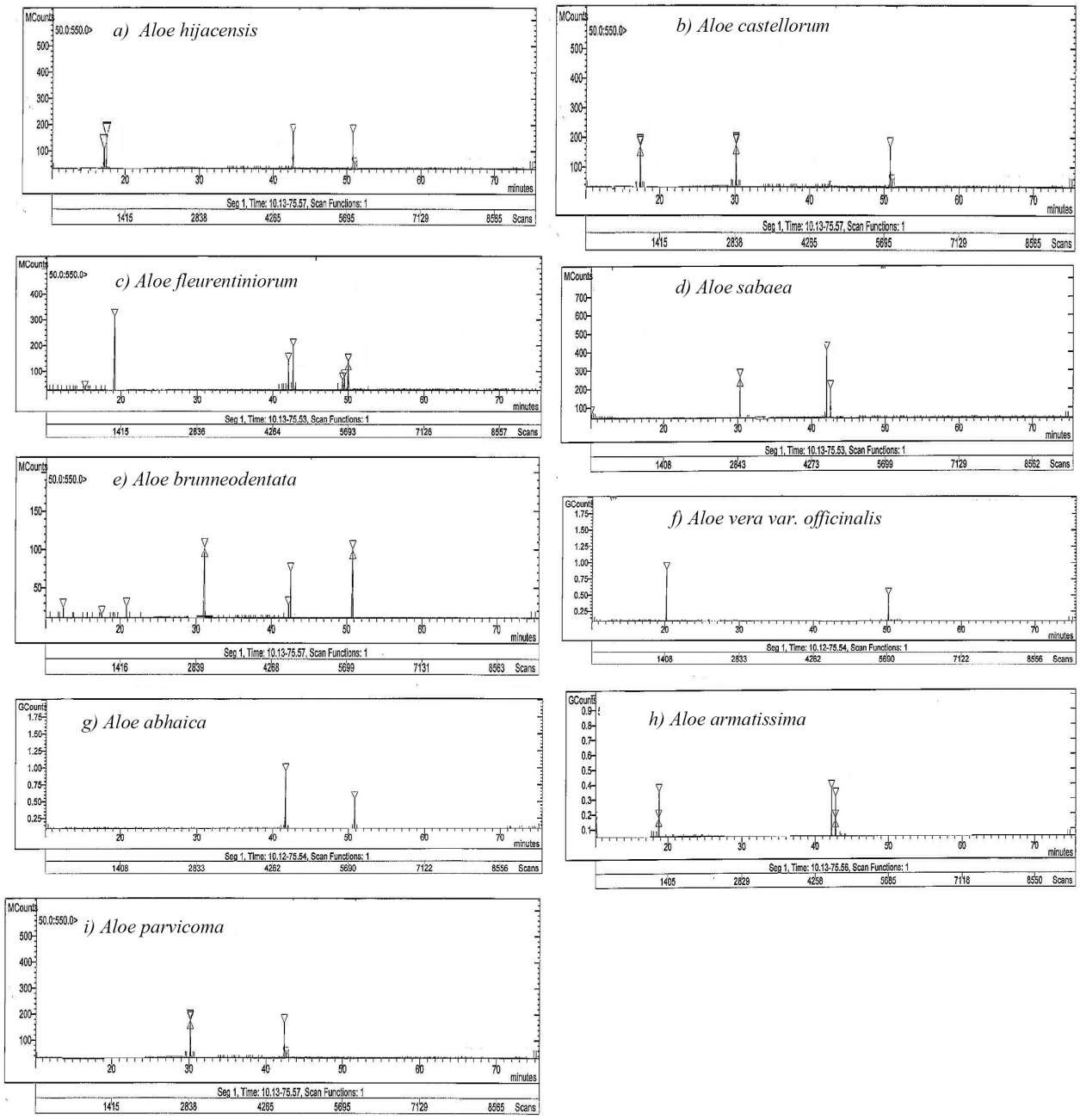

Fig. 7. HPLC profiles of the alkaloid compounds in the leaf exudates from the nine *Aloe* species (a–i). The peaks correspond to the compounds presented in Table 6.

The toxicity to foreign organism cells represents the biological characteristic of alkaloids. Meanwhile, the potential applications of alkaloids in the eradication and decrease of human cancer cell lines have been extensively researched [34]. There were marked variations in the concentrations of the separated alkaloid compounds among the nine studied *Aloe* species (Table 6). *A. fleurentiniorum* had the highest concentration of coniine (6.58 mg/g), while *A. hijazensis* presented the lowest (1.25 mg/g). In addition, *A. abhaica* had the highest concentrations of conhydrine and conmaculatin (5.60 and 4.99 mg/g), while *A. brunneodentata* and *A. fleurentiniorum* had the lowest (1.02 and 0.29 mg/g), respectively. Furthermore, the highest content of 2-methylpiperidine (3.66 mg/g) was recorded in the shoots of *A. sabaea*, while the lowest (1.48 mg/g) was in *A. parvicoma.* Blitzke *et al.* [62] had previously found that *A. sabaea* contains the hazardous piperidine alkaloids, indicating that this plant and other species should be used with caution.

According to [29], the piperidine alkaloid was reported in six *Aloe* species, whereas coniine was found only in *A. viguieri*. Notably, not every kind of *Aloe* species is ed-

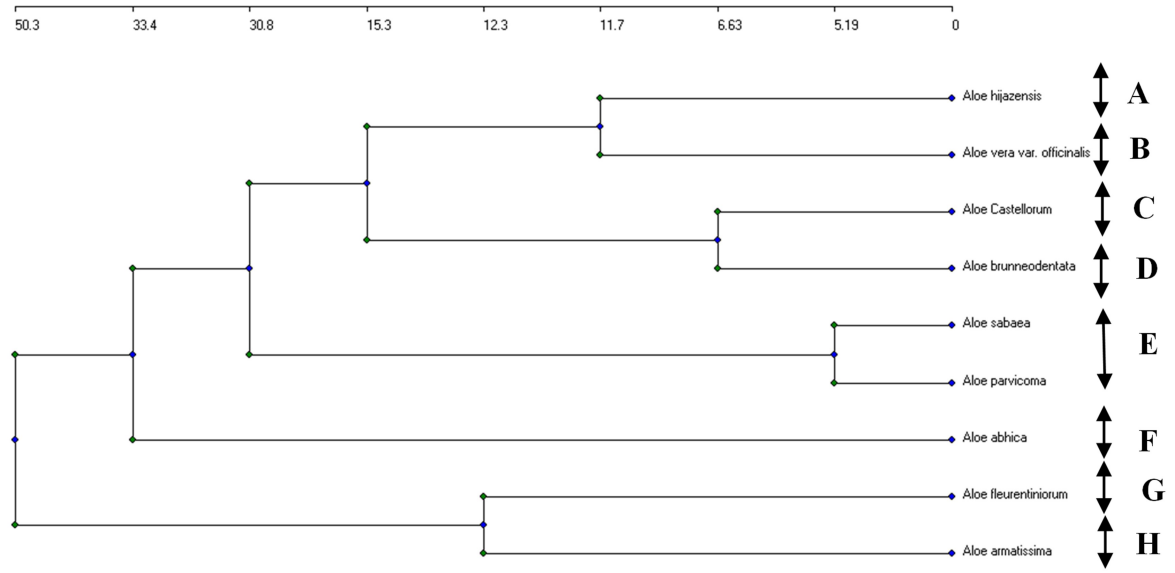

Fig. 8. An agglomerative clustering dendrogram of the concentrations of the four alkaloid compounds identified in the HPLC analysis of the nine *Aloe* species. (A) *A. hijazensis*; (B) *Aloe vera* var. *officinalis*; (C) *A. castellorum*; (D) *A. brunneodentata*; (E) *A. parvicoma* and *A. sabaea*; (F) *A. abhaica*; (G) *A. fleurentiniorum*; (H) *A. armatissima*. Arrows represent five segregated clusters.

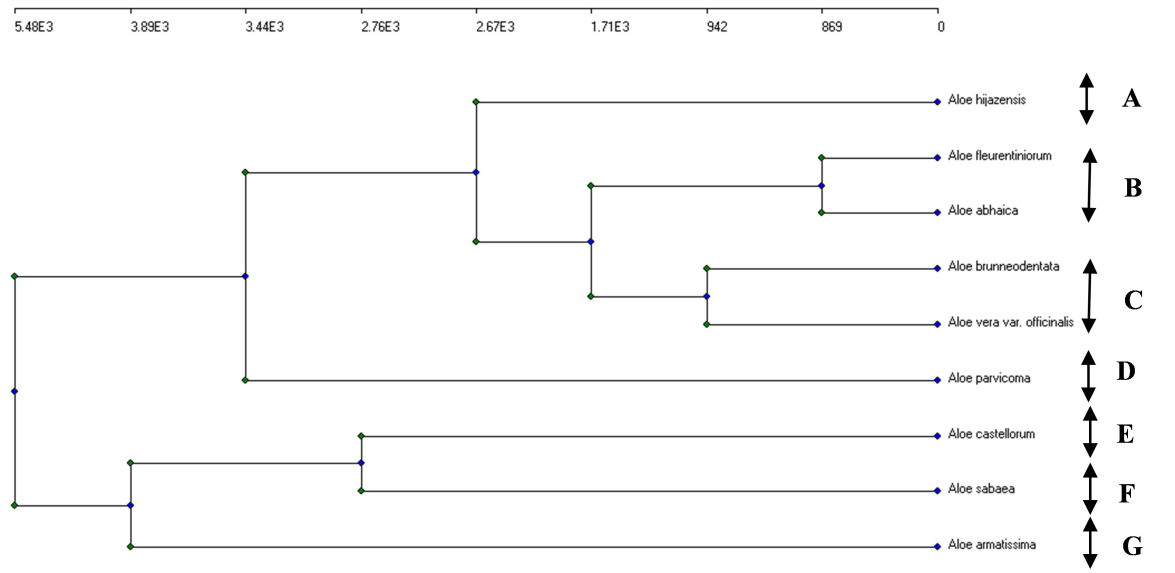

Fig. 9. An agglomerative clustering dendrogram of the concentrations of the 16 compounds identified in the HPLC analysis of the nine studied *Aloe* species. (A) *A. hijacensis*; (B) *A. fleurentiniorum*, *A. sabaea*, and *A. abhaica*; (C) *A. brunneodentata* and *A. Vera* var. *officinalis*; (D) *A. parvicoma*; (E) *A. castellorum*; (F) *A. sabaea*; (G) *A. armatissima*. Arrows represent five segregated clusters.

ible, as some, such as *A. vera* var. *officinalis*, may induce negative reactions, contain hazardous chemicals [63], and promote adverse effects [64].

Based on the alkaloid compounds recorded in the nine *Aloe* species, eight clusters were segregated by applying the agglomerative clustering technique (Fig. 8). The clusters identified were (A) *A. hijazensis*; (B) *Aloe vera* var. *officinalis*; (C) *A. castellorum*; (D) *A. brunneodentata*; (E) *A. parvicoma* and *A. sabaea*; (F) *A. abhaica*; (G) *A. fleurentiniorum*; (H) *A. armatissima*.

Clustering Analysis of the Separated Chemical Compounds

According to the results of the agglomerative clustering applied to the extracted chemical components of the nine *Aloe* species using the UPGAMA clustering analysis, all of the studied species were distinct from one another with lengthy Euclidean distances (Fig. 9). Moreover, seven similarity clusters were recognized according to the most related species: (A) *A. hijacensis*; (B) *A. fleurentiniorum*, *A. sabaea*, and *A. abhaica*; (C) *A. brunneodentata* and *A.*

Vera var. *officinalis*; (D) *A. parvicoma*; (E) *A. castellorum*; (F) *A. sabaea*; (G) *A. armatissima*. These differences may be attributed to variations in the environmental conditions [25].

4. Conclusion

The present study revealed significant variations in the alkaloids, flavonoids, and phenolic compounds among the shoots of the nine studied *Aloe* species. A total of 16 compounds were separated from flavonoids (6), phenolic acids (6), and alkaloids (4). The highest concentration of apigenin was found in *A. parvicoma*, while *A. hijazensis* had the highest concentration of rutin, *A. sabaea* had the highest concentration of kaempferol, and *A. armatissima* contributed the highest concentration of naringin. *A. armatissima* had the highest concentrations of ellagic acid, quercetin, and gallic acid, while *A. hijazensis* had the highest concentration of resorcinol, *A. fleurentiniorum* had the highest concentration of syringic acid, and *A. brunneodentata* had the highest concentration of ferulic acid. The highest concentration of coniine was recorded in *A. fleurentiniorum*, while the highest levels of conhydrine and conmaculatin were found in *A. abhaica*, and 2-methylpiperidine was noted in *A. sabaea*. The nine *Aloe* species exhibited significant divergence, as indicated by the extensive Euclidean distances. The identified compounds and the considerable concentrations of each suggest the potential use of these studied *Aloe* species for various pharmacological purposes. However, caution should be maintained when using *A. fleurentiniorum* and *A. sabaea* for medicinal purposes, as these species contain high concentrations of the toxic alkaloid coniine and 2-methylpiperidine, respectively.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

TG and ME designed the research study. TG, ME and SA performed the research. TG and SA conducted experiments. TG analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The plant materials were gathered from natural habitats in southwestern highlands, Saudi Arabia. The sample collection was done from natural habitats, which requires no permission in Saudi Arabia.

Acknowledgment

The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-171).

Funding

This research was funded by Taif University, Saudi Arabia, project number (TUDSPP-2024-171).

Conflict of Interest

The authors declare no conflict of interest.

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