PRIMARY AND SECONDARY METABOLITE CONTRIBUTING TO THE CHEMOTAXONOMY OF NINE *ALOE* SPECIES GROWN IN SOUTHWESTERN HIGHLANDS OF SAUDI ARABIA

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Abstract. This research was conducted to investigate the primary and secondary metabolites that contribute to the chemosystematics of nine *Aloe* species in the Sarawat Mountains. Nine Aloe species were collected from different locations in the Kingdom of Saudi Arabia (Taif, Al-Baha, Abha and Jazan) to reassess their taxonomic position. *A. armatissima* had the highest protein content (106.98 mg g^{-1}), while *A. brunneodentata* had the highest carbohydrate content (4.05 mg g^{-1}). On the other side, the lowest of protein and carbohydrate contents (35.19 and 0.25 mg g^{-1}) were recorded in the tissues of *A. parvicoma* and *A. fleurentiniorum*, respectively. The results of the analysis of the secondary metabolites indicated significant variations of alkaloids, cardiac glycosides, flavonoids, and phenolic compounds among the shoots of the nine studied *Aloe* species. It was found that *A. hijazensis* had the highest value of phenolic compound, but the lowest of alkaloids (25.02 and 2.30 mg g^{-1}, respectively). On the contrary, *A. parvicoma* had the highest alkaloid content and the lowest phenolic compound content (13.08 and 4.21 mg g^{-1}, respectively). Moreover, the highest cardiac glycoside and flavonoid contents (21.09 and 18.04 mg g^{-1}) were recorded in the shoots of *A. fleurentiniorum* and *A. castellorum*, respectively, while the lowest contents (9.07 and 6.33 mg g^{-1}) were recorded in *A. brunneodentata* and *A. armatissima*. In conclusion, the total chemical constituents of the nine *Aloe* species indicated that all the study species were greatly different except for *A. castellorum* and *A. hijazensis* (cluster A); and *A. fleurentiniorum*, *A. sabaea* and *A. abhaica* (cluster B), which are notably similar in their chemical constituents.
Keywords: *secondary metabolite, carbohydrates, glycosides, phenolic compound, aloes, Sarawat Mountains*

Introduction

The genus *Aloe* (Family Aloaceae) has one of the widest distributions, occurring over most of sub-Saharan Africa as well as on the Arabian Peninsula and Madagascar (Aseeri et al., 2020). Ecologically, aloes are exceptionally heterogeneous, and members of the genus are found in almost every possible habitat, ranging from arid deserts to grassland and savanna to misty coasts and moist, tropical forests (Reynolds, 2004). In these disparate environments, aloes have morphologically become extremely variable and have diversified into a wide spectrum of growth forms including geophytes, small rosette plants, shrubs, climbers and small to large trees (Smith and Van Wyk, 1991). Almost all *Aloe* species are considered to have medicinal and/or cosmetic value, but a few are poisonous (Klopper and Smith, 2013). They have been used as medicinal plants for centuries (Galal et al., 2023), since they have been used for the treatment of ailments (Riaz et al., 2023). In spite of huge progress in pharmaceutical industry, plants are

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major raw materials for synthetic drugs (Shang et al., 2021), and now adays, the world is progressively turning toward effective herbal medicines (Aanouz et al., 2021). Herbal medicines may improve patients' quality of life and reduce the need for polypharmacy because botanical medicines are frequently low in toxicity (Galal et al., 2022).

Aloes has a history stretching back thousands of years, and many ancient texts document its use and therapeutic properties, where the ancient Assyrian people used aloe juice to eliminate the annoying symptoms of eating lousy food and intestinal gas, and was once used in cases of constipation and sluggish bowel (Prisa, 2022). Aloe plants have been used for many centuries for their medicinal properties, particularly as a traditional remedy to treat various health disorders such as heart disease, skin disorders, ulcers, digestive problems and diabetes, in addition to the pharmaceutical industry (Aseeri et al., 2020), it is also used in cosmetics and health products such as creams, shampoos, disinfectants and sunscreens (Prisa, 2022). Numerous studies conducted both in vitro and in vivo have confirmed the biological properties of *Aloe* species, including wound healing, antitumoral, anti-inflammatory, antimicrobial, antimalarial, anticancer, etc. properties. Mostly, these characteristics could not be ascribed to a single class of compounds, but rather to a variety of compounds found in the phytochemical profile of *Aloe* extracts (Andrea et al., 2020).

During the late 19th century, researchers began to isolate, purify, and identify phytobioactive compounds from plants, and their efforts led them to discover vital drugs from plants that are the base of modern medicine (Tanweer et al., 2018). In this view, for the preparation of semi-synthetic drugs, bioactive compounds isolated from medicinal plants have been modified to make them effective (Riaz et al., 2023). The genus Aloe has over the years proved to be one of the most important sources of biologically active compounds, where it has more than 130 compounds belonging to different classes including anthrones, chromones, pyrones, coumarins, alkaloids, glycoproteins, naphthalenes and flavonoids (Dagne et al., 2000). Aloe plants are a unique source of phytochemicals because they can tolerate hot and dry weather, as they store water and vital chemical components in their swollen, succulent leaves (Wójcik et al., 2021). Alkaloids, amino acids, vitamins, hormones, proteins, polyphenols, saccharides, organic acids, and other naturally occurring phytochemicals are abundant in aloes (Sánchez et al., 2020; Yadeta, 2024). Several phenolic compounds have been isolated from Aloe species and it is likely that many more will follow (Yadeta, 2024).

The Kingdom of Saudi Arabia is the largest country in the Arabian Peninsula, which is situated in western Asia. A major portion of the Saudi Arabia is composed of sand desert, with the Rub' Al-Khali being the largest sand desert in the world (Aseeri et al., 2020). However, in the southern and western parts of the country, there are several mountain ranges, some with peaks rising to almost 10,000 feet (3,000 m) above sea level, which, with their higher rainfall and more favorable climates, host diverse ecosystems, in which most of Saudi's *Aloe* species occur (McCoy and Lavranos, 2014). Aloes are among the most familiar of the world's succulent plants occupying a wide range of habitats and assuming various growth forms (Asmelash, 2018). They form an important component of the local flora of many countries from taxonomic, ethnomedicinal, chemical/chemotaxonomic, ecotouristic and horticultural perspectives (Smith et al., 2000). Hence, this unique group of succulent plants provokes wide-ranging interest among both scientists and plant collectors (Smith and VanWyk, 2009).

The genus *Aloe* occupies a central position in the taxonomy of Aloaceae, and this is not only because it is the first described genus with the largest number of species, but it has

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been studied more extensively than other genera both in terms of taxonomy and systematics (Smith and Steyn, 2004). A reassessment of the classification of aloes supports previous studies that have highlighted the need for taxonomic changes to reflect phylogenetic relationships between the core aloes and sister groups (Klopper et al., 2010; Grace et al., 2013). The chemistry of aloe plants has been studied for many years from several viewpoints (Blitzke et al., 2000; Abd-Alla et al., 2009; Adesuyi et al., 2012; Aseeri et al., 2020) rather than chemosystematics. In view of these points, the current study was conducted to comparatively assess the phytochemical constituents of nine *Aloe* species in terms of their primary and secondary metabolites. Such research may help in classifying these species in light of their chemical constituents: perspectives of chemotaxonomy.

Materials and methods

Samples collection

Nine *Aloe* plant species were collected from different locations in the southwestern Saudi Arabia including Taif, Al-Baha, Abha and Jazan, during 2020 to reassess their taxonomic position based on their phytochemical constituents (*Table 1; Fig. 1*). Species identification and nomenclature were carried out according to Migahid (1996), Chaudhary (2001), and Collenette (1999).

Table 1. *Botanical authority and locations of the nine collected study Aloe species*

Species	Location
Aloe parvicoma Lavranos & Collen.	Al-Hawiya, Taif
Aloe x abhaica Lavranos & Collen.	Al-Hawiya, Taif
Aloe brunneodentata Lavranos & Collen.	Wadi Raidah, Asir
Aloe armatissima Lavranos & Collen.	Al-Shafa, Taif
Aloe vera var. *officinalis* (Forssk.) Baker	Taif & Abha
Aloe sabaea Schweinf.	Jabal Shada, Al Bahah
Aloe castellorum Wood	Jabal Fayfa, Jazan
Aloe fleurentiniorum Lavranos Newton	Bani Malik, South Taif
Aloe hijazensis Lavranos & Collen	Al-Bahah

Figure 1. *Location map of the study area showing the sampling sites. 18° 56' 34.57" N and 43° 33' 54.21" E. Source: Google earth 1 January 2021*

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Phytochemical investigations

Three composite leaf samples were collected from each plant species in polyethylene bags and transferred to the laboratory for chemical analysis. Plant materials were rinsed in tap water, then distilled water, and air-dried at room temperature in the shade before being homogenized in a planetary high-energy mill with a hardened chromium steel vial.

Primary metabolites

Soluble carbohydrates

The total soluble carbohydrates were determined using the anthrone method (Sadasivam and Manickam, 2008). About 100 mg of each sample's powder was hydrolyzed in a boiling water bath for 3 h with 5 ml of 2.5 N HCl. The acid digested sample was chilled to room temperature before adding sodium carbonate to neutralize it. With distilled water, dilute the final volume to 100 ml and centrifuge for 15 min at 5000 rpm. The total soluble carbohydrates were then determined by collecting the supernatant.

Total soluble proteins

The total soluble proteins of the leaves of the different study species were determined according to Lowry et al. (1951). About 50 mg sample were taken from each dried and ground plant, hydrolyzed with 1 ml 0.5 NaOH for 60 min at 60°C. After hydrolysis, samples were brought to 10 ml with distilled water and from each two 0.1 ml aliquots were taken for the Lowry–Folin reaction. Bovine serum albumin (BSA) was used as a standard and similarly hydrolyzed. Blanks were included and treated similarly.

Secondary metabolites

Cardiac glycosides

Cardiac glycosides were quantitatively determined according to Solich et al. (1992) and Tofighi et al. (2016). For determination of cardiac glycosides, a 10% ethanol extract were mixed with 10 ml freshly prepared Baljet's reagent (95 ml of 1% picric acid + 5 ml of 10% NaOH). After 1 h, the mixture was diluted with 20 ml distilled water and the absorbance was measured at 495 nm by Shimadzu spectrophotometer model 1050. The standard for cardiac glycosides determination was securidaside.

Total flavonoid contents (TFC)

The TFC was determined according to the methods described by Solich et al. (1992) and Tofighi et al. (2016). The plant was extracted under reflux conditions (80°C) with 20.0 ml water-ethanol solution 60% (v/v) (pH = 5.06) during 60 min. The extract was cooled to room temperature and filtered. The residue was re-extracted under equivalent conditions. Both hydro-alcoholic extract and re-extract were combined, and the volume was completed to 50 ml of water-ethanol solution 60% (v/v), resulting in the stock solution. An aliquot of the stock solution was transferred to a 10.0 ml volumetric flask and made to volume with methanol, resulting in the blank solution. A second aliquot of the stock solution was transferred to another 10.0 ml volumetric flask, a volume of the 2% $AlCl_3$ was added and made to volume with methanol, which was named test

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solution. After 25 min the absorbance of the test solution was measured at 430 nm against blank solution.

The $TFC_{herbal\ material}$ results were calculated, as quercetin, and it represents the average of three-determinations. The results were expressed as the amount of flavonoid mg g^{-1} of herbal material (corrected for moisture content): $TFC_{herbal\ material} = (TFC_{tested\ solution} \times 1.25 \times 50) / (w - ld)$, 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 cardiac glycosides determination was quercetin.

Total phenolic contents

The concentration of phenolics in the plant ethanol extract was determined using spectrophotometric method (Singleton et al., 1999; Tofighi et al., 2016). The reaction mixture was prepared by mixing 0.5 ml of solution of extract, 2.5 ml of 10% Folin–Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin–Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at λ_{max} = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line. The standard for cardiac glycosides determination was gallic acid.

Total alkaloid contents

Total alkaloids determination: Bromocresol green (BCG, Aldrich chemicals) dye was used to estimate total alkaloids (Li et al., 2015). BCG solution was prepared by heating 69.8 mg BCG with 3 ml NaOH (2N) and 5 ml of distilled water until completely dissolving. The solution was then completed to 1 liter with distilled water. Ten microliters of the crude extracts were thoroughly mixed with 3 ml of BCG solution. Thirty minutes later, 5 ml of chloroform were added, and shaken for 2 min. The lower layer was separated after 30 min. The extraction was continued for three times. A set of reference standard solutions of 0.1% atropine (Merck, Dermstadt) was prepared, and followed the steps described above. The absorbance of color was read at 418 nm. The total alkaloid content was expressed as Atropine equivalent (AE)/gm crude extract.

Data analysis

The matrix of chemical constituents of the different study plant species were analyzed by PAST (free programs on web) software (Hampl et al., 2001). Similarity of quantitative data was calculated using the Nei and Li/Dice similarity index (Nei and Li, 1979), and similarity estimates were analyzed using UPGMA (unweighted pair group method using arithmetic averages). The matrices of mutual coefficients of similarity calculated by PAST were clustered (Agglomerative Clustering) and resulting clusters were expressed as dendrogram. Moreover, Wisconsin polar ordination was applied to ordinate the study species based on the Euclidian distance (Bray and Curtis, 1957).

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Statistical analysis

The variation in the chemical characteristics among the different study species were assessed using one-way analysis of variance (ANOVA 1), after testing the data for normality according to SPSS software (SPSS, 2012). A post-hoc test was applied according to (Duncan's test) when differences are significant.

Results

Primary metabolites

The results of the primary metabolites indicated significant variation in the total soluble carbohydrates among the tissues of the nine study *Aloe* species (*Table 2*). In addition, the post-hoc test (Duncan's test) indicated significant differences between each pair of the target species. *A. armatissima* had the highest content of proteins (106.98 mg g^{-1}), while *A. brunneodentata* had the highest carbohydrates' content (4.05 mg g^{-1}). On the other side, the lowest contents of proteins and carbohydrates (35.19 and 0.25 mg g^{-1}) were recorded in the tissues of *A. parvicoma* and *A. fleurentiniorum*, respectively.

Table 2. *Variations in the primary metabolites' content (Mean ± SD) in the tissues of the nine Aloe species. Maximum and minimum values are underlined*

Species	Primary metabolites	
	Proteins	Carbohydrates
	mg g^{-1} DW	
Aloe hijazensis	63.88 ± 1.25f	1.07 ± 0.09f
Aloe castellorum	59.04 ± 1.34g	2.13 ± 0.20d
Aloe fleurentiniorum	82.56 ± 2.52c	0.25 ± 0.01i
Aloe sabaea	71.73 ± 2.94e	0.56 ± 0.03g
Aloe brunneodentata	91.29 ± 6.41b	4.05 ± 0.71a
Aloe vera var. *officinalis*	50.81 ± 1.16h	3.12 ± 0.23c
Aloe x abhaica	77.46 ± 3.95d	0.44 ± 0.12h
Aloe armatissima	106.98 ± 8.48a	1.46 ± 0.23e
Aloe parvicoma	35.19 ± 1.90i	3.62 ± 0.74b
F-value	**267.6***	**283.8***

Means with the same letters are not significant according to Duncan's test. *P < 0.05

Based on the contents of the primary metabolites, the nine *Aloe* species were classified, using the UPGAMA clustering analysis, into 6 clusters (*Fig. 2*): (A) included *A. castellorum* and *A. hijazensis;* (B) included *A. vera* var. *officinalis;* (C) included *A. parvicoma;* (D) comprised *A. fleurentiniorum*, *A. sabaea;* and *A. abhaica;* (E) comprised *A. brunneodentata;* and (F) comprised *A. armatissima.* Moreover, the Wisconsin polar ordination confirmed the segregation of the study species into the same 6 clusters (*Fig. 3*).

Secondary metabolites

The results of the analysis of the secondary metabolites indicated significant variation in the alkaloids, cardiac glycosides, flavonoids, and phenolic compounds

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among the shoots of the nine study *Aloe* species (*Table 3*). In addition, the post-hoc test (Duncan's test) indicated significant variations in the investigated metabolites among the target species. Besides, there is a difference between each species pairs in the investigated secondary metabolites, except flavonoids. It was found that *A. hijazensis* had the highest value of phenolic compound (25.02 mg g^{-1}), and the lowest of alkaloids (2.30 mg g^{-1}), in contrast with *A. parvicoma*, which had the highest alkaloids' content and the lowest phenolic compounds (13.08 and 4.21 mg g^{-1}, respectively). Moreover, the highest cardiac glycosides and flavonoids' contents (21.09 and 18.04 mg g^{-1}) were recorded in the leaves of *A. fleurentiniorum* and *A. castellorum*, respectively, while the lowest contents (9.07 and 6.33 mg g^{-1}) were recorded in *A. brunneodentata* and *A. armatissima*.

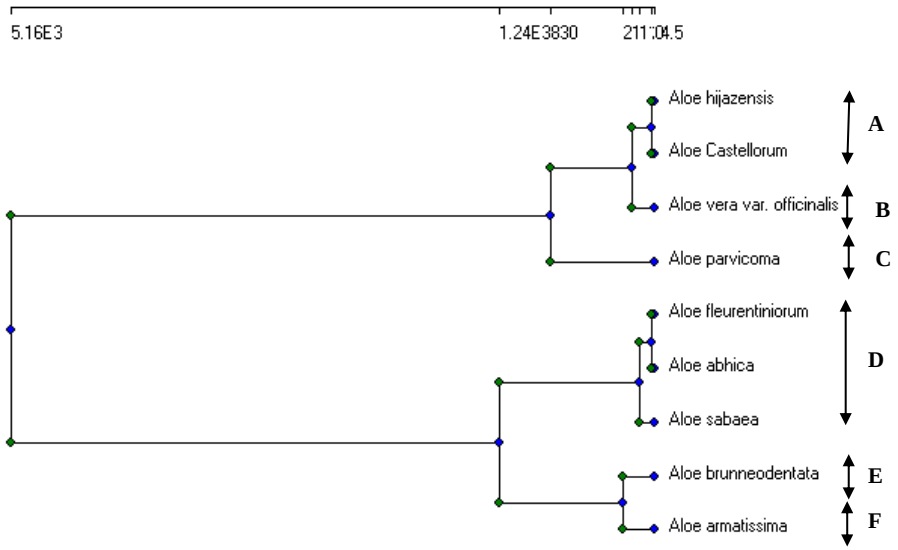

Figure 2. *The dendrogram resulting from the application of the agglomerative clustering technique on the contents of the primary metabolites of the nine study Aloe species*

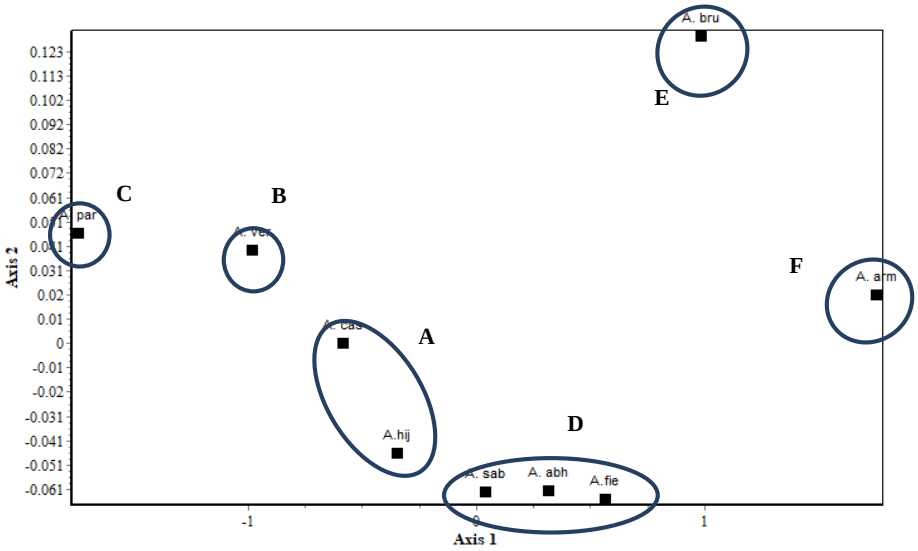

Figure 3. *Similarity ordination resulting from the application of non-metric similarity analysis on the primary metabolites of the nine study Aloe species*

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Table 3. *Variations in the secondary metabolites' content (mean ± SD) in the tissues of the nine Aloe species. Maximum and minimum values are underlined*

Species	Secondary metabolites			
	Alkaloids	Cardiac glycosides	Flavonoids	Phenols
	mg g^{-1} DW			
Aloe hijazensis	2.30 ± 0.19i	11.49 ± 1.78f	14.03 ± 2.59b	25.02 ± 2.68a
Aloe castellorum	4.09 ± 0.70g	12.10 ± 0.47e	18.04 ± 2.56a	21.99 ± 1.50b
Aloe fleurentiniorum	11.06 ± 0.75c	21.09 ± 0.58a	9.02 ± 2.08d	13.05 ± 1.33f
Aloe sabaea	8.21 ± 0.13d	17.08 ± 1.81c	11.08 ± 1.53c	13.15 ± 1.33e
Aloe brunneodentata	12.11 ± 1.33b	9.07 ± 1.53i	9.17 ± 1.52d	19.13 ± 0.58c
Aloe vera var. *officinalis*	6.27 ± 0.68f	16.14 ± 0.56d	14.11 ± 1.01b	5.05 ± 0.05h
Aloe x abhaica	2.88 ± 0.02h	9.23 ± 1.10h	7.42 ± 0.21f	9.86 ± 0.21g
Aloe armatissima	7.68 ± 0.22e	10.63 ± 1.53g	6.33 ± 0.32g	16.15 ± 2.68d
Aloe parvicoma	13.08 ± 0.95a	17.22 ± 1.71b	8.18 ± 1.51e	4.21 ± 0.10i
F-value	**301.1***	**510.4***	**270.3***	**917.2***

Means with the same letters are not significant according to Duncan's test. *P < 0.05

Based on the contents of the secondary metabolites, the nine *Aloe* species were classified, using the UPGAMA clustering analysis, into four clusters (*Fig. 4*): (A) included *A. castellorum* and *A. hijazensis;* (B) included *A. brunneodentata* and *A. armatissima; (*C) included *A. abhaica;* (D) comprised *A. fleurentiniorum* and *A. sabaea;* and (E) comprised *A. parvicoma* and *A. vera* var. *officinalis.* Moreover, the Wisconsin polar ordination confirmed the segregation of the study species into the same 5 clusters (*Fig. 5*).

Clustering analysis of the total chemical constituents

The application of the agglomerative clustering technique, using the UPGAMA clustering analysis, on the total chemical constituents of the nine *Aloe* species indicated that all the study species were different from each other with long Eucladian distances (*Fig. 6*). According the most related species, 6 similarity clusters were recognized: (A) included *A. castellorum* and *A. hijazensis;* (B) included *A. fleurentiniorum, A. sabaea* and *A. abhaica;* (C) comprised *A. brunneodentata;* (D) included *A. armatissima;* (E) comprised *A. vera* var. *officinalis;* and (F) comprised *A. parvicoma.* Moreover, the Wisconsin polar ordination confirmed the segregation of the study species into the same 6 clusters (*Fig. 7*).

Discussion

Phytocompounds are responsible for the potent biological activities of the *Aloe* species (Babu and Noor, 2020). Primary metabolites such as carbohydrates and proteins, and secondary metabolites including alkaloids, flavonoids, sterols, saponins, coumarins, and phenolic acids are among the phytoconstituents found in the *Aloe* genus (Singh et al., 2022). The biosynthesis of plant metabolites although controlled by genetic factors is affected by environmental influences, plant type, and functionally

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different plant parts (El-Bakry et al., 2014). As revealed by the present study, *Aloe* species showed significant variations in the concentration of primary and secondary metabolites. *A. armatissima* had the highest content of proteins (106.98 mg g^{-1}), while *A. brunneodentata* had the highest carbohydrates' content (4.05 mg g^{-1}). On the other side, the lowest contents of proteins and carbohydrates (35.19 and 0.25 mg g^{-1}) were recorded in the tissues of *A. parvicoma* and *A. fleurentiniorum*, respectively. The accumulation of carbohydrates may be due to reduction in their utilization, either as a source of energy or for the formation of new cells and tissues (Harish and Murugan, 2011). Carbohydrates are energy-rich molecules that play an important role in the immune system, pathogenesis, blood clotting, fertilization, and protein folding and placement (Galal et al., 2022**)**. However, the protein pattern changes are accompanied by the biological changes in the adaptation process, which makes the organism more fit in an altered environment (El-Bakry et al., 2014).

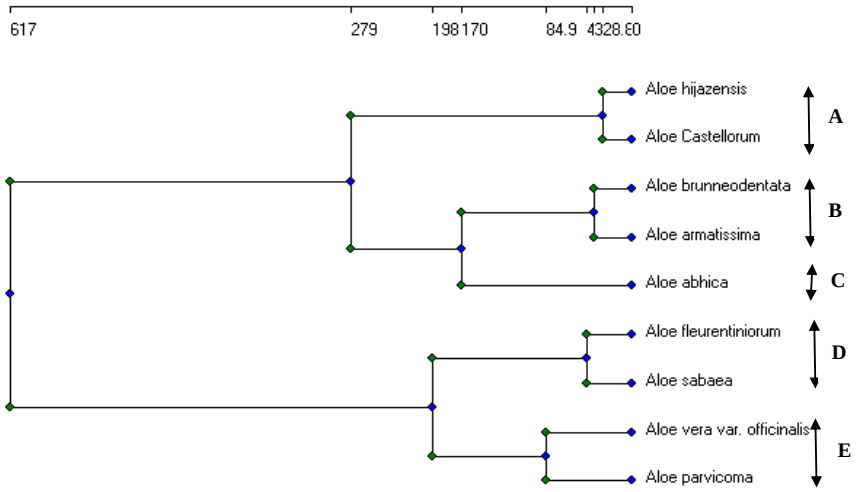

Figure 4. *The dendrogram resulting from the application of the agglomerative clustering technique on the contents of the secondary metabolites of the nine study Aloe species*

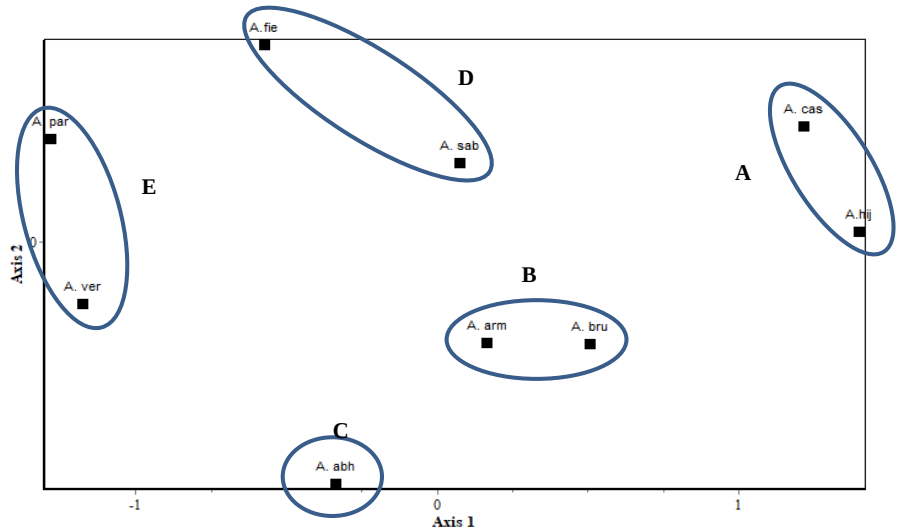

Figure 5. *Similarity ordination resulting from the application of non-metric similarity analysis on the secondary metabolites of the nine Aloe species*

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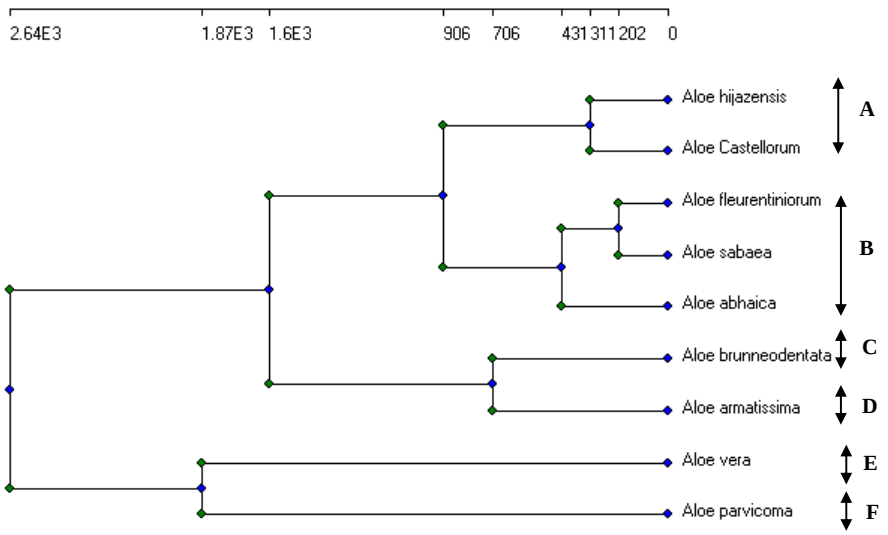

Figure 6. *The dendrogram resulting from the application of the agglomerative clustering technique on the total chemical constituents of the nine study Aloe species*

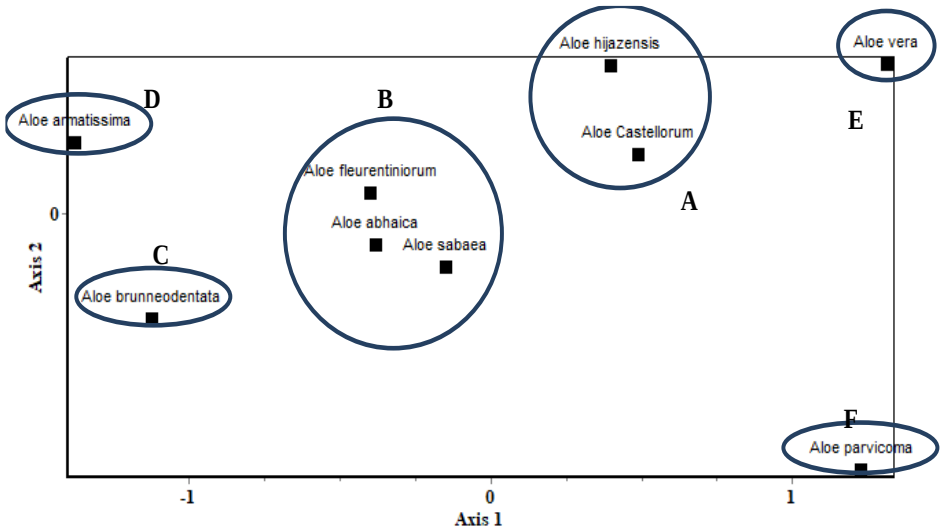

Figure 7. *Similarity ordination resulting from the application of non-metric similarity analysis on the total chemical constituents of the nine Aloe species*

Secondary metabolites are organic chemicals created by organisms such as plants, fungi, or bacteria as a result of secondary metabolic processes that result in the creation and accumulation of different chemical compounds (Galal et al., 2022). Secondary metabolites are created near the end of the growth phase and hence are not directly engaged in the organism's typical physiologic activities such as growth, development or reproduction (Zandavar and Afshari Babazad, 2023). Phytochemical studies on the genus *Aloe* have shown that the plants from this genus are rich sources of different classes of compounds such as flavonoids; alkaloids, and phenolic compounds. These classes of compounds have been shown to possess antiviral, anti-tumor and antibacterial activities; thus, plants from *Aloe* genus should be explored further as an alternative source of medicine (Ombito et al., 2015). The results of the analysis of the secondary

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metabolites indicated significant variation in the alkaloids, cardiac glycosides, flavonoids, and phenolic compounds among the shoots of the nine study *Aloe* species. According to Dagne et al. (2000), the leaves of *Aloe* species are store houses of many interesting secondary metabolites belonging to different classes of compounds including alkaloids, anthraquinones, flavonoids, coumarins and phenolic acids.

The previous phytochemical screening of *Aloe* species revealed the presence polysaccharides, flavonoids, carbohydrates, coumarins, tannins, chromones, alkaloids, anthraquinones, organic compounds, pyrones, phytosterols, anthrones, sterols, vitamins, proteins, and mineral constituents in the different plant organs (Salehi et al., 2018; Nalimu et al., 2021; Singh et al., 2022). The results of the analysis of the secondary metabolites in the current study indicated significant variation in the alkaloids, cardiac glycosides, flavonoids, and phenolic compounds among the leaves of the nine study *Aloe* species. Phenols are major group of compounds acting as primary antioxidants or free radical scavenger (Adesuyi et al., 2012), while alkaloids have the potential use in the elimination and reduction of human cancer cell lines (Nobori et al., 1994) and have powerful pain killer medications (Kam and Liew, 2002). In addition, flavonoids are antioxidants and has been proved to exhibit a wide range of biological activities like antimicrobial, anti-inflammatory, antiangionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Gouda, 2018).

Tizazu and Bekele (2024) recorded flavonoids, simple and complex polysaccharides, minerals, vitamins, enzymes, hydrocarbons, fatty acids, indoles, pyrimidines, aldehydes, ketones, dicarboxylic acids, and alkaloids in the different tissues of *Aloe* species. These chemical constituents were present in the different *Aloe* species but at varying concentrations (Nalimu et al., 2021). In the present study, the highest cardiac glycosides and flavonoids' contents (21.09 and 18.04 mg g^{-1}) were recorded in the shoots of *A. fleurentiniorum* and *A. castellorum,* respectively, while the lowest contents (9.07 and 6.33 mg g^{-1}) were recorded in *A. brunneodentata* and *A. armatissima.* Cardiac glycosides are a class of secondary metabolites that are traditionally used to increase cardiac contractile force in patients with congestive heart failure and cardiac arrhythmias (Abarquez, 2001). According to Nalado and Abduljabbar (2023), cardiac glycosides have antimicrobial potential and also play a great role in curing a variety of diseases such as heart arrhythmia, anti-inflammatory effect and heart congestion. Moreover, *A. hijazensis* had the highest value of phenolic compound, but the lowest of alkaloids (25.02 and 2.30 mg g^{-1}, respectively). On the contrary, *A. parvicoma* had the highest alkaloids' content and the lowest phenolic compounds (13.08 and 4.21 mg g^{-1}, respectively). Di Scala et al. (2013) recorded 0.37 mg g^{-1} total phenolic, while Adesuyi et al. (2012) recorded 24.71, 32.46 and 2.32 mg g^{-1} alkaloid, flavonoid and phenolic contents, respectively in the tissues of *A. vera*. Flavonoids and alkaloids were higher, but phenolics were lower than those recorded in the investigated *Aloe* species. Alkaloids contain antiproliferative, antimicrobial, and antioxidant properties that can be exploited in medication development (Zandavar and Afshari Babazad, 2023). Alkaloids have been analyzed from *Aloe* species quantitatively and qualitatively (Usman et al., 2020). They were recorded in the gel, latex, skin, whole leaf (Salehi et al., 2018) and roots, and flowers (Sánchez et al., 2020; Yadeta, 2024) of various *Aloe* species such as *A. adigratana, A. barbadensis, A. calidophila, A. ferox, A. vera, A. turkanensis,* and *A. Gilbertii.*

In view point of chemosystematics, the analysis of primary metabolites indicated a great similarity between *A. castellorum* and *A. hijazensis* on one hand and *A.*

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fleurentiniorum, A. sabaea; and *A. abhaica* on the other hand. Additionally, *A. vera* var. *officinalis, A. parvicoma, A. brunneodentata,* and *A. armatissima* were significantly different. However, the secondary metabolites revealed great similarity between *A. brunneodentata* and *A. armatissima; A. castellorum* and *A. hijazensis; A. fleurentiniorum* and *A. sabaea;* and *A. parvicoma* and *A. vera* var. *officinalis;* while *A. abhaica* was remarkedly different. Furthermore, using the UPGAMA clustering analysis, on the total chemical constituents of the nine *Aloe* species indicated that all the study species were greatly different except *A. castellorum* and *A. hijazensis;* and *A. fleurentiniorum, A. sabaea* and *A. abhaica,* which are notably similar.

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Conflict of interests. The authors declare that they have no competing interests.

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