

Research Article

Chemical and Biochemical Evaluation of *Aloe vacillans* Forsskål forms and their Taxonomical Significance Cultivated in Amran Governorate-YemenKhlood A. A. H. Almahzoum*¹, Maher Ali Almaqtari ² ¹Department of Chemistry, University of Aden, Aden,-Yemen²Department of Chemistry, Faculty of Sciences, Sana'a University, Yemen.<https://doi.org/10.47372/uajnas.2025.n2.a02>

ARTICLE INFO	Abstract
Received: 04/01/ 2026 Accepted: 19/02/ 2026	The present study aimed to evaluate the chemical, biochemical, and phytochemical properties of the endemic <i>Aloe vacillans</i> Forsskål leaf extracts, focusing on populations cultivated in the Amran Governorate, Yemen, and determining their taxonomical significance. A total of 59 characteristics (26 qualitative and 33 quantitative) were analyzed. Numerical and statistical analyses confirmed the existence of two distinct morphological forms: red-flowered and yellow-flowered. Phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, tannins, and saponins. (FT-IR) spectroscopy identified key functional groups, including hydroxyl (OH), C-H, C=C, and carbonyl (C=O) groups, in both forms. The antioxidant activity, assessed via the ferric-bipyridine reduction (FBRC) method, revealed high capacities of $244 \pm 5.2 \mu\text{g ASA/mg}$ for the red form and $232 \pm 4.8 \mu\text{g ASA/mg}$ for the yellow form, with statistically significant differences ($P < 0.05$). Antimicrobial potential was evaluated against four human pathogens (<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , and <i>C. albicans</i>) using the agar well diffusion method. The red form consistently exhibited superior antimicrobial activity compared to the yellow form across all tested concentrations. These findings provide robust evidence for the taxonomical distinction between the two forms and highlight their potential as natural sources for pharmaceutical applications.
Keywords: <i>Aloe vacillans</i> ; Amran-Yemen; Phytochemical screening, Antioxidant, Taxonomical significance; Antimicrobial activity.	

1. Introduction

The word Aloe, which means a bitter shining material, comes from Arabic (alloe). Africa is where this plant genus first appeared, and there are almost 200 species of this genus [1]. Aloe, historically used as a laxative in a large dose or a stomachic in a small dose in the form of juice (or whole leaf) extract and as a healing agent of burns skin ailments, and wounds in the form of fresh juice for more than 20 centuries, fell into disuse in the West as the seat of civilization moved to the temperate zones where the tropical plant could not survive the freezing winters [2]. The genus Aloe exhibits typical monocotyledonous characteristics, with identification relying on morphological traits such as rosette leaf arrangement, compact racemose inflorescence, predominant flower coloration, and thickened cuticle enhancing adaptation to harsh environmental conditions [3]. The genus *Aloe L.* encompasses over 600 taxa, including subspecies and varieties [4,5], mainly

found in eastern and southern Africa, with limited presence in southwestern Arabia and Socotra Island [6,7]. Southern, eastern, and northeastern Africa exhibit the highest species density of aloes. Historically, aloe has been used as a medicinal plant, with *Aloe vera* noted in ancient herbal texts [8]. The leaves of *A. vera* are used for various medicinal purposes, including the treatment of mild fevers, wounds, burns, digestive disorders, diabetes, cancer, beauty health, and skin ailments [9,10]. *Aloe (Liliaceae)* is a tropical plant species with longstanding significance in traditional medicinal practices across diverse cultural contexts. Its gel, derived from thin-walled parenchyma within the foliar tissue, demonstrates extensive biological activities applicable across food processing, pharmacology, and dermatological product development sectors. Numerous studies have elucidated *Aloe* therapeutic potential in cosmetics, attributable to its high content of bioactive - including vitamins, enzymes,

polysaccharides, and amino acids -that confer beneficial attributes such as hydration enhancement, inflammation reduction, microbial inhibition, and wound repair promotion. This renders *Aloe* an invaluable component in contemporary skin-care preparations [11].

The leaf extract of *Aloe* is renowned for its calming effect on the skin and alleviation of localized inflammation. These properties are attributed to the wide range of biologically active substances present in the leaf extract. Notably, the significant anti-inflammatory activity of *Aloe* extract stems from the enzyme bradykinase. Additionally, aloin and vitamins C and E found in the extract provide protection against harmful ultraviolet radiation (UVB), environmental pollution, and chemical irritants. Phytosterols from *Aloe* enhance collagen synthesis and improve skin elasticity through their estrogen-like action on ER α and β receptors in dermal fibroblasts. Furthermore, *Aloe* contains several compounds capable of inhibiting fungal, viral, and bacterial growth [12].

A. vacillans is more variable than most *Aloe* in Yemen, like *A. vera* var. *officinalis*; it has two distinct morphological forms, which have been described as two separate species. The red form, with red flowers, was described as *A. audhalica* by Lavranos [13], and the yellow form, with yellow flowers, is described as *A. dhalensis*[13]. Moreover, the distribution of these two morphological forms is of particular interest since the yellow form (Figure 1B) is mainly found in the southern part of the country, while the red form (Figure 1A) is found in the northern part. Both forms occur with approximately equal frequency in Ibb Governorate and along the Sana'a-Amran Road [7,14]. As previously mentioned, chemical and biochemical properties, including primary and secondary metabolites, are important characteristics for identifying and distinguishing different plant taxa at the genus and species level. Among the endemic flora of Yemen, *Aloe vacillans* Forsskål stands out as a significant medicinal plant. This study focuses on populations cultivated in the Amran Governorate, a region characterized by unique environmental conditions that may influence the phytochemical profile of this species. By investigating these specific endemic forms, this research contributes to the geographical and environmental documentation of Yemen's biodiversity.

Furthermore, *A. vacillans* is represented in Yemen by two morphological forms, which are considered distinct species. *A. vacillans* Forsskål is an endemic species to the southwestern Arabian Peninsula. The study aimed to evaluate the chemical and biochemical properties of these two forms of *A. vacillans* in Yemen and to determine their taxonomical relationship based on these properties.

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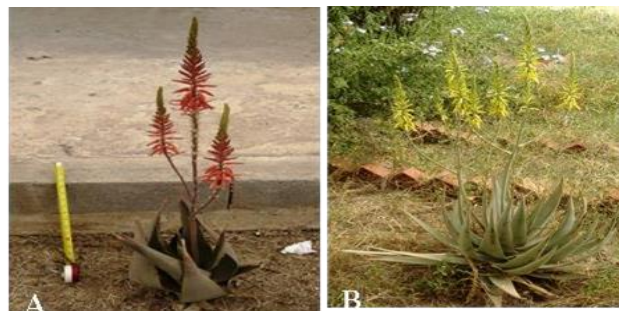


Figure 1. General view of *A. vacillans*: A - Red form; B - Yellow form.

2. Materials and methods:

2.1. Study Area and Plant Collection

Samples of *Aloe vacillans* Forsskål (both red and yellow-flowered forms) were collected from their natural habitats in the Amran Governorate in September–November, Yemen (Latitude: 15.65° N, Longitude: 43.94° E). This region is characterized by a high-altitude semi-arid climate, with an elevation ranging from 2,000 to 2,500 meters above sea level (figure 2). The unique environmental stressors in this mountainous area, including significant temperature fluctuations and specific soil compositions, play a vital role in shaping the phytochemical profile and secondary metabolite accumulation of this endemic species. The plant material was identified and authenticated at the Department of Biology, Faculty of Science, Sana'a University. Botanical identification was performed by Dr. Hassan Ibrahim, Biology Department, Faculty of Science, Sana'a University. All chemicals and reagents used in this study were obtained from Sigma-Aldrich (USA) and Himedia (India).



Figure 2. Geographical location of the study area: Amran Governorate, Yemen, showing the sampling sites (●) for *Aloe vacillans* forms.

2.2. Leaves Treatment

Fresh leaves of each form of *A. vacillans* were treated as follows:

1. Washing: Fresh leaves of each form of *A. vacillans* were thoroughly washed with tap water to remove any dirt, debris, or contaminants.
2. Cutting: The cleaned leaves were cut into small pieces to facilitate the drying process.
3. Drying Method: The cut leaves were dried using Air Drying: The pieces were spread out in a single layer on clean, dry trays or screens in a well-ventilated area, away from direct sunlight, for several days until they were completely dried.
4. Grinding: Once fully dried, the leaves were ground into a fine powder using an electric grinder.
5. Sieving: The powdered samples were then sieved through a 300 μm mesh to ensure uniform particle size.
6. Storage: The sieved powder of each sample was stored in air-tight cellophane bags and kept in a refrigerator until further analysis.

2.3. Extraction of essential oil

For essential oil extraction, 25 g of *A. vacillans* (two forms, individually) powder was extracted with 250 mL of petroleum ether (60-80 °C) using a Soxhlet apparatus [15,16] at the National Center of Public Health Laboratories. After each extraction, the solvent was completely removed by evaporation using a water bath and oven at 40-50 °C and finally at room temperature [17].

2.3.1 Acetone extraction

The process of extraction using acetone included some significant steps. To ensure uniformity, two 10-gram samples of *A. vacillans* were selected. Acetone was utilized as a solvent because it is very efficient in terms of dissolving organic molecules. Both samples were mixed with 100 mL of acetone and agitated for 3 hours at a rate of 220-230 rpm [17] to enhance the extraction of soluble molecules. After shaking, the mixture was filtered with Whatman filter paper No. 1 to differentially isolate the solid and liquid extract. The filtered extract was transferred to a fresh evaporation flask for the acetone evaporation step. The acetone was then evaporated in a rotary evaporator under vacuum conditions with a temperature below 56 °C. This evaporation was conducted until all the acetone was removed, resulting in a concentrated extract, which was kept for further analysis of proteins and enzymes in the Physical Chemistry and Biochemistry Research laboratory, Department of Chemistry, Faculty of Science, Sana'a University.

2.3.2 Aqueous extraction

To prepare the aqueous extract, 10 g of *A. vacillans* powder is combined with 100 mL of distilled water. The mixture is continuously stirred at a speed of 220–230 rpm for 9 hours using a magnetic stirrer, to ensure uniform mixing and effective extraction of soluble compounds from the *Aloe vacillans* powder. After stirring, the mixture is filtered through Whatman No. 1 filter paper to separate the solid residue, yielding a clear aqueous extract. Although not explicitly detailed, the evaporation step typically involves gentle heating via rotary evaporation or simple thermal methods to reduce water content and concentrate the extract. The resulting product of *Aloe vacillans* is a potent aqueous extract, suitable for subsequent applications or analysis [17].

2.3.3 Ethanol extraction:

Preparation of the ethanol extract: 10 g of *Aloe vacillans* powder (from two different forms) were extracted with 100 mL of ethanol (95% v/v). The mixture was stirred continuously at 220–230 rpm for 6 hours using a magnetic stirrer to facilitate the dissolution of active constituents. Following the extraction period, the mixture was filtered through Whatman filter paper No. 1 to separate the solid residues. The ethanol in the filtrate was then removed by rotary evaporation under reduced pressure, yielding a concentrated extract. The final product was stored in a cool, dark environment until further biochemical analysis.

2.4 UV-vis spectroscopy

The UV-Vis spectra of both the petroleum ether and aqueous extracts derived from the two morphological forms of *Aloe vacillans* leaves were recorded in the wavelength range of 200 to 400 nm by a UV-3101PC spectrophotometer at the Yemen Standardization Metrology and Quality Control Organization, Sana'a, Yemen [18].

2.5 FT-IR spectroscopy

The FT-IR spectra of the petroleum ether extract and aqueous extract from *A. vacillans* of the two forms of *A. vacillans* leaves extract were recorded on the FT-IR-8400 S spectrometer at Yemen Standardization Metrology and Quality Control Organization, Sana'a, Yemen, employing an attenuated total reflectance (ATR) crystal (ZnSe). In total, 32 scans were carried out, and a resolution of 4 cm^{-1} was used [18].

2.6 GC-MS analysis

The petroleum ether extract of *Aloe vacillans* leaf samples was analyzed using a GCMS-QP2010 Plus (Shimadzu, Japan) at the Yemen Standardization, Metrology, and Quality Control Organization in Sana'a, Yemen. The instrument was equipped with a Shimadzu AOC-20i GC auto-sampler and an Alltech Heliflex® AT™-502.2 capillary column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness). Operating Parameters (Column Name: Heliflex® AT™ 502.2, Length: 30 meters,

Inner Diameter: 0.25 mm, Film Thickness: 0.25 μm). The injection parameters were set as follows: injection volume of 2.0 μL , injection temperature profile starting at 80 $^{\circ}\text{C}$ (held for 1 min), ramping to 200 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C}/\text{min}$, followed by further increase to 250 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$. The analytical column operated under a constant pressure of 108.0 kPa, maintaining a flow rate of 1.58 mL/min within the column and a total system flow rate of 6.2 mL/min, resulting in a linear velocity of 46.3 cm/s. Helium was the carrier gas and the temperature was programmed at 80.0 $^{\circ}\text{C}$ (hold time, 2 min), to 200.0 $^{\circ}\text{C}$ (4.00 min) to 280 $^{\circ}\text{C}$ (10 min). The ACQ of the MS was set to scan between 40.00 m/z to 800.00 m/z at a scan speed of 1666 with a start time of 3.00 min and an end time of 30.00 min [19]. Compound identification was carried out using the NIST Mass Spectral Library and the Wiley Registry of Mass Spectral Data, facilitating the profiling of volatile phytochemicals present in the petroleum ether extract [19]. "ACQ" refers to "data acquisition process" denoting the process of collecting ionized mass data during analysis.

3. Results and Discussions

Firstly, the judicious selection of extracts based on solubility, polarity, and compatibility with analysis ensured complete analysis of *A. vacillans* phytochemical content. In so doing, it ensured maximum extraction of targeted compounds, enabling exact and reliable analysis across methods.

3.1. Determination of qualitative constituent of *A. vacillans* leaf extract

A. vacillans showed the presence of various phytochemical groups of constituents; From the results, the aqueous extract of both species of *A. vacillans* contains alkaloids, flavonoids, phenolic compounds, tannins, proteins, carbohydrates, saponins and siphoning. This agrees with [38,39] compared to results of similar analyses obtained in the aqueous extract of *A. tororoana* and *A. barbadensis*, respectively. Moreover, aqueous extract of *A. barbadensis* was found to be positive for protein and carbohydrate content [38]. These phytochemicals have been documented to exhibit varied biological activities, such as antioxidant, antimicrobial, and medicinal activities. The exact composition may vary depending upon the extraction methods and environmental conditions, indicating the need for a complete phytochemical study in independent research.

3.2. UV-Vis spectroscopy

UV-Vis spectroscopy is a valuable technique for identifying the presence of specific phytochemicals in plant extracts due to its ability to probe electron distribution in conjugated systems. The absorbance bands observed in the UV spectra of *A. vacillans* extracts can be directly correlated with the presence of phytochemicals identified in qualitative tests.

Observations from UV-Vis Spectra:

- Red Form: Absorbance bands at 310 nm (broad), 370 nm (medium), and 340 nm (small).
- Yellow Form: A large band at 340 nm and a small band at 320 nm.
- Aqueous Extracts: Red form showed absorbance at 270.40 nm (0.6516 A), while the yellow form had bands at 311.60 nm (0.7778 A) and 267.40 nm (1.0223 A).

Correlation with Phytochemicals:

1. Flavonoids: Typically exhibit absorbance around 270 nm and 310 nm, correlating with the peaks observed in the aqueous extracts. This suggests their presence in both forms of *A. vacillans*.
2. Phenolic Compounds: Often show absorbance in the range of 280-320 nm. The bands in the UV spectra align with this range, indicating the potential presence of phenolic compounds.
3. Alkaloids: While alkaloids have a broader range of absorbance, the UV spectra can indicate their presence through specific peaks, particularly around 300 nm.

The observed, spectrum of the red form of *A. vacillans* presented a band to a longer wavelength than those of the yellow form (Figures 3-5) for the petroleum ether extract and aqueous extract, respectively, of the two forms of *A. vacillans*, according to [40-42].

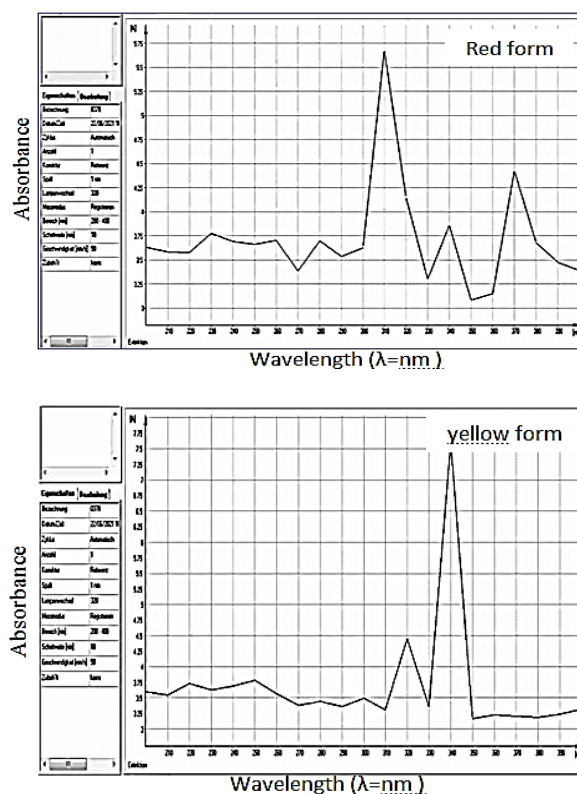


Figure 3. UV-vis spectra of the petroleum ether extract of *A. vacillans*

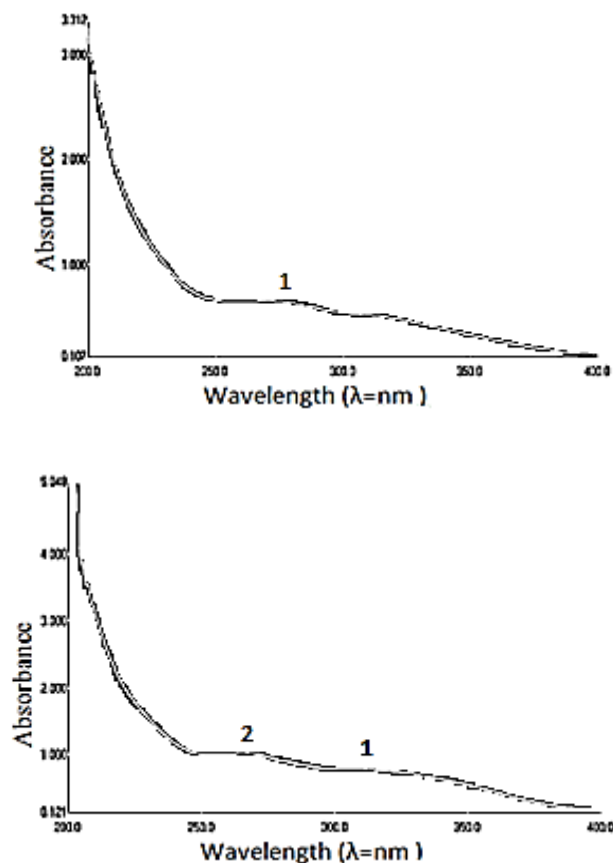


Figure 4. UV-vis spectra of the aqueous extracts of *A. vacillans*: (up) - red form; right - yellow form

The FT-IR analysis of the petroleum ether extracts of the two forms of *A. vacillans* (red and yellow flowers) confirmed the presence of various functional groups associated with important chemical compounds.

These included hydrogen-bonded –OH groups of alcohols, phenols, and carboxylic acids appears at (3568–3283 cm^{-1} red, 3568–3210 cm^{-1} yellow)^[42-45], while the bands at (2994–2852 cm^{-1} red) due to sp^3 of CH_3 symmetric and asymmetric stretches; the presence bands at (1261–1242 cm^{-1} red, 1270–1245 cm^{-1} yellow) show group C–O (ether, ester, phenol, alcohol, and carboxylic acids)^[43-45]. Also, there are other bands showing some groups, such as hydrogen-bonded carboxylic acids, aliphatic CH_2 and CH_3 bending.

The spectra revealed the presence of C–H alkanes, C–C stretches, and aromatic –C=C–H groups (Table 1, Figure 5). The detected functional groups align well with earlier studies that reported the presence of similar bonds in alkaloids, flavones, and other compounds extracted from related species [42–46]. This outcome further demonstrates that the sample is entirely composed of calcite. According to the literature, the bands at around 3100 cm^{-1} and 3457 cm^{-1} are attributed to the symmetric and anti-symmetric stretching of the water's O–H bond, respectively.[23][24].

Fig.2a shows a tiny peak at 200°C belongs to the loss of ore's moisture content while the second very sharp small narrow peak may result from the loss of crystallization water content. The calcination of the lime-stone mineral calcite is the cause of the last broad, wide major endothermic peak. This peak, which begins at 550°C and finishes at around 650°C, represents the breakdown of Y-LSM-Nak (**Error! Reference source not found.**c) into carbon dioxide (CO_2) and calcium oxide (CaO). **Error! Reference source not found.**c's data indicates that the weight remained constant at 56.06 percent. It is assumed that the sample is made up entirely of CaCO_3 based on the mass loss related to CaCO_3 breakdown.

Table 1. Relevant composition chemical composition of the limestone samples

Functional groups	Wavenumbers (cm^{-1}) Red form	Wavenumbers (cm^{-1}) Yellow form
OH	3568-3283	3568-3210
CH_3	2994-2852	2995-2849
Hydrogen-bonded carboxylic acids	2591-2546	2584-2563
C-H alkanes, C-C stretches bands, aliphatic CH_2 bending (scissoring) and aliphatic CH_3 bending (symmetrical)	1436-1309	1436-1309
C-O	1261-1242	1270-1245
–C=C–H	951-896	951-896

The FTIR spectra of the aqueous extracts of the two forms of *A. vacillans* (red and yellow flowers) confirmed the presence of key functional groups associated with various bioactive compounds (Table 2, Figure 5). Both extracts exhibited absorption bands characteristic of OH stretch at (3566-3209 cm⁻¹ red, 3587-3192 cm⁻¹ yellow), sp³ of CH₃ symmetric and asymmetric stretch appears its bands at (2954-2921 cm⁻¹ red, 2955-2922 cm⁻¹ yellow), bands C-O group of (alcohols, ethers, esters, phenol, and carboxylic acids) appear at (1260-1180 cm⁻¹ red, 1273-1246 cm⁻¹ yellow). Additionally, other groups as C-H stretching (sp² and aromatic absorption), Fermi doublet (aldehydes), aromatic C=C stretching, and alkene, these groups are consistent with previous studies reporting similar functional groups in plant extracts [42-46]. These results highlight the potential bioactive nature of the aqueous extracts from both forms of *A. vacillans*.

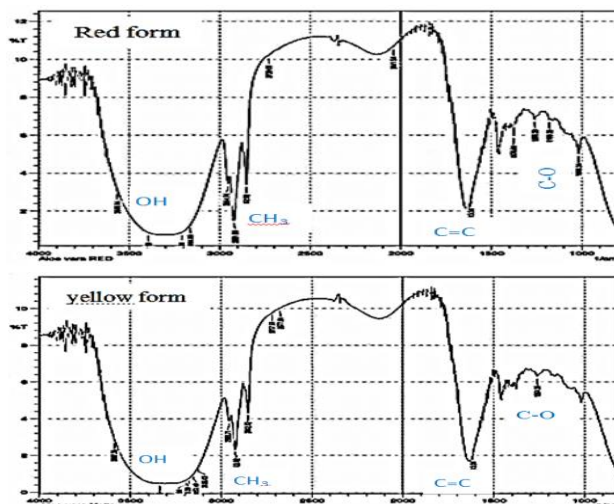


Figure 6. FT-IR spectra of the aqueous extracts of *A.*

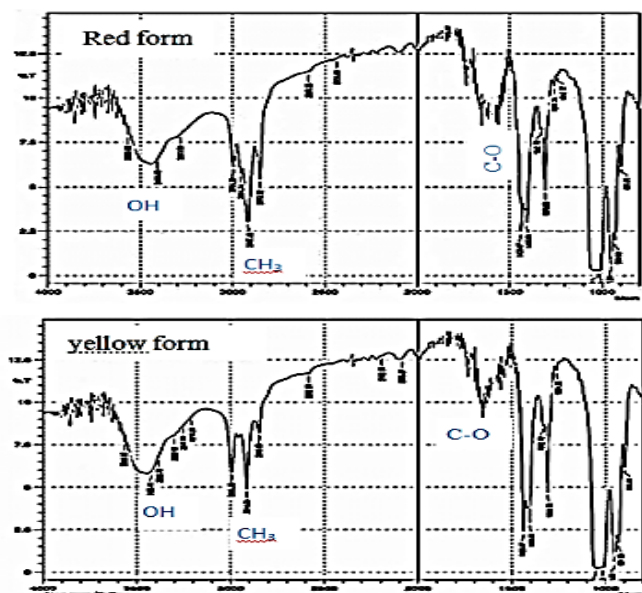


Figure 5. FT-IR spectra of the petroleum ether extracts of *A. vacillans*

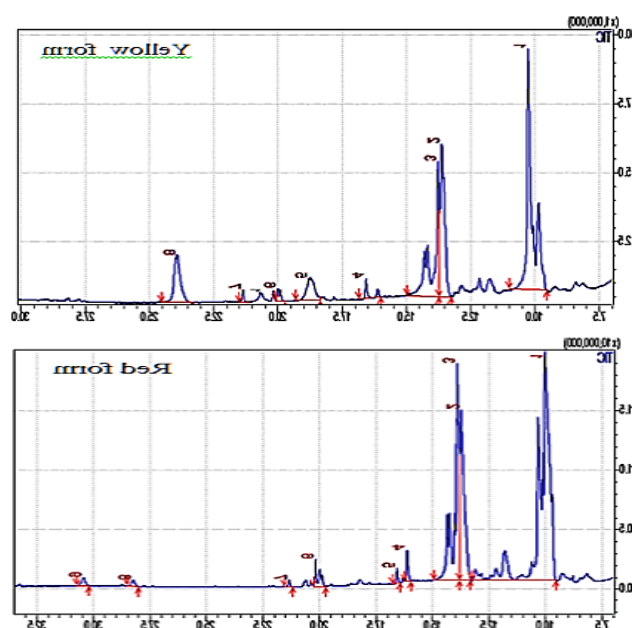


Figure 7. GC-MS spectrum of *A. vacillans*

Table 2. FT-IR results of aqueous two forms extract of *A. vacillans* with their functional groups

Functional groups	Wavenumbers (cm ⁻¹) Red form	Wavenumbers (cm ⁻¹) Yellow form
OH	3566-3209	3587-3192
C-H	3164-2989	3175-3125
CH ₃	2954-2921	2955-2922
Aldehydes -C-H	2852-2729	2852-2717
C=C	1614-1579	1614-1578
C-O	1260-1180	1273-1246

3.3. Gas chromatography-mass spectrometry (GC-MS) analysis of the extract:

The GC-MS analysis of the petroleum ether extract from *A. vacillans* (both red and yellow forms) revealed the presence of several bioactive compounds (Table 3, Figure 7). The red form consisted of prominent compounds such as oleic acid (54.64%), linolenic acid (17.70%), and 2-octylcyclopropene-1-heptanol (22.27%), while the yellow form contained oleic acid (38.73%), linolenic acid (20.86%), and 2-octylcyclopropene-1-heptanol (20.51%) as the dominant compounds.

The percentage values not only depict the relative abundance of these compounds but also denote their existence, which is crucial for our taxonomic comparisons between the two forms. The two fractions contained other compounds like 5-alpha-androstan-16-one, palmitic acid vinyl ester, and other sterols (Table 3). The findings support previous reports [47], which showed the presence of palmitic acid and linolenic acid in methanolic extracts of *A. vacillans* [19], in which oleic acid was shown in an aqueous extract of *A. vera*.

3.4 Quantitative determination of phytochemical constituents of the extract of the two forms of *A. vacillans* leaves by visible spectrophotometry:

Visible spectrophotometry was employed to derive quantitative data on the phytochemical content of *Aloe vacillans* with high levels of alkaloids, carbohydrates, flavonoids, proteins, phenolic compounds, and tannins. The calibration curves derived assisted in quantitative determination, accurately confirming the bioactive potential of the extracts.

Absorbance Measurements:

The absorbance values as illustrated in (Figure 8). For the extracts were recorded at specific wavelengths corresponding to each phytochemical:

- **Alkaloids:** A high absorbance of 5.00.
- **Carbohydrates:** Absorbance values were 2.486 (red form) and 2.948 (yellow form).
- **Flavonoids:** Absorbance values were 1.0392 (red form) and 1.2269 (yellow form).
- **Proteins:** Absorbance values were 0.511 (red form) and 0.489 (yellow form).
- **Phenols and Tannins:** Absorbance values were 0.476 (red) and 0.421 (yellow).

Results Interpretation:

- **Alkaloids:** The high absorbance indicates a significant concentration, corroborating findings from previous studies [48] regarding the presence of alkaloids in *A. vera*.
- **Carbohydrates:** The absorbance values align with literature [38], suggesting high carbohydrate content in both forms of *A. vacillans*.
- **Flavonoids:** The absorbance results for flavonoids are consistent with previous research [38], indicating their presence in the aqueous extracts.
- **Phenolic Compounds and Tannins:** The lower absorbance values correspond to their lesser concentrations relative to other phytochemicals but still suggest notable presence.
- **Proteins:** The absorbance values for proteins support the findings from earlier studies [38] regarding protein content in *A. vacillans*.

Table 3. Gas Chromatography-mass spectrometry (GC-MS) results of *A. Vacillans*

Compound Name	RT (min)		Area		Area (%)	
	Red	Yellow	Red	Yellow	Red	Yellow
Oleic acid	10.033.	10.268	556551930	119504214	54.64	38.73
linolenic acid	13.752	13.631	180315095	64345159	17.70	20.86
2-octylcyclopropene-1-heptanol	13.925	778.13	226838919	63258778	22.27	20.51
16-octadecenyl acetate	16.139	-	12569246	-	1.23	-
Palmitic acid vinyl ester	16.591	160575	6014056	5392155	0.59	1.75
5-alpha-androstan-16-one	20.174	20.008	21371615	4396825	2.10	1.43
Beta-sitosterol	28.258	-	5066018	-	0.50	-
lupeol	30.463	-	7199736	-	0.71	-
Fagarasterol	-	23.939	-	30767000	-	9.97
Gamma-sitosterol	-	180785	-	18529522	-	6.01

3.4. Nutritional value assessment:

To determine the nutritional value as a percentage, we use (mass of part/ mass of all * 100, m/m * 100). *A. vacillans* (red and yellow form) showed a high carbohydrate content percentage (43.55 and 46.75%, respectively), which made the plant a good source of carbohydrates [38,49,50]. Furthermore, regular moisture content was the second highest parameter noted with an average moisture content of 17.43% (red) and 16.84% (yellow), which agreed with [38]. Ash content became important because certain plant samples were high in minerals [51], which were essential for the proper functioning of tissues and acted as second messengers in some biological cascade mechanisms [50,52]. The percentage of crude fiber where also detected represents the fourth highest parameter noted in *A. vacillans* (red and yellow form) leaves; 13.17 and 12.262 % for the red and yellow forms of *A. vacillans*, this result agrees with [49,51,52]. Lipids are generally stowed systems of energy in living organisms, they are major structural elements of biological membranes as phospholipids and sterols [53]. Crude Lipid (Fat) content was found to be the fifth highest constraint origination, 8.86 and 7.45% in the red and yellow forms of *A. vacillans*, respectively. The lowest restriction parameter in *A. vacillans* leaves was the crude protein with a regular amount of 0.0712% for red and 0.0624% for yellow. This result agreed with the finding of [38]. All these nutritional values are shown in the (Table 4).

3.5. Determination of ash:

Ash content was determined as a percentage by mass (% m/m). The ash content was calculated by incinerating the sample at a specified temperature until all organic matter had been completely combusted, leaving behind the inorganic residue. The percentage ash was determined using the equation: Ash content was involved because some of the plant samples had high mineral content [51], which were required in tissue functioning as well as acted as second messengers in some biological cascade systems [52].

From (Table 5) ash content was the third highest with average content of 16.92 and 16.64% in the red and yellow varieties, respectively. This was supported by [50,51], who cited that the mean ash content was among the highest parameters that had been recorded in *Aloe barbadensis* and *Aloe vera* leaves; 16.88 and 19.50%, respectively.

3.6. Protein measurement:

The absorbance of different concentrations of BSA and the two forms of *A. vacillans* is given in (Table 5, Figure 9). The lowest restriction parameter in *A. vacillans* leaves was the crude protein with a regular amount of 0.0712% for red and 0.0624% for yellow. This result agreed with the finding of [38] where the crude protein was one of the lowest parameters in *A. barbadensis* leaves.

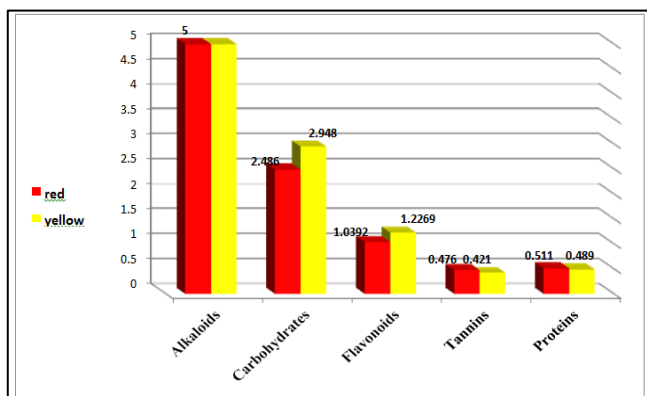


Figure 8. The quantitative results of *A. vacillans* red and yellow absorption spectra

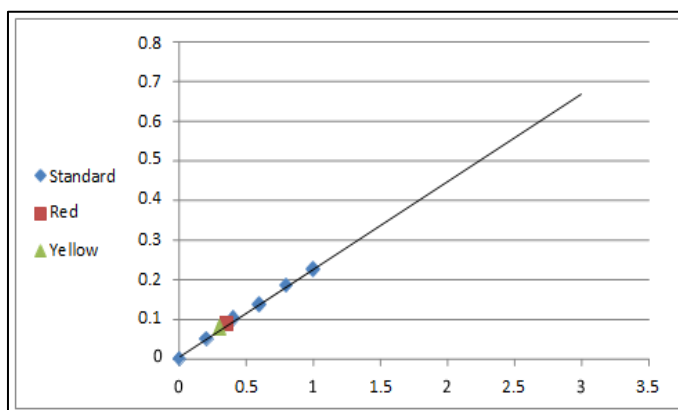


Figure 8. Standard curve of BSA

Table 4. Chemical composition of *A. vacillans* leaves

Composition	Carbohydrates, %	Moisture, %	Ash, %	Fiber,%	Fat, %	Protein, %
Red	43.550±0.003	17.42±0.01	6.920±0.002	13.170±0.003	8.86±0.06	0.071±0.002
Yellow	46.750±0.004	16.84±0.05	16.640±0.005	12.262±0.002	7.447±0.004	0.062±0.003

3.7. Total phenolic content (gallic acid):

The total phenolic content (TPC) of the ethanolic extract of the two forms of *A. vacillans*. 44.4 mg of GA/100 g for the yellow form and the red form, with TPC of 40.4 mg of GA/100 g. These results were close to the findings of [54] where the total phenolic content of *Aloe vera* of high hydrostatic pressure gel was 37.70 mg of GA/100 g. Moreover, the high total phenolic content (TPC) may be due to the high absorption value of flavonoids (1.2269 A) in the yellow form when compared with the red form (1.0392 A).

The argument that the yellow variant contains a greater total phenolic content due to its greater absorption value of flavonoids (1.2269 A) in comparison to that of the red variant (1.0392 A) is a plausible one. Flavonoids are known to have antioxidant activity and are responsible for the total phenolic content. The absorbance values, in general, are greater with greater concentrations of phenolic compounds, and it seems that there is some relationship between absorbance and TPC.

Research evidence supports the idea that phenolic content can be influenced by a variety of factors, such as plant color, environmental factors, and extraction methods. Overall, the findings regarding the TPC of *Aloe vacillans* agree with the literature, and the relationship between flavonoid uptake and TPC provides a logical basis for the disparity observed between the yellow and red types. More research could explore the individual flavonoid compositions of each group in more detail to clarify the sources of these variations in TPC.

3.8. Ascorbic acid measurement:

Determination of Ascorbic acid in the two forms of *A. vacillans* ethanol leaf extract using the developed Ferric-Bipyridine reducing capacity (FBRC) method. The ascorbic equivalent antioxidant capacities (scavenging activity) of the two *Aloe* forms were 244 µg ASA/mg (red form) and 232 µg ASA/mg (yellow form). These results were according with previous studies [55,56], which recorded that *Aloe vera* ethanolic and methanolic leaf gel extracts and *A. vacillans* methanolic leaf gel extracts showed a DPPH scavenging activity (antioxidant activity). The two extracts of *A. vacillans* showed different ranges of antioxidant activity, and this could be related to the different amounts of flavonoids, phenolic compounds [57] and oleic acid [58] in the leaf extract of the two forms of *A. vacillans*.

The antioxidant activity values were indeed very close, indicating that both forms exhibit high antioxidant capacity [49]. Although this small difference may not be biologically significant, it holds considerable importance within the context of this study. Differences in antioxidant activity can be attributed to variations in flavonoid and phenolic compound contents, as well as oleic acid concentrations in the extracts. These compounds are known to account for antioxidant

activity. Despite results showing that both analogs function equally effectively as antioxidants, this minor disparity could become critical in certain applications, such as nutraceutical or pharmaceutical products. Further investigation into the constituents responsible for antioxidant activity might provide insights into optimizing their use in specific scenarios. This finding aligns with previous research findings, validating their reliability and confirming that both forms possess high antioxidant activity similar to other *Aloe* species. Overall, while both varieties of *A. vacillans* demonstrate good antioxidant activity, the slight variation underscores the importance of understanding phytochemical compositions and how they influence efficacy. In comparison with other regional *Aloe* species, the antioxidant capacity observed in *A. vacillans* aligns with findings reported for *Aloe vera* and *Aloe arborescens* [59,60], yet it exhibits a unique chemical profile characterized by specific phenolic concentrations. This comparative analysis underlines the taxonomical distinctiveness of the Yemeni endemic forms (red and yellow) relative to other Arabian Peninsula flora.

3.9. Biochemical analysis:

Antioxidant enzymes play a crucial role in plant adaptation to stress factors by neutralizing excessive reactive oxygen species (ROS). ROS generation increases in response to stressful conditions and can damage cellular components, including DNA, proteins, and lipids, leading to reduced cell viability and tissue functionality. Superoxide dismutase (SOD) neutralizes superoxide radical to form hydrogen peroxide. The result of biochemical analysis of the two forms of *A. vacillans* (Table 6) showed that SOD in red and yellow were 0.275 and 0.273 U/mg, respectively, while the catalase enzyme activity was reported as 3.50 in red form and 5.55 U/mg in yellow form.

Catalase is an oxidoreductase that catalyzes the reaction of converting hydrogen peroxide into oxygen and water and works effectively at high concentrations of hydrogen peroxide. Peroxidase is an oxidoreductase that catalyzes the oxidation of natural compounds and xenobiotics with hydrogen peroxide to form various oxidation products. It operates effectively at low concentrations of hydrogen peroxide. Peroxide performs important functions in plant cells: protecting cells from reactive oxygen species; participating in lignin synthesis; regulating growth processes; and being part of the regulatory system for biotic and abiotic stress; and participating in the formation of auxin and ethylene, as well as the reduction of nitrates and nitrites, thus contributing to nitrogen metabolism, growth, and respiration[61]. Furthermore, peroxidase activity was perceived as 0.0011 U/mg for red and 0.0049 U/mg for yellow; however, amylase activity in the red and yellow was 0.122 U/mg and 0.263 U/mg, respectively.

On the other hand, the biochemical analysis for the two forms of *A. vacillans* showed a reducing biochemical activity of 0.105 mg/mL and 0.145 mg/mL for the red and yellow, respectively. The role of amylase in plants is that with its participation, alpha-1,4-glycosidic bonds are hydrolyzed from storage polysaccharides (amylose, amylopectin, glycogen) to form oligomers and reducing disaccharides, which are used to synthesize biologically active compounds, antioxidants and ATP [62]. These results were close to the biochemical activity (Superoxide dismutase, Peroxidase, Amylase and reducing sugars) recorded for *A. vera* by [51]. The variance in the biochemical activities between *A. vera* and *A. vacillans* may be due to the difference in the methodology of extraction and /or to the difference in the plant material.

3.9. Antimicrobial activity of the essential oil from *A. vacillans*:

Results in (Table 7) showed the antibacterial activity of petroleum ether extract of *A. vacillans* two forms (4 mg/mL) on the selected microorganisms (*E. coli*, *S. aureus*, *P. aeruginosa* and *C. albicans*). The minimum inhibitory concentration of *A. vacillans* (red and yellow forms) petroleum ether extracted against *P. aeruginosa* (1 mm red and yellow) and *S. aureus* (0.5 mm red and yellow) was 5µg (Table 8). While the *A. vacillans* (red and yellow forms) petroleum ether extract at lower concentrations did not show any antibacterial activity against *E. coli*. Moreover, the *A. vacillans* (red and yellow forms) petroleum ether extract (60 µg/ 15 µL) showed (Table 8) a high antibacterial activity against *P. aeruginosa* (7.6 mm red and 7.3 mm yellow, respectively), compared with Ampicillin (zero) and Gentamicin (zero) antibiotics, however, the Ampicillin (14 mm) and Gentamicin (20 mm) showed a high antibacterial activity against *S. aureus* in comparison with *A. vacillans* (red and yellow forms) of petroleum ether extract (60 µg/15 µL), where 6 mm for the red form and 5.6 mm for the yellow form. On the other hand, the *A. vacillans* (red and yellow forms) petroleum ether extract (60 µg/15µL) showed high antibacterial activity against *E. coli*; 5.3 mm for the red and yellow form in comparison with Ampicillin antibiotic (zero).

However, Gentamicin antibiotic (15 mm) showed a high antibacterial activity against *E. coli*, compared with *A. vacillans* (red and yellow forms) petroleum ether extract (60 µg/15 µL); 5.3 mm for the red form and 5.3 mm for the yellow form. Moreover [63], mentioned that different concentrations of *A. vacillans* methanolic extract (10, 20 and 30 mg/µL) showed an antibacterial activity against *P. aeruginosa*, *S. aureus* and *E. coli*, but its activity was lower than the antibacterial activity of *Aloe Lanata* Methanolic Extract. Therefore, the earlier results were closely compatible with the findings of [63] and the differences in the antibacterial activities of the *A. vacillans* extracts may be due to the dissimilarity in the methodology of extraction. On the other hand, The Minimum Inhibitory Concentration of *A. vacillans* (red and yellow forms) petroleum ether extracted against *C. albicans* (2.3, 2 mm red and yellow, respectively) was 200 µg/50 µL (Table 9). The previous results were closely compatible with the findings of [62]. While the *A. vacillans* (red and yellow forms) petroleum ether extract (60µg / 15µL) showed (Table 8) a high antibacterial activity against *C. albicans* (6.6, 6.3 mm red and yellow, respectively). This result agreed with [47], reporting that fresh and dry gels of *A. vacillans* showed strong antimicrobial activity against *C. albicans*. However, the antibiotics Fluconazole 25 µg/dis (6 mm) showed a low antifungal activity against *C. albicans* in comparison with *A. vacillans* (red and yellow forms) petroleum ether extract (60 µg/15 µL); 6.6 mm for the red form and 6.3 mm for the yellow form. On the other hand, the antifungal activity of *A. vacillans* (red and yellow forms) petroleum ether extract (60 µg/15 µL) against *C. albicans* is lower than the activity of Nystatin (100 Units/disc), Ketoconazole (10 µg/dis) and Clotrimazole (10 µg/dis) antibiotic (23, 15 and 15 mm, respectively) against *C. albicans*. Based on earlier studies [58,63,64] and previous results the antimicrobial activities of *A. vacillans* (red and yellow forms) may be due to the presence of Oleic acid, lupeol and β-sitosterol, which showed high antimicrobial activities against selected microbes. The presented data specifies the Minimum Inhibitory Concentration (MIC) for different strains, enabling a straightforward comparison of activity levels.

Table 6. Biochemical activity of *A. vacillans* leaves

Name	SOD, U/mg	CAT, U/mg	POx, U/mg	Amylase, U/mg	Reducing sugars, mg/mL
Volume Extract, mL	0.08	0.32	0.60	1.5	0.6
Red	0.275	3.50	0.0011	0.122	0.105
Yellow	0.273	5.55	0.0049	0.263	0.145

However, determining if these MIC values are relatively high or low requires careful consideration. It is essential to reference them against concentrations reported in comparable studies utilizing the same species. Such comparative analysis is vital because differences in extraction techniques, concentration measurements, and environmental influences can significantly impact outcomes. Although references to prior studies offer contextual insight, the interpretation of results must proceed cautiously [63]. Variability in methodologies and specific *Aloe* species used across studies complicates direct comparisons. While comparative analyses are informative, drawing definitive conclusions about relative effectiveness should be avoided without sufficient supporting evidence. The discussion appropriately notes that antibiotics have well-established efficacy profiles, thereby serving primarily as positive controls rather than focal elements. This positioning helps integrate the findings concerning *A. vacillans* extracts more meaningfully into existing literature on antimicrobials.

3.10. Minimum inhibitory concentration of *A. vacillans* two forms crude essential oil against test microorganism:

The findings on the minimum inhibitory concentrations (MIC) of *A. vacillans* essential oil against *S. aureus* and *P. aeruginosa* provide valuable insights into the antimicrobial potential of this species (Table 8).

1. Comparison with Literature: The MIC values reported (6.25, 12.5, and 25 μ L) for both *S. aureus* and *P. aeruginosa* highlight a moderate level of activity. Previous studies on other *Aloe* species have reported varying MICs, often influenced by factors like extraction methods and the specific chemical composition of the essential oils. For instance, some studies have found lower MICs for *S. aureus* in other *Aloe* species, suggesting that while *A. vacillans* shows promise; it may not be the most potent among its peers.

2. Contributions to the Genus and Species: These results contribute to the understanding of *A. vacillans* within the *Aloe* genus by demonstrating its potential as an antimicrobial agent. The ability to inhibit both gram-positive *S. aureus* and gram-negative *P. aeruginosa* bacteria suggests a broad-spectrum efficacy, which is particularly valuable in the context of rising antibiotic resistance.

3. Implications for Future Research: This study underscores the need for further exploration of the phytochemical constituents of *A. vacillans* essential oil. Identifying the active compounds responsible for antimicrobial activity could lead to the development of natural alternatives to conventional antibiotics, especially as resistance grows.

Plants have traditionally served as the primary source of natural products for drug development. Consequently, exploring the chemical space of yet unexplored plants represents a promising approach to discovering new bioactive

compounds and fully unlocking the medical potential of this diverse group of organisms. Secondary metabolites exhibit substantial chemical diversity, often being specific to taxonomic groups due to their formation through conserved metabolic processes. Therefore, one strategy in investigating phytochemical space involves mapping the distribution of secondary metabolites across different taxonomic groups. This can help identify understudied or poorly explored areas, rendering it an effective method for research endeavors [67]. The results demonstrate that *A. vacillans* leaf extracts possess significant potential as natural antioxidant and antimicrobial agents. While the extracts show promising antimicrobial and antioxidant activities, further toxicological evaluations are necessary to ensure their safety for clinical use, as seen in similar *Aloe* evaluations [68].

The observed antioxidant and antimicrobial activities in both forms of *A. vacillans* can be attributed to the synergistic effect of its bioactive constituents. Specifically, the presence of Flavonoids and Phenolic compounds plays a crucial role in scavenging free radicals due to their hydroxyl groups. Furthermore, the identification of Oleic acid via GC-MS is significant—where it reached 54.64% in the red form and 38.73% in the yellow form—as this fatty acid is well-known for its ability to disrupt microbial cell membranes, thereby enhancing the antimicrobial potential of the extracts.

Statistical analysis using ANOVA and t-tests confirmed that while both forms possess significant bioactivity, the slight variations in chemical concentrations between the red and yellow forms were statistically significant ($P < 0.05$). This is particularly evident in the higher antioxidant capacity (244 μ g ASA/mg) and antimicrobial activity of the red form compared to the yellow form (232 μ g ASA/mg). These findings suggest that flower color variation in *A. vacillans* may correlate with specific biosynthetic pathways, providing further insight into the taxonomical relationship between these two morphological forms.

Taxonomical analysis is a method used to classify and understand the relationships among different biological forms based on specific characteristics. According to the preceding results, 59 chemical characteristics - 26 qualitative and 33 quantitative - along with the antimicrobial activity of the two investigated forms of *A. vacillans*, demonstrate significant taxonomic value for distinguishing them into two different infra-specific taxa under the species *A. vacillans*. The primary principle behind this analysis is to quantify similarities and differences among forms, allowing researchers to delineate distinct taxa.

Table 7. Zone inhibition in mm of the selected microorganism against crude essential oil of *A. vacillans* two forms

Test organisms	Extract <i>A. vacillans</i>						Antibiotics			
	20 µg/5µL		40 µg/10 µL		60 µg/15 µL		AMP		GEN	
Antibacterial	R	Y	R	Y	R	Y				
<i>E. coli</i>	0	0	3.3±0.2	3±0.1	6±0.4	5.3±0.3	0		15	
<i>S. aureus</i>	3±0.1	3±0.2	4.6±0.3	4.6±0.2	6±0.5	5.6±0.4	14		20	
<i>Ps. aeruginosa</i>	3.6±0.2	3.3±0.1	6±0.4	6±0.3	7.6±0.6	7.3±0.5	0		0	
Antifungal							NS	KT	CC	FLC
<i>C. albicans</i>	2.3±0.1	2±0.1	4±0.3	3.6±0.2	6.6±0.5	6.3±0.4	23	15	15	6

Note: Values are presented as mean ± standard deviation of three independent replicate (n=3).R: red flowered form , Y: yellow flowered form .AMP: Ampicillin. GEN: Gentamicin. NS: Nystatin, KT: Ketoconazole.

Table 8. Minimum inhibitory concentration of *A. vacillans* two forms crude extracts against microorganisms' test

Test organisms	Extract <i>A. vacillans</i>							
	20 µg/5 µL		10 µg/2.5 µL		5 µg/1.25 µL		2.5 µg/0.625 µL	
Antibacterial	R	Y	R	Y	R	Y	R	Y
<i>E. coli</i>	0	0	-	-	-	-	-	-
<i>S. aureus</i>	3±0.1	3±0.1	1±0.05	1±0.05	0.5±0.02	0.5±0.02	-	-
<i>Ps. aeruginosa</i>	3.6±0.2	3.3±0.1	2±0.1	2±0.1	1±0.05	1±0.05	-	-
Antifungal								
<i>C. albicans</i>	2.3±0.1	2±0.1	0	0	0	0	0	0

*Measurements Zone inhibition without wells R: *A. vacillans* (red) and Y: *A. vacillans* (yellow).

The value "zero" indicates that no antibacterial activity was observed for the specified antibiotics (Ampicillin and Gentamicin) against the tested microorganisms at the concentration used in this study. (-) indicates no activity.

3.11. Methodology:

1. Data Collection: Chemical characteristics and antimicrobial properties were meticulously recorded for the two forms of *A. vacillans*.

2. Numerical Analysis: A similarity matrix was created based on the collected data, enabling mathematical comparisons.

3. Dendrogram Construction: The resulting dendrogram illustrated the relationships among the studied forms, revealing a clear division into two series (SI and SII) at a similarity level of 91.44%.

- Series I: Red form (*A. audhalica* Lavranos and Hardy)
- Series II: Yellow form (*A. dhalensis* Lavranos)

This division underscores significant distinctions between the two forms, despite their morphological similarities in leaf structure and spines.

3.12. Results and Relevance of Infra specific Taxa:

The analysis demonstrated that the red and yellow forms of *A. vacillans* are indeed two different infra specific taxa. This classification aligns with previous studies that suggest the two forms are synonymously recognized as *A. audhalica* and *A. dhalensis*, respectively.

The chemical analysis revealed distinct compounds associated with each form:

- Yellow Form: Contains gamma-sitosterol and Fagarasterol.
- Red Form: Exhibits beta-sitosterol, lupeol, and 16-octadecyl acetate.

These chemical differences are crucial for understanding the ecological roles and potential applications of each form. For example, variations in antimicrobial properties could indicate differences in medicinal uses. Additionally, recognizing these infra specific taxa enhances biodiversity understanding and conservation efforts, ensuring that both forms of *A. vacillans* are adequately represented and preserved within their native habitats.

Numerical analysis was employed to construct a similar matrix illustrating relationships between the two studied forms. The resulting dendrogram (Fig. 9) demonstrated that the investigated forms of *A. vacillans* clustered into two series (SI and SII) with a relative similarity level of 91.44%. Series I comprise the red-colored form of *A. vacillans*, while Series II includes the yellow-colored form of *A. vacillans* [13]. The two forms of *A. vacillans* were described by [13] as *A. audhalica* Lavranos and Hardy (red flowers) and *A. dhalensis* Lavranos (yellow flowers), but according to previous studies [36,69], *A. audhalica* Lavranos and Hardy and *A. dhalensis* were synonyms for *A. vacillans*. Furthermore, the two forms have many similarities in their morphological structures, such as leaf shape, color, marginal spines shape and color [5]. However, our results illustrated that they have different chemical compositions; gamma-sitosterol and Fagarasterol, were found in the yellow form, while beta-sitosterol, lupeol and 16-octadecyl acetate were recorded in the red form. Moreover, the

cluster analysis based on chemical characters (26 qualitative and 33 quantitative) and antimicrobial activity of the two forms clarified that there were two different taxa of *A. vacillans* under investigation, which were of similar level, 91.44%.

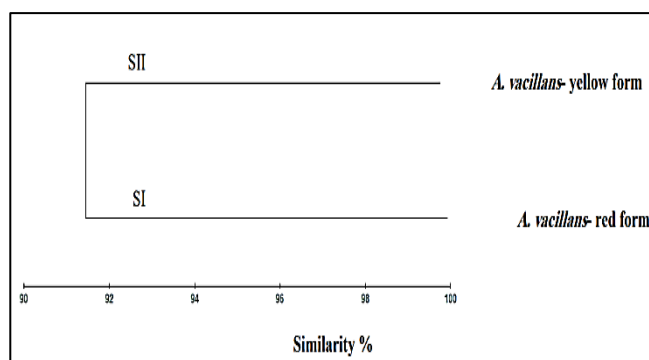


Figure 9. Visualization of cluster analysis of the relationship between two plant forms *A. vacillans*

The results obtained in this study align with findings reported by [70], who examined epidermal properties of *A. vacillans* leaves and their taxonomic significance for classifying two distinct forms. These authors also identified two forms of *A. vacillans* - a red and a yellow form - in Yemen, categorizing them as separate intra-specific taxa within the subspecies *A. vacillans*, described as *A. audhalica* Lavranos & Hardy and *A. dhalensis* Lavranos. These chemical differences are crucial for understanding ecological roles and potential applications of each form. For instance, variations in antimicrobial properties may indicate differential uses in medicine. Furthermore, recognizing these intra-specific taxa contributes to comprehending biodiversity and conservation efforts, ensuring both forms of *A. vacillans* are adequately represented and preserved in their natural habitats. This study investigates the endemic plant *A. vacillans* to elucidate its phytochemical and nutritional attributes, particularly focusing on medicinally relevant compounds present in its red and yellow forms.

Future Perspectives

Future studies should focus on conducting detailed cytotoxicity assays to evaluate the safety profile of these extracts for pharmacological applications. Furthermore, the isolation and characterization of individual active compounds remain a priority for upcoming research phases to fully elucidate the therapeutic potential of *A. vacillans*.

Conclusion

Yemen possesses a remarkably diverse flora, with endemic species like *Aloe vacillans* Forsskål representing a significant but understudied medicinal resource. This study provides a comprehensive chemical, nutritional, and biological characterization of the two morphological forms (red and yellow-flowered) of *A. vacillans* cultivated in the Amran Governorate.

Phytochemical and FT-IR analyses confirmed the presence of key bioactive secondary metabolites, including flavonoids, phenolics, and alkaloids, which underpin the plant's therapeutic potential. Quantitatively, the red form exhibited a superior antioxidant capacity (244 µg ASA/mg) compared to the yellow form (232 µg ASA/mg), while the yellow form showed higher catalase (CAT) activity (5.55 U/mg). Nutritionally, carbohydrates were identified as the predominant component (>43%), with significant crude fiber and lipid contents in both forms.

The antimicrobial evaluation demonstrated potent effectiveness, particularly against *Pseudomonas aeruginosa*, with a minimum inhibitory concentration (MIC) of 6.25 µL. Furthermore, the detailed assessment of 59 chemical and biochemical characteristics provided robust evidence for distinguishing these two forms as distinct infraspecific taxa, thereby contributing to the taxonomic documentation of Yemen's endemic biodiversity.

While these results highlight *A. vacillans* as a promising source for natural antioxidants and antimicrobial agents, further research is warranted. Future studies should focus on conducting detailed cytotoxicity assays to evaluate the safety profile of these extracts for clinical applications. Additionally, the isolation and characterization of individual active compounds remain a priority for upcoming research phases to fully elucidate the specific therapeutic mechanisms of this endemic species.

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Disclosure

The authors declare that they have no known competing financial interests.

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بحث علمي

التقييم الكيميائي والكيميائي الحيوي لأشكال نبات *Aloe vacillans* (فورسكال) وأهميتها التصنيفية المزروعة في

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الملخص

مفاتيح البحث

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كلمات مفتاحية:

المسح الكيميائي النباتي، مضادات الأكسدة، الأهمية التصنيفية، النشاط المضاد للميكروبات

هدفت الدراسة الحالية إلى تقييم الخصائص الكيميائية، والكيميائية الحيوية، والنباتية لمستخلصات أوراق نبات الصبار المتوطن (*Aloe vacillans* Forsskål)، مع التركيز على المجموعات النباتية التي تنمو في محافظة عمران بالجمهورية اليمنية، وتحديد أهميتها التصنيفية. ومن خلال تحليل 59 صفة (26 نوعية و33 كمية)، أكدت التحليلات العددية والإحصائية وجود شكلين مورفولوجيين متميزين: أحدهما ذو أزهار حمراء والآخر ذو أزهار صفراء. وقد أظهر المسح الكيميائي النباتي وجود مركبات حيوية فعالة مثل القلويدات، والفلافونيدات، والفينولات، والعفص، والصابونين، كما حدد تحليل الأشعة تحت الحمراء (FT-IR) المجموعات الوظيفية الرئيسية في كلا الشكلين، بما في ذلك مجموعات الهيدروكسيل، والكربونيل، والروابط الكربونية. وفيما يخص النشاط الحيوي، كشف تقييم القدرة المضادة للأكسدة بطريقة (FBRC) عن كفاءة عالية بلغت $5.2 \pm 244 \mu\text{g ASA}/\text{mg}$ للشكل الأحمر و $4.8 \pm 232 \mu\text{g ASA}/\text{mg}$ للشكل الأصفر، مع وجود فروق ذات دلالة إحصائية ($P > 0.05$). كما أظهرت نتائج اختبار الفعالية المضادة للميكروبات ضد أربع ممرضات بشرية توفراً مستمراً للشكل الأحمر في التثبيط مقارنة بالشكل الأصفر عند جميع التركيزات. تخلص الدراسة إلى أن هذه النتائج تقدم دليلاً قوياً على التمايز التصنيفي بين الشكلين، وتؤكد إمكانتهما كونهما مصادر طبيعية واعدة للتطبيقات الصيدلانية.