# Variation in Phytochemical Composition Reveals Distinct Divergence of *Aloe vera* (L.) Burm.f. From Other *Aloe* Species: Rationale Behind Selective Preference of *Aloe vera* in Nutritional and Therapeutic Use

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## Abstract

In the present study, we have phytochemically characterized 5 different abundant *Aloe* species, including *Aloe vera* (L.) Burm.f., using silylation followed by Gas Chromatography-Mass Spectrometry technique and compared the data using multivariate statistical analysis. The results demonstrated clear distinction of the overall phytochemical profile of *A vera*, highlighted by its divergent spatial arrangement in the component plot. Lowest correlation of the phytochemical profiles were found between *A vera* and *A aristata* Haw. (−0.626), whereas highest correlation resided between *A aristata* and *A aspera* Haw. (0.899). Among the individual phytochemicals, palmitic acid was identified in highest abundance cumulatively, and carboxylic acids were the most predominant phytochemical species in all the *Aloe* species. Compared to *A vera*, linear correlation analysis revealed highest and lowest correlation with *A aspera* ($R^2 = 0.9162$) and *A aristata* ($R^2 = 0.6745$), respectively. Therefore, *A vera* demonstrated distinct spatial allocation, reflecting its greater phytochemical variability.

*Aloe vera* (L.) Burm.f. is a medicinal herb known for its diverse pharmacological activities. It is used in traditional medicinal systems of different parts of the world time immemorial. *Aloe vera* is used in Indian traditional medicinal systems for the treatment of skin disease, infections, diabetes, inflammation, and also as an immunostimulant.[1,2] *Aloe vera* extract is used as antidiabetic and antihypertensive in Trinidad and Tobago[3]; as antifungal and antiworm in Chinese traditional medicine[4]; as antifever, antiinfection, and wound healing in Persian medicine[5-7]; and to provide relief from various pathological complications in traditional medicinal systems of Egypt, Japan, Mexican, African, and so on.[4] Moreover, *A vera* is also globally recognized as a functional nutritional supplement due to its health benefit.[8] *Aloe vera* is one of the few plants whose nutritional and medicinal utilities are found in traditional and home-made practices throughout the world. Since the past 3 decades (Supplementary Data, available at http://chp.sagepub.com/supplemental), the rise of pharmacognostic studies has

demonstrated several medicinal properties of *A vera* such as anticancer, antidiabetic, hepatoprotective, anti-inflammatory, antimicrobial, immunomodulatory, wound healing, and antioxidant properties.[9-11] Phytochemical analysis also revealed the presence of several nutritional ingredients like complex carbohydrates (mannan, galactan, xylan, etc), amino acids (glutamic acid, arginine, valine, tyrosine, etc), lectin-like protein constituents, vitamins (folic acid, $B_2$, $B_6$, β-carotene, etc), anthraquinones (cinnamic acid esters, aloe-emodin, aloetic-acid, etc), enzymes (lipase, carboxylase, superoxide dismutase, etc),

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inorganic nutrients (calcium, potassium, phosphorous, iron, etc), and other diverse classes of phytochemicals (steroids, γ-linolenic acid, phenolics, etc), which not only provide nutrition and energy but are also responsible for prevention of diseases.[12] With 167 book records, 3470 PubMed Central entries, 5524 protein sequences, and 1191 nucleic acid sequences till date, *A vera* is one of the highly investigated medicinal plant that has also been a commercial success as pharmacological, nutraceutical, and skin care products.[13]

Interestingly, the genus *Aloe* (family Asparagaceae) comprises 516 individual species[14]; however, the primary focus of most nutritional, pharmacological, and natural product studies are concentrated on *A vera* only. This might be because the rationale of pharmacognostic studies is rooted down in the traditional alternative and complementary medicines, where mostly *A vera* is used in the treatment of diverse ailments. However, in traditional medicine, as the pharmacological activities are the sum total of the properties of the bioactive constituents where synergy plays the crucial role, a comprehensive and comparative account of the phytochemical fingerprint of *A vera* with other *Aloe* species are lacking. Interestingly, other than *A vera*, many other *Aloe* species are known for their diverse bioactivities.[15,16] Several bioactive constituents have been isolated and characterized from *A vera* nevertheless; however, the basic phytometabolomic profiles of most of the *Aloe* species remain completely unknown. A systematic comparison of the common chemical constituents of *A vera* with other *Aloe* species was missing.

Therefore, here we have chemically characterized 4 major Aloe species, namely, *Aloe aristata* Haw., *Aloe jucunda* Reynolds, *Aloe albiflora* Guillaumin, and *Aloe aspera* Haw., using gas chromatography-mass spectrometry and compared their phytochemical profiles with that of *A vera* (L.) Burm.f. The phytochemical profiles of each *Aloe* species with several identified phytochemicals were further subjected to multivariate statistics. This resulted in data reduction and highlighted the interrelated correlation patterns of the 5 *Aloe* species, reflecting the extent of divergence of *A vera* phytochemical fingerprint as a single variable.

## Material and Methods

### Chemicals

All the required chemicals and solvents were procured from HiMedia Laboratories Pvt Ltd (Mumbai, India), unless otherwise indicated. N,O-bis(trimethylsilyl)trifluoroacetamide + trimethylchlorosilane (99:1, v/v) was obtained from Sigma-Aldrich (St Louis, MA). Milli-Q ultrapure water was obtained from the central facility of the Department of Zoology, University of North Bengal. All solvents used in the analysis were high-performance liquid chromatography grade.

### Plant Material

Healthy and disease-free leaves of *A vera*, *A aristata*, *A jucunda*, *A albiflora*, and *A aspera* were collected form the Medicinal Plant Garden of University of North Bengal (26.71°N, 88.35°E) on September 22, 23, and 26, 2015. The plant samples were authenticated and the

following accession numbers were given, respectively: 9781, 09766, 09767, 09753, and 09779. Voucher specimens of all the samples were stored at the herbarium of the Department of Botany, University of North Bengal.

### Sample Preparation and Silylation Derivation for Gas Chromatography-Mass Spectrometry

The leaves of the 5 different *Aloe* species were washed properly with water to remove any dirt or foreign matter and then cut into pieces (~0.5 cm) using a sterile scalpel and shade dried (dehydrated) at laboratory temperature (25°C) for 3 weeks. The dried leaves were then ground to powder using a pestle and mortar. The resultants (50 mg) were then mixed with 2 mL *n*-hexane and incubated at 25°C with occasional shaking. After 24 hours, 20 μL of BSTFA + TMCS (99:1, v/v) mixture was added to the previous solution and incubated for 60 minutes at laboratory temperature with occasional shaking. The mixtures then was centrifuged for 20 minutes at 15 000 rpm at 25°C and the clear supernatants were collected. The supernatants were passed through anhydrous $Na_2SO_4$ and activated charcoal (2:1, w/w) in a 10-cm minicolumn 1/10th packed with nonabsorbent cotton to remove any trace of moisture and color. The eluted clear liquid was recentrifuged at 15 000 rpm for 20 minutes and passed through Whatman filter paper No. 1 (11 μm). The resultant clear solutions were used for gas chromatography-mass spectrometry analysis.

### Gas Chromatography-Mass Spectrometry Analysis

The samples were analyzed using a Thermo-Scientific Trace 1300 gas chromatography instrument attached with Thermo-Scientific ISQ QD single quadrupole mass spectrophotometer following a previously standardized method.[17-20] The gas chromatography system was equipped with TG-5MS column (30 m × 0.25 mm × 0.25 μm). The inlet temperature was maintained at 250°C. The initial temperature was set at 60°C (solvent delay 5 minutes) with a hold of 4 minutes, followed by a ramp of 5°C/min to 290°C with a hold of 10 minutes (60-minute program). Samples (1 μL) were injected in a splitless mode (split flow 50 mL/min) with splitless time of 0.80 minutes, using a Thermo-Scientific AI-1310 auto-sampler. The carrier gas was helium (99.99%), with a constant flow of 1 mL/min, passed through hydrocarbon and dehydrating columns. Mass spectrometry transfer line temperature was set at 290°C with an ion source temperature of 230°C (electron ionization). The individual samples were analyzed at electron energy 70 eV (vacuum pressure = 2.21e-0.5 Torr). The mass analyzer range was set to 50 to 650 atomic mass unit (amu). All samples were analyzed thrice for confirmation.

### Gas Chromatography-Mass Spectrometry Data Analysis

MS data analysis was performed by Automated Mass Spectral Deconvolution and Identification System (AMDIS), version 2.70. The major and essential compounds were identified by mass fragmentation patterns (*m/z*) in terms of molecular peak ($M^+$) and base peak (* representing mass fragment with optimum abundance) of the reference of parent compound using MS Interpreter, Version 2.0, and by matching with the reference database of the National Institute Standards and Technology (NIST) with a MS library version 2011.

## Statistical Analysis

Data were statistically analyzed using IBM SPSS statistics version 20.0 software package for Windows. Data reduction technique was employed following previously reported methods.[18,21,22] The interrelationship between the metabolomic profiles of the 5 different plants were analyzed by Principal Component Analysis (PCA) based on the correlation matrix. Under descriptive statistics, KMO and Bartley's tests were performed to study the underlying dimensions of the 5 variables. Two factors were extracted under Varimax method. Data were further analyzed by employing a Hierarchical Cluster Analysis (HCA). The cluster method employed was between group linkages with interval of square Euclidian distance. Transform values of variables (average zero and SD 1), called $Z$ scores, was carried out as a pretreatment of the data. Bivariate correlation analysis and proximity heat map were generated using Microsoft Excel 2010. Relative abundance was calculated (mean $\pm$ SD) from the data of 3 parallel runs. $P < .05$ was considered statistically significant.

## Results and Discussion

The complete phytochemical composition of medicinal plants consists of a complex mixture of chemical species with varying polarity and volatile properties. Here, the hydrogen bonding in the component phytochemicals were reduced by silylation in order to transform them into much more volatile, nonpolar, and thermally stable form, appropriate for the gas chromatography analysis. The replacement of an active terminal proton from the functional groups (–OH, –COOH, =NH, –NH$_2$, –SH) with an alkylsilyl group, such as in most of the cases trimethylsilyl group, caused elevation of volatility and polarity shift, resulting in identification of the compounds in their trimethylsilyl (C$_3$H$_9$Si) form with an increase of $m/z$ 73.

The gas chromatography-mass spectrometry analysis (see Figure 1 and Supplementary Data, available at http://chp.sage pub.com/supplemental) demonstrated quite distinct yet significantly overlapping phytochemical profiles of the 5 Aloe species, which revealed presence of several bioactive compounds, many of which are well established for their potent pharmacognostic properties. A total of 63, 39, 49, 37, and 42 compounds were identified, respectively, in A vera, A aristata, A jucunda, A albiflora, and A aspera. Among these, several compounds were identified as signature compounds in individual plants, such as 4-ethylbenzaldehyde (M$^+$* 134, $m/z$ 119, 105), 4-hydroxybutyric acid derivative (M$^+$ 248, $m/z$ 147*, 117), 2-hydroxyoctanoic acid derivative (M$^+$ 232, $m/z$ 117, 97, 73*), β-copaene (M$^+$ 204, $m/z$ 161, 119*, 105), adipic acid (M$^+$ 290, $m/z$ 141, 111, 73*), pimelic acid derivative (M$^+$ 304, $m/z$ 155, 125, 73*), suberic acid derivative (M$^+$ 318, $m/z$ 169, 129, 73*), protocatechuic acid derivative (M$^+$ 370, $m/z$ 281, 193, 73*), phytol (M$^+$ 296, $m/z$ 123, 71*), and so on, in A vera; 3-ethylphenol derivative (M$^+$ 174, $m/z$ 179*), p-ethylguaiacol (M$^+$ 152, $m/z$ 137*, 122), vanillin (M$^+$ 224, $m/z$ 209, 194*, 163, 147) in A aristata; phenylacetic acid (M$^+$ 208, $m/z$ 193, 91, 73*), benzene, 4-ethyl-1,2-dimethoxy- (M$^+$ 166, $m/z$ 151*, 135, 95), 4-vinylveratrole (M$^+$ 164*, $m/z$ 149, 91), 2-allyl-1,4-dimethoxy-3-methyl-benzene (M$^+$ 91*, $m/z$ 177, 149, 91), phenol,3,5-bis(1,1-dimethylethyl)- (M$^+$ 206, $m/z$ 191*, 57),

estragole (M$^+$ 147*, $m/z$ 133, 121, 77), bumetrizole (M$^+$ 115, $m/z$ 300*, 272, 147) in A jucunda; hydrocinnamic acid derivative (M$^+$ 297, $m/z$ 207*, 91, 75), 1,2-ethanediol, phenyl (M$^+$ 281, $m/z$ 179*, 147) in A albiflora; m-pyrol (M$^+$ 98*, $m/z$ 71), oleic acid derivative (M$^+$ 253, $m/z$ 339, 117, 73*), campesterol derivative (H$^+$ 472, $m/z$ 382, 343, 129*), and stigmasterol derivative (M$^+$ 484, $m/z$ 394, 255, 129 83*) were only detected in A aspera.

The relative abundance of squalene (98.26 $\pm$ 2.83) was optimum in A vera, whereas the presence of α-linolenic acid was highest in A aristata (93.22 $\pm$ 2.48) and A jucunda (91.69 $\pm$ 2.53). Similarly, palmitic acid was present in highest abundance in both A albiflora (97.63 $\pm$ 2.48) and A aspera (98.15 $\pm$ 2.56). Considering cumulative relative abundance in all the 5 Aloe species (Figure 2), the 5 major constituents with optimum relative abundance were palmitic acid (91.65 $\pm$ 11.42), octadecanoic acid (81.30 $\pm$ 12.06), linolenic acid 58.13 $\pm$ 37.31), linoleic acid derivative (42.61 $\pm$ 16.96), and squalene (39.19 $\pm$ 35.96). Alternatively, benzeneacetaldehyde (0.34 $\pm$ 0.16), benzoic acid (0.38 $\pm$ 0.09), octanoic acid derivative (0.52 $\pm$ 0.01), hexadecane derivative (0.64 $\pm$ 0.51), and phenol,2,4-bis(1,1-dimethylethyl)- (0.72 $\pm$ 0.46) were detected at the lowest abundance. On the other hand, linolenic acid (58.13 $\pm$ 37.31), squalene (39.19 $\pm$ 35.96), sitosterol (20.18 $\pm$ 22.93), linoleic acid derivative (42.61 $\pm$ 16.96), and ethyl-9,12,15-octadecatrienoate (20.81 $\pm$ 15.88) demonstrated highest variability in their abundance.

A detailed account of individual chemical species are presented in Figure 3, which demonstrated predominance of phytochemicals with carboxylic acid groups in all the plants. In fact, the carboxylic acid compounds demonstrated greatest degree of variation in their abundance (22.8 $\pm$ 8.67). A methylanthraquinone derivative (H$^+$ 310; $m/z$) in A vera was identified by $m/z$ 295* (C$_{17}$H$_{15}$O$_3$Si; loss of CH$_3$) and 265 (C$_{16}$H$_{13}$O$_2$Si; loss of C$_2$H$_3$ + H$_2$O) as the single ketone compound. Benzaldehyde derivatives are the only aldehydes detected in all 5 plants.

The multivariate statistical analysis (Figure 4) revealed quite distinct yet interconnected phytochemical profiles of the 5 Aloe species, reflecting on the significantly diverse phytochemical profile of A vera. Principal component analysis causes dimensional data reduction and thereby reflected the underlying correlation patterns of the given set of variables. Here variables of the experimental data are arranged in the most simplified pattern to reflect the overall picture of the phytochemical profile. Similarly, the hierarchical clustering represented stepwise similarities of the phytochemical profiles in a relatively simplified form. The Scree plot (Figure 4A) demonstrated greater variability of A vera located at eigenvalue <1 compared to other Aloe species located at eigenvalue <1. The near linear formation generated by A aristata, A jucunda, A albiflora, and A aspera represents very small fraction of variability among them. The spatial arrangements of the 5 Aloe species in the loading plot (Figure 4B) were based on the presence or absence of individual phytochemicals. For example, compounds such as benzaldehyde derivative, tetradecane
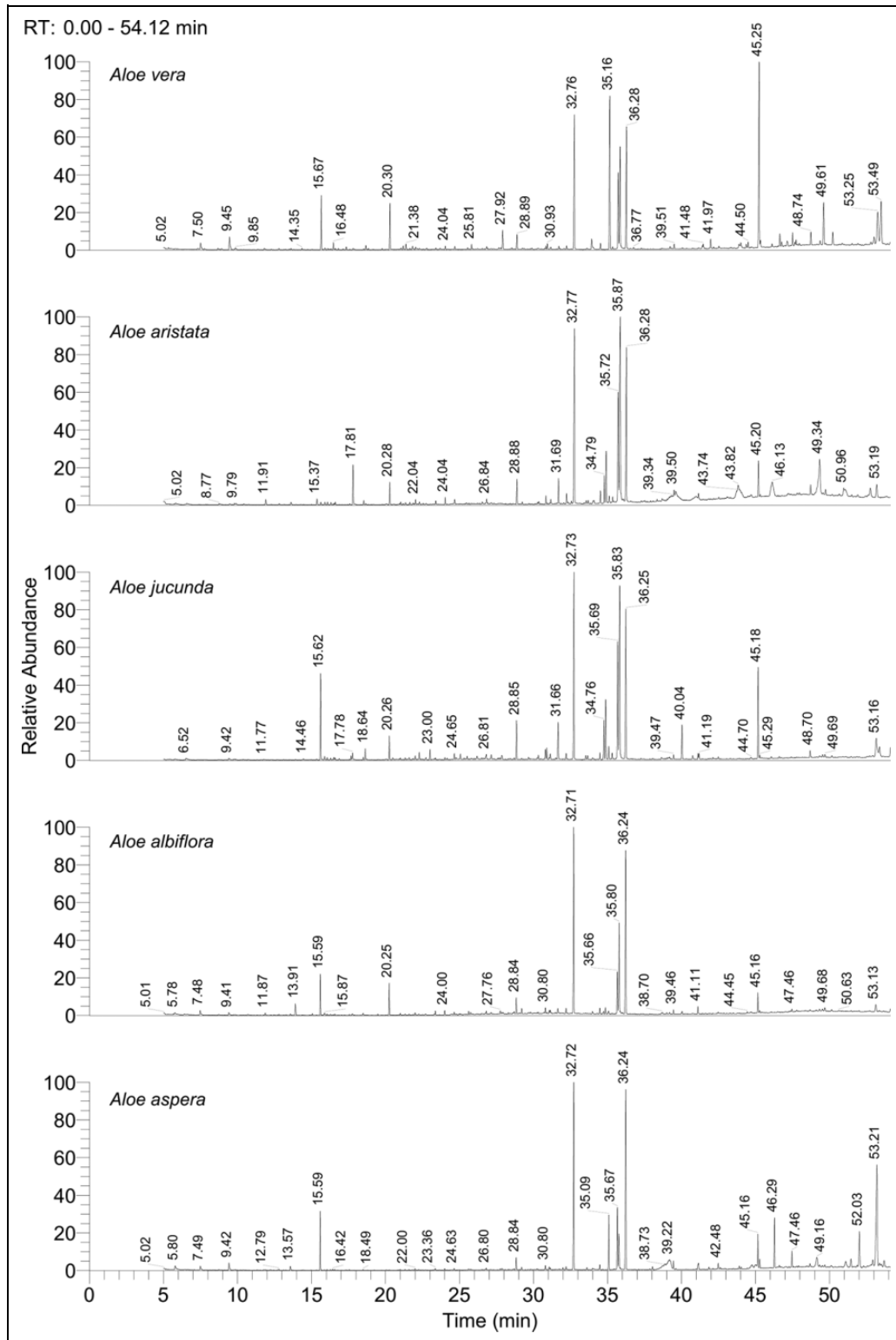
**Figure 1.** Gas chromatogram of *Aloe vera*, *Aloe aristata*, *Aloe jucunda*, *Aloe albiflora*, and *Aloe aspera*. Data represented as retention time (RT;=0-54 minutes) at *x*-axes versus relative abundance (RA; max 100) at *y*-axes. Phytochemical profiling was performed by silylation followed by gas chromatography-mass spectrometry analysis form the leaves of 5 *Aloe* species. Individual compounds were identified by mass fragment analysis and matching with the reference data form NIST MS library version 2011. The details of individual compounds identified are provided in Supplementary Data, available at http://chp.sagepub.com/supplemental.
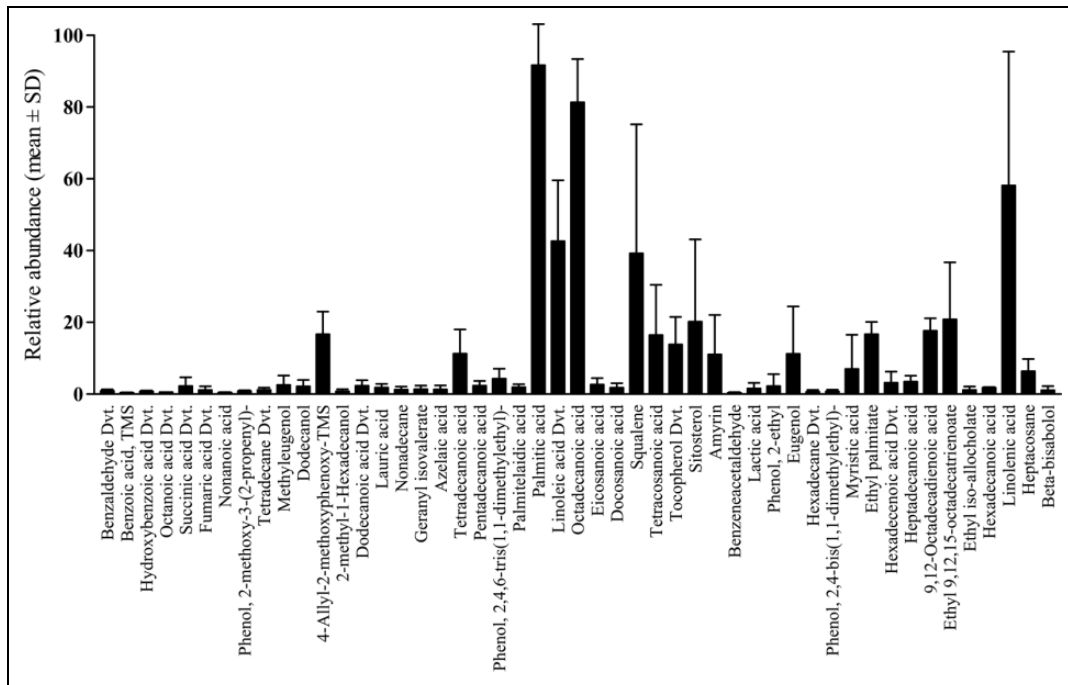
**Figure 2.** The variation in relative abundance of different phytochemicals commonly found in the 5 *Aloe* species. The phytochemicals were selected based on their presence in 2 or more *Aloe* species. Palmitic acid, octadecanoic acid, and linolenic acid were the 3 most abundant phytochemicals, whereas the abundance of squalene, sitosterol, and linoleic acid were highly variable. Data represented as mean $\pm$ SD of relative abundance in 2 to 5 *Aloe* species.
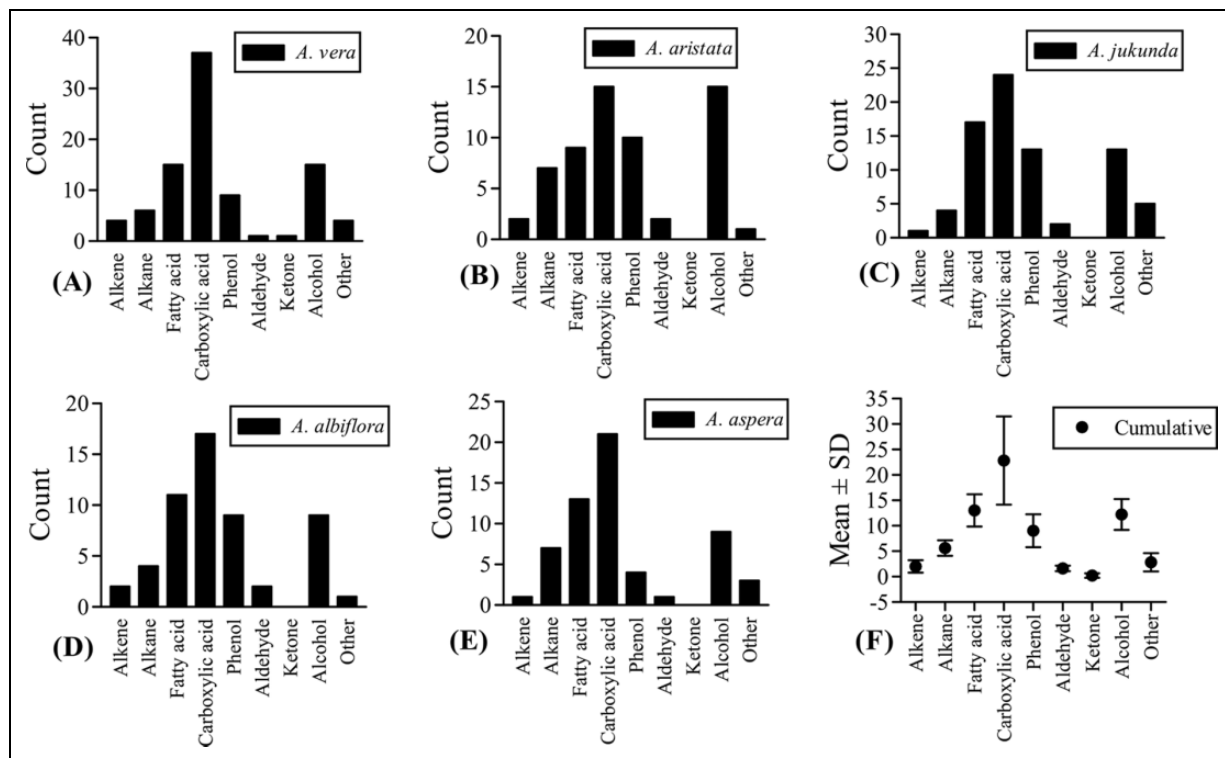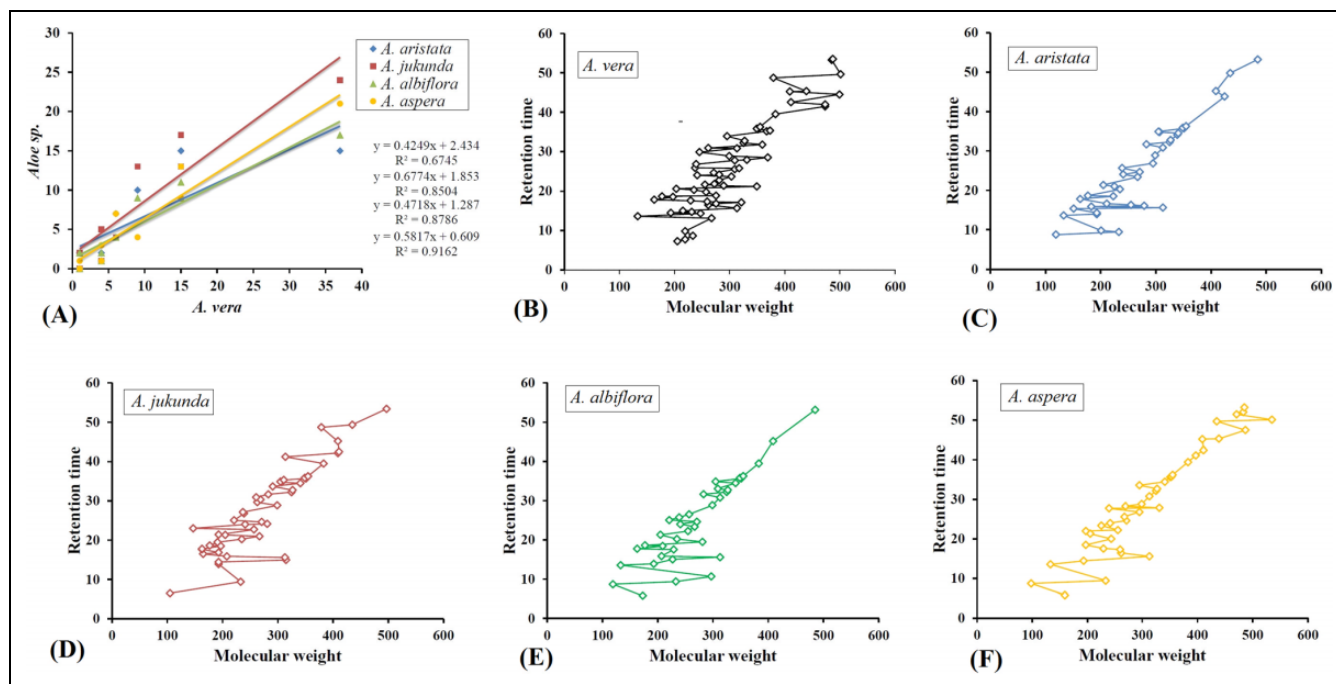


**Figure 3.** The count of different phytochemicals of the major phytochemical species based on functional groups. (A) *Aloe vera*, (B) *Aloe aristata*, (C) *Aloe jukunda*, (D) *Aloe albiflora*, (E) *Aloe aspera*, and (F) cumulative count of all 5 *Aloe* species. The carboxylic acid group also includes fatty acid compounds. The "other" group includes identified minor chemical species. Several organic acids were identified contributing to the greatest share of all phytochemicals and with highest variability. A huge variety of phenolic compounds were identified, which are known for diverse pharmacological activities.
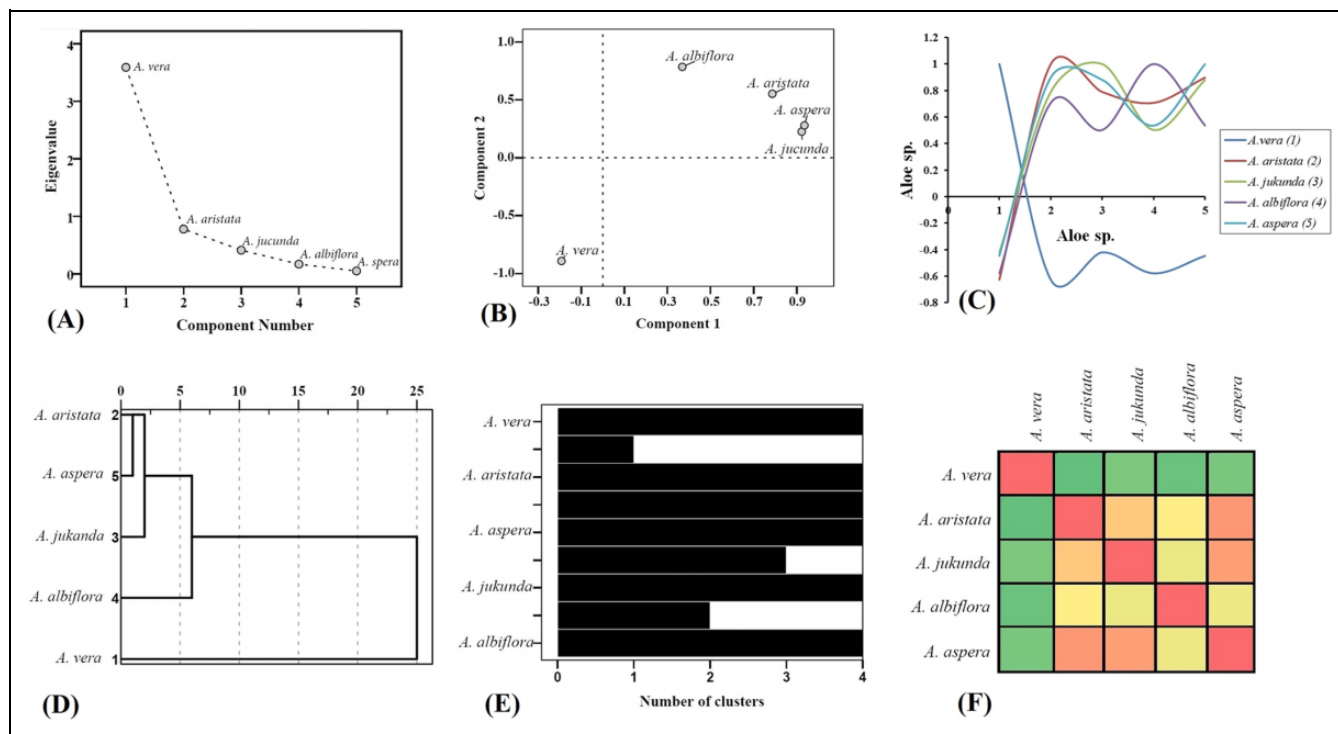
**Figure 4.** Multivariate statistical analysis of the phytochemical profiles of the 5 *Aloe* species. The phytochemical profiles of the 5 *Aloe* species obtained from the gas chromatography-mass spectrometry study were subjected to multivariate correlation, principal component, and clustering analysis to visualize the intercorrelation patterns and extent of similarities in their phytochemical profiles. (A) The scree plot demonstrates that the phytochemical profile of *Aloe vera* (eigenvalue >1) represents the greatest variability compared to other *Aloe* species (eigenvalue <1). (B) The clustered spatial arrangement of *Aloe aristata*, *Aloe jucunda*, *Aloe albiflora*, *Aloe aspera* in the double-positive quadrant, opposite to *Aloe vera* in the double-negative quadrant of the principal component loading plot clearly reflects nonequivalent phytochemical profile of *Aloe vera*. (C) The extent of correlation patterns (*P* < .001, 1-tailed) are reflected through the scatter plot, generated from the correlation matrix, obtained from principal component analysis. Here, least correlation resided between *Aloe vera* and *Aloe aristata* (r = −0.626), whereas greatest correlation was found between *Aloe aspera* and *Aloe aristata* (r = 0.899). The absolute correlation trend of *Aloe vera* remains negative to other *Aloe* species, signifying deviation in phytochemical profile. (D) Dendrogram representing the hierarchical clustering of the phytochemical profiles of 5 *Aloe* species, which remains in accordance with the principal component analysis, demonstrating 2 separate clusters. Cluster 1 comprises *Aloe vera* and cluster 2 comprises *Aloe aristata*, *Aloe jucunda*, *Aloe albiflora*, and *Aloe aspera*. This further illustrates step-wise divergence of the phytochemical profiles with terminal branching of *Aloe aspera* and *Aloe aristata*. (E) The Icicle diagram (corresponding to agglomeration schedule table in Supplementary data, available at http://chp.sagepub.com/supplemental) represents the progression of clustering, that is, at which step the individual phytochemical profiles of 5 different *Aloe* species were diverted. (F) Proximity heat map generated from proximity scores (Supplementary Data) corresponding to "D." Shades of green to red color demonstrates high to low proximity scores. Highest phytochemical proximity resided between *Aloe aspera* and *Aloe aristata*, whereas lowest proximity was between *Aloe vera* and *Aloe aristata*.

derivative, dodecanoic acid derivative, lauric acid, palmitic acid, linoleic acid derivative, squalene, linolenic acid, and so on, were detected in all the 5 plants. However, compounds such as glyceric acid, β-copaene, adipic acid, protocatechuic acid, p-ethylguaiacol, vanillin, 4-vinylveratrole, estragole, bumetrizole, campesterol, stigmasterol, and so on, were identified only in individual *Aloe* species. The loading plot (Figure 4B) of first (PC1) and second (PC2) principal components cumulatively accounted for 71.81% and 87.36% variance, respectively. The completely separate and isolated location of *A vera* in the double-negative quadrant clearly indicates significant (*P* < .001) variance of its phytochemical constituents form other *Aloe* species located in the double-positive quadrant. *Aloe vera* was loaded highly negative on PC2 (PC1: −0.192; PC2: −0.893), whereas *A aspera* was loaded highly positive on component 1 (PC1: 0.936; PC2: 0.279). Interestingly,

partial overlapping of *A aspera* (PC1: 0.936; PC2: 0.279) and *A jucunda* (PC1: 0.923; PC2: 0.226) resulted due to common primary and secondary metabolic pathways, giving rise to detection of common phytochemicals or their chemical derivatives. The extent of similarity of the phytochemical profiles were calculated using scatter plot generated from the correlation matrix (Figure 4C) that showed significantly (*P* < .001) highest correlation (0.899) between *A aspera* and *A aristata* and least correlation (−0.626) between *A vera* and *A aristata*. Cluster analysis was further performed using hierarchical cluster analysis method to distinguish the associations of the phytochemical profiles and also to validate the component analysis data. The extent of similarity of the phytochemical profiles were supported by the denodgram of the hierarchical cluster analysis (Figure 4D) and the proximity heat-map (Figure 4F), generated using the proximity data of hierarchical cluster analysis. *Aloe vera*

**Figure 5.** The results of various linear bivariate correlation analysis. (A) Correlation analysis of different phytochemical species (alkane, alkene, fatty acid, carboxylic acid, phenol, aldehyde, ketone, alcohol, others) identified in *Aloe aristata*, *Aloe jucunda*, *Aloe albiflora*, *Aloe aspera* versus *Aloe vera*. Compared to *Aloe vera*, optimum ($R^2 = 0.9162$) and minimum ($R^2 = 0.6745$) correlation of phytochemical species were found in case of *Aloe aspera* and *Aloe aristata*, respectively. $R^2$ = coefficient of determination, representing fraction of the variance within the dependent variables that could be predicted from the independent variables. (B-F) Scatter plots representing distribution of phytochemicals in the order of increasing molecular weight against retention time. Among the 5 *Aloe* species, 1-heptatriacotanol and M-pyrol were identified as the highest (536) and lowest (99) molecular weight compounds, both of which were detected in *Aloe aspera*.

located at the completely separate arm signifies its highly diverse phytochemical profile, followed by divergence from *A albiflora, A jucunda, A aspera*, and *A aristata*, respectively. Among the 5 *Aloe* species, lowest proximity resided between *A aspera* and *A aristata* (12.139), whereas greatest proximity was detected between *A vera* and *A aristata* (195.082; see Supplementary Data, available at http://chp.sagepub.com/supplemental).

Based on the results of multivariate analysis, *A aristata, A jucunda, A albiflora*, and *A aspera* were compared with *A vera*, based on the abundance of phytochemical species (Figure 5A). *Aloe aspera* was found to be highly comparable ($R^2 = 0.9162$) and *A aristata* ($R^2 = 0.6745$) was least equivalent to that of *A vera*. The individual phytochemicals were further plotted to visualize the pattern of distribution based on their retention time versus molecular weight, which revealed metabolomic gaps and scarcity of high molecular weight compounds at higher retention time (Figure 5B-F). This may have resulted due to the fact that the volatile nature of compounds trends to decrease with increase of molecular weight. For example, only 5 compounds were identified between molecular weight 355 and 485 in *A aristata*, 3 compounds between molecular weight 409 and 497 in *A jucunda*, and 4 compounds between molecular weight 355 and 485 in *A albiflora*. Within the given mass range (50-650 amu) in the mass analyzer, the broadest range of compounds were identified in *A aspera*, where M-Pyrol (molecular weight 99) and 1-heptatriacotanol (molecular

weight 536) were the compounds with lowest and highest molecular weight, respectively.

## Conclusion

The pharmacological properties in traditional herbal medicines are dependent on the synergy of individual components, cumulatively accounting for the overall bioactivities. Interestingly, this is clear from the present study where several phytochemicals were commonly identified in different *Aloe* species. However, significant difference were observed in their relative abundance, resulting in scattering of the 5 *Aloe* species based on their phytochemical profiles. The present phytochemical analysis of 5 different *Aloe* species followed by multivariate statistical analysis clearly depicts distinct phytochemical profile of *A vera*. Synergy of phytochemicals, being a relative term, cannot be measure in an absolute degree. Yet the present study reflects how the overall phytochemical profile may play a key role in selective preference of using *A vera* over other *Aloe* species in traditional medicine.

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