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Full Length Research Paper

Evaluation of genetic diversity using morphological markers and gas chromatography-mass spectrometry (GC/MS) analysis in some *Aloe* sp. accessions

Mansour Saljooghianpour[1]*, Mansour Omidi[2], Shamsali Rezazadeh[3] and Mohammadreza Naghavi[2]

[1]Department of Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, P. O. Box 14155-4933, Iran.
[2]Department of Plant Breeding, Tehran University, Karaj, Iran.
[3]Institute of Medicinal Plants, ACECR, Karaj, Iran.

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Thirty three *Aloe* accessions were collected from different geographical regions of Iran. Morphological evaluation and gas chromatography-mass spectrometry (GC/MS) analysis were applied for showing genetic variations among the studied accessions. Morphological analysis indicated that all the studied characters have a significant difference of P < 0.01 among *Aloe* accessions. Cluster analysis indicated that there were differences among all accessions and commercial accessions performed better than wild accessions and were ranked higher; while the most wild-accessions performed worse than commercial accessions and were ranked lower. According to the results, 26 bioactive phytochemical compounds were identified. Furthermore, results indicated that these compounds in accordance with identified bioactive phytochemical compounds in wild *Aloe* plants by other researchers. With attention to the obtained results of GC/MS analysis, these compounds have fluctuated within selective *Aloe* accessions. Morphological analysis together with GC/MS analysis indicated that there are remarkable genetic variations among selective *Aloe* accessions.

Key words: Aloe medicinal plant, genetic diversity, phytochemical components, morphological markers, gas chromatography-mass spectrometry (GC/MS) analysis.

INTRODUCTION

Aloe vera is a medicinal, cosmetic and ornamental plant. Aloe genusis is a perennial succulent herb grown in tropical and subtropical parts of the world. The different species have somewhat different concentrations of active ingredients (Yagiet al., 1998). At least, a quarter of Aloe genera is valued for traditional medicine (Grace et al., 2009) while a small number is wild harvested or cultivated for natural products prepared from the bitter leaf exudates or gel-like leaf mesophyll. Aloe gel is 99% water with a pH of 4.5 and is a common ingredient in many non-prescription skin salves. Aloe extracts have been used to treat canker sores, stomach ulcers and even AIDS. The gel contains an emollient polysaccharide, glucomannan, which is a good moisturizer utilized in many cosmetics. Acemannan, the major carbohydrate fraction in the gel demonstrates antineoplastic and antiviral effects. Other important pharmacological activities of *Aloe vera* are anti-diabetic, antiseptic, anti-tumor and wound and burn healing effect (Rajasekaran et al., 2006). The sticky latex liquid is derived from the yellowish-green pericyclic tubules that line the leaf (rind); this is the part that yields the laxative anthraquinones. The leaf lining (latex, resin or sap) contains anthraquinone glycosides (aloin, aloe-emodin and barbaloin) and these are potent stimulant laxatives.

*Corresponding author. E-mail: m.saljooghian@iauiranshahr.ac.ir.

In nature, *Aloe* sp. is propagated through lateral buds (Meyer and Staden, 1991). There are morphological variations in some economically important Aloe species (Darokaret al., 2003) and leaf phenolic constitution (Van et al., 1995; Viljoen, 1999). However, due to lack of expressions for reproductive characters in some of the species, it is impossible to distinguish them (Reynolds, 1990). Since morpho-chemical characters are dependent on age and environment, it is essential to characterize this medicinally and economically important genus genetically. The success of any genetic conservation and breeding program depends largely on the identification of the amount and distribution of genetic diversity in the gene pool of the concerned plant. Knowledge on the genetic diversity and relationships among plant varieties is important to recognize gene pools, to identify gaps in germplasm collections and to develop effective conservation and management strategies. In this way, morpho-chemical evaluations can provide insights into the genetic structure and diversity within and among varieties from different geographical origins, producers and distributors (Nayanakantha et al., 2010). Without this information, breeders have no means of selecting appropriate plant material for the participation in screening and breeding programs, with a view to the introduction of novel varieties into a country (Russell et al., 1997).

Gas chromatography-mass spectrometry (GC/MS) can provide meaningful information for components that are volatile, non-ionic and thermally stable and have relatively low molecular weight. In this present study, phytochemical components were analyzed by GC/MS in selective aloe accessions. Information about the genetic diversity of aloe germplasm in Iran is particularly important for variety identification in enhancing the classification of germplasm collections and exploiting them in breeding programs and for the development and introduction of new accessions. Thus, the present study was taken to characterize the aloe germplasm accessions collected from different geographical regions of Iran and maintained in Islamic Azad University for investigating genetic diversity in Some *Aloe* sp. accessions using morphological markers and GC/MS analysis.

MATERIALS AND METHODS

Plant material

Thirty three Aloe accessions were collected from different geographical regions of Iran and were planted in the farmland of Iranshahr Islamic Azad University. These accessions included 31 wild genotypes and 2 commercial genotypes.

Morphological characteristics

Morphological characters such as sprig number, plant height, leaf length, leaf width, leaf thickness, leaf fresh weight, leaf dry weight, number of burr in leaf, number of leaf, mean of leaf burr length, mean of inter-burr length, stem length, stem diameter, mean of internode length, leaf cuticle thickness, leaf mesophyll thickness, leaf gel weight, the gel weight ratios to leaf through weight were recorded in all aloe accessions for comparative studies. Morphological data were analyzed by SPSS version 19, MSTATC and SAS software version 9.1. Data matrices were constructed with individuals (33) as columns and characters (18) as rows. The data matrices were then subjected to multivariate methods as cluster analysis (using average linkage between groups) by SPSS program version 19.

Preparation of plant extract

The selective aloe accessions were washed with distilled water and kept at room temperature and air-dried. Dried plants were crushed to small pieces and powdered and were kept in polythene bags for further use. Aqueous extract of studied samples were used to carry out the qualitative and quantitative analysis using standard procedures to identify the bioactive phytochemical components as described by Sofowara (1993) and Trease and Evans (1989).

Gas chromatography-mass spectrometry (GC/MS) is a method that combines the features of gas liquid chromatography and mass spectrometry to identify different substances within a test sample. GC/MS can provide meaningful information for components that are volatile, non-ionic and thermally stable and have relatively low molecular weight.

Extracts of *Aloe* accessions were analyzed by GC/MS. GC analysis were performed using a Hewlett-Packard 6890 chromatograph equipped with a flame ionization detector and injector MS transfer line temperature of 280°C respectively. A fused silica capillary column Hp- 5 ms (5% Phenyl: 95% dimethyl siloxane 30 M × 0.25 mm Film thickness 0.32 Lm) was used. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. The carrier gas, helium was at a flow rate of 1 ml/min. GC/MS analyses were carried out on an Agilent Technologies Network mass spectrometer (model 5973) coupled to H.P. gas chromatograph (model 6890) equipped with NBS 75K Library Software database. The capillary column and GC conditions were as described earlier. Mass spectra were taken at 70 eV and the scanning rate of 1 scan/s and the run time was 90 min. Compound identification was accomplished by comparing the GC relative retention times and mass spectra to those of authentic substances analyzed under the same conditions by their retention indices (RI) and by comparison to reference components.

RESULTS AND DISCUSSION

Morphological analysis

In the present study, thirty three aloe accessions were studied with some morphological traits that some of those were easily separated from other accessions by their distinct phenotypic characteristics stem from development. Morphological analysis indicated that all the studied various characters have a significant difference at $P < 0.01$ among aloe accessions. Sarbaz-6 accession was found to be the tallest (77 cm) as it possesses a distinct stem (calescent) with long internodes. Minimum leaf thickness (1.23 cm) and wideness (1.680 cm) was recorded for zehkalot accessions and chanf-2 accessions. Therefore, these accessions may be said to contain a lesser amount of gel. Maximum amounts for

Table 1. Aloe accessions name and their given label.

Accession's name	Label	Accession's name	Label
Chanf-1	1	Segar	18
Gotij-1	2	Zaboli-3	19
Phanouch-1	3	Zaboli-4	20
Sarbaz-1	4	Chanf-2	21
Sarbaz-2	5	Spakeh-2	22
Sarbaz-3	6	Sarbaz-5	23
Sarbaz-4	7	Chabahar-1	24
Spakeh-1	8	Chabahar-2	25
Iranshahr-1	9	Iranshahr-2	26
Bent	10	Gotij-3	27
Zaboli-1	11	Spakeh-3	28
Zaboli-2	12	Sarbaz-6	29
Lashar	13	Khash	30
Saravan	14	Zehkalot	31
Nikshahr	15	Control-1	32
Phanouch-2	16	Control-2	33
Gotij-2	17	-	-

leaf fresh weight, leaf dry weight, gel weight, number of leaf, stem length. The mean of internode length, leaf mesophyll thickness, leaf length and leaf width characteristics which were recorded for Sarbaz-6 showed that it was a wild accession. Maximum sprig number and gel weight ratio to leaf through weight for Chanf-1 accession and maximum mean of inter burr length in the Zaboli-2 accessions were measured. Furthermore, maximum leaf cuticle thickness and number of burr in leaf were found in the Iranshahr-1 accession and maximum mean of leaf burr length and Minimum stem diameter were recorded in the Sarbaz-2 accession. In this paper, we used accession label instead of accessions name for comfortable explanation (Table 1).

Genetic diversity studied was based on morphological various characters using analysis of the cluster among aloe accessions. Dendrogram using average linkage (between groups) indicated that all accessions were divided into three major groups. There were commercial accessions (31 and 32 numbers) in the third group, and the best wild accession (number of 29) was in this group. This clustering indicated that there were differences among all accessions. Commercial accessions performed better than wild accessions and were ranked higher; whereas, the most wild-accessions performed worse than commercial accessions and were ranked lower (Figure 1). According to them, the similarity mean between commercial accessions (31 and 32 numbers) was 97% while the similarity mean among wild accessions was 79%. Thus, the observed morphological differences and similarity values of *A. vera* strains maintained at Iran suggest that they all possess a useable genetic diversity.

Phenotypic correlations among morphological various characters indicated that there were highly significant correlations at $P < 0.05$ or $P < 0.01$ among the most studied characters. For example, a highly significant positive correlation at $P < 0.01$ among the gel weight and all the studied characters except the numbers of burr in leaf were observed. Other correlations among morphological various characters are available in Table 2. Phenotypic correlations among characters indicated that some associated factors correlating with each other contribute to the occurrence of these characters (saljooghianpour et al., 2010). Aloe accessions were more separated with the help of their leaf morphology. Although, common aloe accessions exhibited some variations in height, leaf size and gel weight and these could be attributed to the adaptations of their original geographical and environmental conditions. Complete phenotypic expression of vegetative characters that show variations makes the identification more difficult. Moreover, traditional morphological observations and chemical characters alone cannot determine the roles of phenotypic plasticity and genetic differentiation on population variation and adaptation (Gepts, 1993). Hence, they lack the resolving power needed to identify individual genotypes (Nayanakantha et al., 2010). There are other Aloe species, which resemble *A. vera* accessions in growth habits and morphological traits (Reynolds, 1966). More, identifying Aloe accessions at its early stage is also sometimes ambiguous. These accessions were further subjected to GC-MS analysis for the assessment of variation at the molecular level. Thus, in order to determine genetic variations of these accessions, GC-MS analysis were used in the present study.

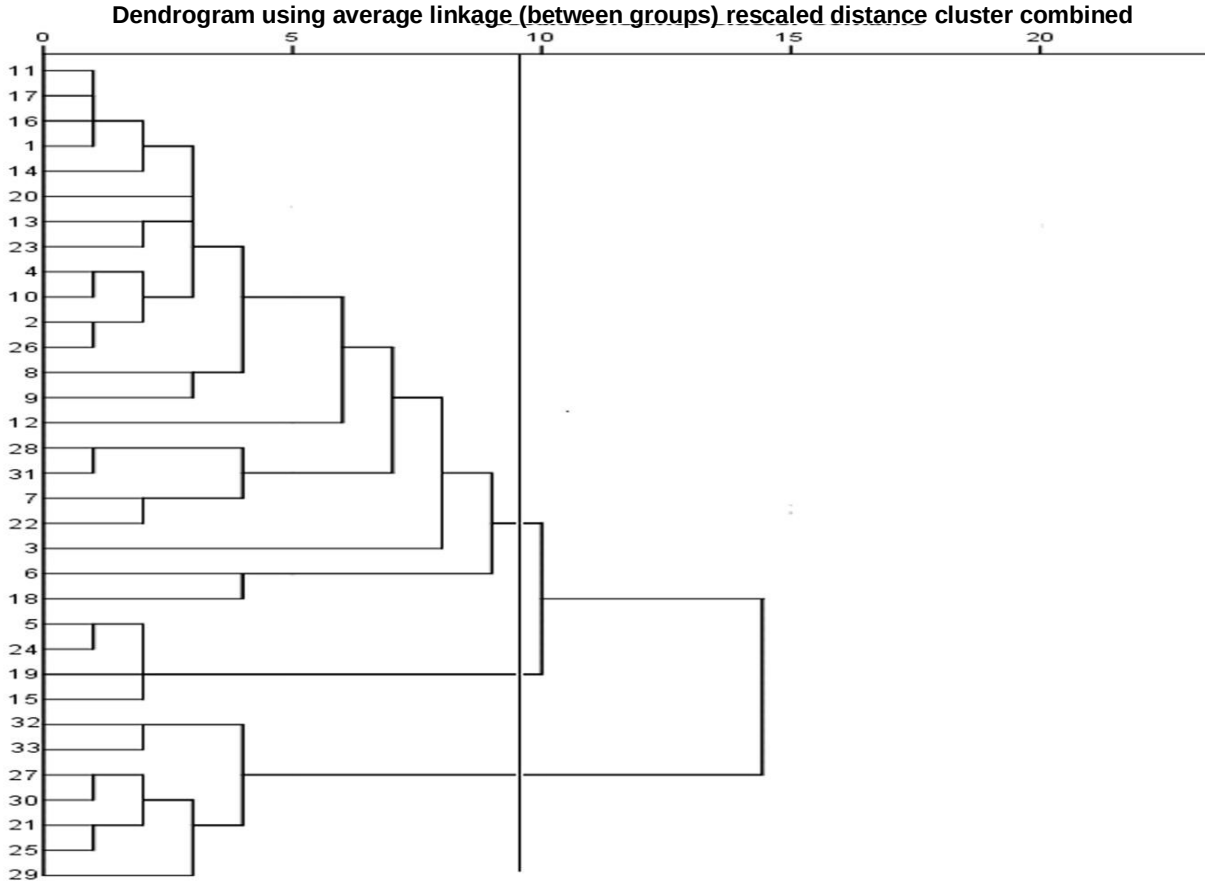

Figure 1. Cluster analysis of 33 Aloe accessions based on morphological characters.

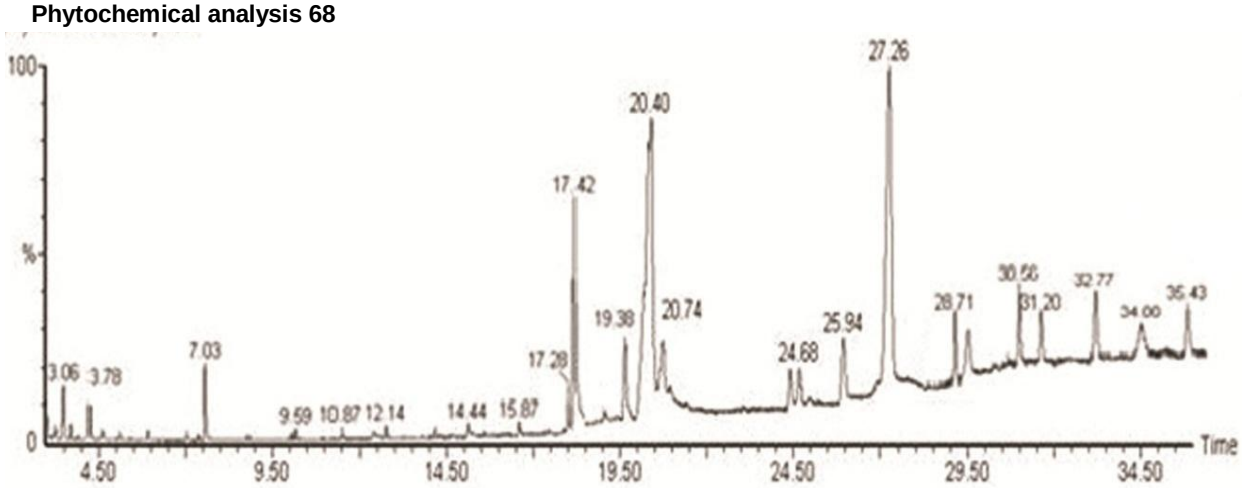

Figure 2. GC/MS chromatogram of selective Aloe accession (24).

The utilization of GC-MS was effective and useful for the identification of the bioactive compounds in Aloe plant. According to the results, 26 bio-active phytochemical compounds were identified in the GC-MS analysis of selective Aloe accessions. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula (Figure 2).

Results indicated that identified bioactive phytochemical compounds of selective Aloe accessions were in accordance with identified bioactive phytochemical

Table 2. Phenotypic correlation of studied characters in aloe accessions.

Variable	Mean of interburr length	Sprig number	Leaf length	Leaf width	Leaf thickness	Leaf fresh weight	Leaf dry weight	Number of burr in leaf	Number of leaf	Mean of leaf burr length	Plant height	Stem length	Stem diameter	Mean of internode length	gel weight ratios to leaf through weight	Leaf gel weight	Leaf cuticle thickness	Leaf mesophyll thickness
Mean of interburr length	1																	
Sprig number	-0.247*	1																
Leaf length	0.645**	-0.335**	1															
Leaf width	0.727**	-0.344**	0.682**	1														
Leaf thickness	0.539**	-0.292**	0.565**	0.632**	1													
Leaf fresh weight	0.769**	-0.368**	0.879**	0.796**	0.559**	1												
Leaf dry weight	0.760**	-0.361**	0.872**	0.791**	0.550**	1.000**	1											
Number of burr in leaf	0.047	0.03	.0346**	0.181	0.032	0.221*	0.220*	1										
Number of leaf	0.647**	-0.297**	0.582**	0.808**	0.643**	0.680**	0.685**	0.176	1									
Mean of leaf burr length	0.200*	-0.143	0.240*	0.439**	0.102	0.384**	0.388**	0.156	0.364**	1								
Plant height	0.696**	-0.398**	0.931**	0.731**	0.510**	0.880**	0.889**	0.264**	0.629**	0.199*	1							
Stem length	0.628**	-0.336**	0.626**	0.632**	0.486**	0.688**	0.680**	0.05	0.604**	-0.011	0.760**	1						
Stem diameter	0-.429**	0.01	-0.354**	-0.546**	-0.348**	-0.446**	-0.441**	-0.004	-0.470**	-0.361**	-0.315**	-0.255*	1					
Mean of internode length	0.514**	-0.272**	0.458**	0.459**	0.602**	0.507**	0.500**	-0.09	0.502**	0.052	0.482**	0.650**	-0.374**	1				
gel weight ratios to leaf through weight	-0.111	0.006	-0.157	-0.164	-0.158	-0.14	-0.148	-0.229*	-0.215*	-0.240*	-0.116	-0.072	0.032	0.017	1			
Leaf gel weight	0.767**	0.370**	0.874**	0.791**	0.551**	0.998**	0.990**	0.206	0.671**	0.369**	0.878**	0.688**	0.447**	0.513**	0.770**	1		
Leaf cuticle thickness	0.558**	-0.202*	0.579**	0.751**	0.540**	0.639**	0.632**	0.122	0.688**	0.401**	0.619**	0.591**	-0.454**	0.443**	-0.05	0.641**	1	
Leaf mesophyll thickness	0.521**	-0.287**	0.546**	0.604**	0.999**	0.536**	0.533**	0.024	0.620**	0.079	0.487**	0.464**	-0.330**	0.593**	-0.16	0.528**	0.496**	1

compounds by other researchers (Sathyaprabha et al., 2010; Lakshmi et al., 2011). Ten compounds with its biological activities were found in *Aloe* accessions. The prevailing compounds were oleic acid; 11,14-Eicosadienoic acid, methyl ester; n-Hexadecanoic acid; 1,2-benzenedicarboxylic acid, butyloctyl ester; hexadecanoic acid, methyl ester; Tetradecanoic acid; (4,7-Dinitronaphthalen-1-yl)-(4-methoxyphenyl) diazene; 1- Heptanol, 2-propyl-; 1,2-Benzenedicarboxylic acid, diisooctyl ester and Squalene. Major compounds of Aloe were shown to have the activity as anti-cancer and anti-microbial, etc. The composition of identified active compounds in this paper has been the subject of future research studies. Based on the obtained results of GC-MS analysis, bioactive phytochemical compounds have fluctuated within selective Aloe accessions. The phytochemical profiles of individual plants will change depending on a variety of other environmental and growth conditions. Wild plants may produce secondary metabolites, which have no apparent role in primary plant growth or development processes. These molecules are often unique to plants from a single species and increase during times of high stress such as drought, fire and bacterial infection (Cock, 2011). So, further studies need to emphasize the role of different types of stress in the production metabolites.

Results of GC/MS analysis indicated that there are genetic variations in selective Aloe accessions (Figure 3). However, morphological analysis and GC/MS analysis indicated that more remarkable genetic variations are observed among the selective Aloe accessions. Therefore, the Aloe genetic background in Iran is much more limited than other regions in the world. However, this should be further evaluated and confirmed based on a larger number of accessions from geographically more diversified regions. Hence, GC/MS analysis combined with morphological analysis provided a better relationship to identify these accessions. However, the more researches on these accessions using molecular markers could be useful for genetic resource identification

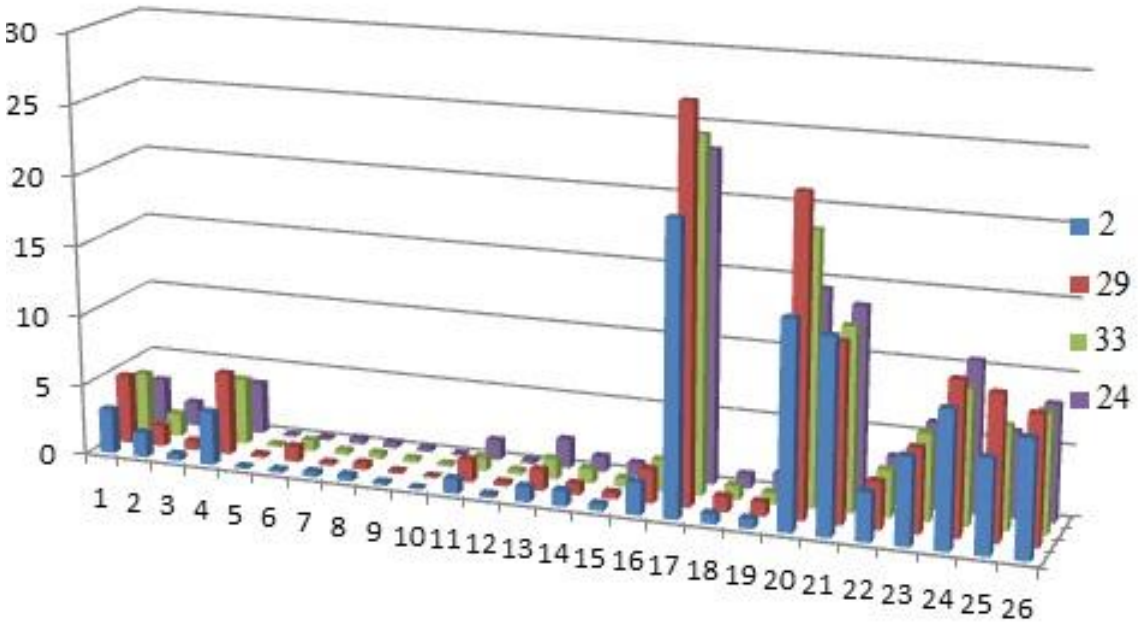

Figure 3. GC/MS graph of selective Aloe accessions (2, 29, 33 and 24 accessions).

and introduction.

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