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Identification of phytochemical components of aloe plantlets by gas chromatography-mass spectrometry

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Aloe vera **plants were collected from Blochestan, Iran and were transferred to tissue culture laboratory. Shoot tip explants were inoculated on solid MS medium supplemented with 0.5 mgl^{-1} benzyl adenine + 0.5 mgl^{-1} α-naphthalene acetic acid and sub-cultured on the same medium for plantlet production and propagation once every four weeks. After plantlets production, extracts of *A. vera* plantlet were analyzed by gas chromatography-mass spectrometry (GC-MS). According to the results, 26 phytochemical compounds were identified. Results indicate that these compounds of micropropagated plantlets are similar to the phytochemical compounds identified by other researchers in aloe plants. With attention on the obtained results of GC-MS analysis, the obtained compounds of micropropagated plantlets did not vary in relation to aloe plants. These results also indicate that the use of propagated plantlets by tissue culture to produce and extract phytochemical compounds is useful and efficient, as was observed and expected. So, we can use this method (tissue culture) instead of aloe cultivation which is limited in some regions of the world.**

Key words: Aloe medicinal plant, phytochemical components, micropropagation, tissue culture, gas chromatography-mass spectrometry (GC-MS) analysis.

INTRODUCTION

Aloe vera is a medicinal, cosmetic and ornamental plant. The genus Aloe is a perennial succulent herb growing in tropical and subtropical parts of the world. Therefore, aloe cultivation is limited in these regions. There are over 300 species of aloe; most of them are native to South Africa, Madagascar and Arabia.

The different species have somewhat different concentrations of active ingredients (Yagi et al., 1998; Van Wyk et al., 1995). 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 exudate or gel-like leaf mesophyll; *A. vera* is commonly cultivated and supports a global natural products industry. Today, *A. vera* gel is an active ingredient in hundreds of skin lotions, sun blocks and cosmetics (Grindlay et al., 1986). 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 (Henry, 1979). Acemannan, the major carbohydrate fraction in the gel demonstrates antineoplastic and antiviral effects (Mc Daniel et al., 1990). The gel also contains bradykininase, an anti-inflammatory agent, which prevents itching, and salicylic acid as well as other antiprostaglandin compounds that relieve inflammation (Yagi et al., 1982). Other important pharmacological activities of *A. vera* are anti-diabetic (Rajasekaran et al., 2006), antiseptic (Capasso et al., 1998), anti-tumor (Winter et al., 1981), and wound and burn healing effect (Heggers et al.,

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Abbreviations: GC-MS, Gas chromatography-mass spectrometry; **MS,** Murashige and Skoog medium.

Figure 1. *Aloe vera* plant.

1993). The sticky liquid latex is derived from the yellowish-green pericyclic tubules that line the leaf (rind); this is the part that yields laxative anthraquinones. The leaf lining (latex, resin or sap) contains anthraquinone glycolsides (aloe-emodin and barbaloin) which are potent stimulant laxatives.

Sexual reproduction by seeds due to male sterility in aloe plants is almost not effective and vegetative propagation through lateral shoots or lateral buds is only possible during growing seasons (Nayanakantha et al., 2010), and is slow and very expensive for commercial plant production (Meyer and Staden, 1991). To overcome slow propagation rate, micro propagation will be a very useful technique for mass production of aloe.

A. vera has been cultured *in vitro* by various researchers (Natali et al., 1990; Roy and Sarkar, 1991; Abrie and Staden, 2001). The technique of tissue and organ culture is used for rapid multiplication of plants, for genetic improvement of crops, for obtaining disease-free clones and for preserving valuable germplasm. One of the major applications of plant tissue culture is micropropagation or rapid multiplication. As compared to conventional propagation, micropropagation has the advantage of allowing rapid propagation in limited time and space.

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, thermally stable and have relatively low molecular weight.

In this present study, we used micropropagated plantlets of *A. vera* for evaluation of phytochemical components by GC-MS analysis.

MATERIALS AND METHODS

A. vera plants (Figure 1) were collected from Blochestan farmland in Iran and were transferred to Tissue Culture Laboratory of Karaj Agricultural Biotechnology Research Institute in September 2010. Shoot tip explants containing one to two buds were cut and washed with tap water for 10 min, and after surface sterilization using 2% (w/v) NaOCl for 20 min, they were thoroughly rinsed with sterile water. The explants were thoroughly washed with sterile double distilled water for four to five times to remove any trace of the sterilant. Then, the explants were inoculated on solid Murashige and Skoog (MS) (Murashige and Skoog, 1962) medium supplemented with 0.5 mgl^{-1} benzyl adenine + 0.5 mgl^{-1} α-naphthalene acetic acids into jars (250 ml capacity) containing 40 ml of the above-mentioned medium. Samples were sub-cultured every four weeks once on the same medium for plantlet production and propagation (Figure 2).

Preparation of plant extract

The micropropagated *A. vera* plantlets were washed with distilled water and were kept in-room temperature to be dried by air. Dried

Figure 2. Micropropagated plantlets of *A. vera*.

plantlets were crushed to the small pieces and were powdered and kept in polythene bags for further use. Aqueous extract of the studied samples were used to carry out the qualitative and quantitative analysis using standard procedures to identify the phytochemical components as described by Sofowara (1993) and Trease and Evans (1989).

Extracts of *A. vera* plantlets were analyzed by GC-MS. GC analysis was performed using a Hewlett-Packard 6890 chromatograph equipped with a flame ionization detector and injector MS transfer line with temperature of 280°C, respectively. A fused silica capillary column Hp- 5ms (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 above. Mass spectra were taken at 70 eV; the scanning rate was 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 with reference components.

RESULTS AND DISCUSSION

The utilization of GC-MS was effective and useful for the identification of the bioactive compounds in *A. vera*. According to the results, 26 bio-active phytochemical compounds were identified in the GC-MS analysis of *A. vera* plantlets. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula (Table 1).

Results indicate that these compounds of micropropagated plantlets are similar to the phytochemical compounds identified by other researchers in aloe plants (Sathyaprabha et al., 2010; Lakshmi et al., 2011). 10 compounds with biological activities were found in *Aloe vera*. The main compounds include oleic acid (14.49), 11,14-eicosadienoic acid, methyl ester (2.71), n-hexadecanoic acid (20.41), 1,2-benzenedicarboxylic acid, butyloctyl ester (2.28), hexadecanoic acid, methyl ester (1.45), tetradecanoic acid (1.03), (4,7-dinitronaphthalen-1-yl)-(4-methoxyphenyl)diazene (0.09), 1-heptanol, 2-propyl- (3.77), 1,2-benzenedicarboxylic acid, diisooctyl ester (13.56) and squalene (6.57). These compounds of *A. vera* were shown to have the activity of anticancer, antimicrobial, etc. These results also indicate that the use of propagated plantlets by tissue culture to produce and extract phytochemical compounds is useful and efficient, as was observed and expected. So, we can use this method (tissue culture) instead of aloe cultivation which is limited in some regions of the world for production and extraction of phytochemical compounds.

The composition of identified active compounds in *A. vera* is the subject of future research studies. With attention on the obtained results of GC-MS analysis, the obtained compounds of micropropagated plantlets do not vary in relation to aloe plants, and the bioactive phytochemical compounds have not changed within micropropagated plantlets in relation to wild plant (Figures 3 and 4). With regards to the variations of environmental and growth conditions, the phytochemical profiles of individual plants changes. Wild plants may produce secondary metabolites, which have no apparent

Table 1. Identified components of *A. vera* plantlet by GC-MS.

RT	Name of the compound	Molecular Formula	Molecular Weight	Peak area (%)
3.06	p-Xylene	C_8H_{10}	106	3.13
3.78	1,5-Heptadien4-one,3,3,6-trimethyle-	$C_{10}H_{16}O$	152	1.69
7.03	1- Heptanol, 2- propyl-	$C_{10}H_{22}O$	158	3.77
8.25	Tridecane	$C_{13}H_{28}$	184	0.1
9.59	7- Tetradecane, (z)-	C14H28	196	0.17
10.87	Tetradecane	$C_{14}H_{30}$	198	0.32
12.14	Hexadecane	$C_{16}H_{34}$	226	0.38
13.64	12,15-Octadecadiynoic acid,methyle ester	$C_{19}H_{30}O_2$	290	0.18
13.96	(4,7-Dinitronaphthalen-1-yl)-(4-methoxyphenyl)diazene	$C_{17}H_{12}N_4O_5$	352	0.09
14.44	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.03
15.87	Octadecane, 3- ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	0.21
17.28	Undecane	$C_{11}H_{24}$	156	0.45
17.42	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	13.56
19.38	9-Octadecenoic acid, (2-phenyl-1,3dioxolan-4-yl)methyle ester.cis-	$C_{28}H_{44}O_4$	444	3.04
20.74	9,12,15- Octadecatrienoic acid, 2-((trimethylsilyl)oxy)-1-(((trimethylsilyl)oxy)methyl) ethyl ester, (ZZZ)-	$C_{27}H_{52}O_4Si_2$	496	2.08
20.40	Oleic acid	$C_{18}H_{34}O_2$	282	14.49
24.68	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.45
25.94	1,2-Benzenedicarboxylic acid, butyloctyl ester	$C_{20}H_{30}O_4$	334	2.28
27.26	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256	20.41
28.71	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	2.71
29.11	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	2.63
30.48	Eicosane	$C_{20}H_{42}$	282	3.36
31.20	Heptacosane	$C_{27}H_{56}$	380	6.08
32.77	Octacosane	$C_{28}H_{58}$	394	9.52
34	Squalene	$C_{30}H_{50}$	410	6.57
35.43	Hentriacontane	$C_{31}H_{64}$	436	8.14

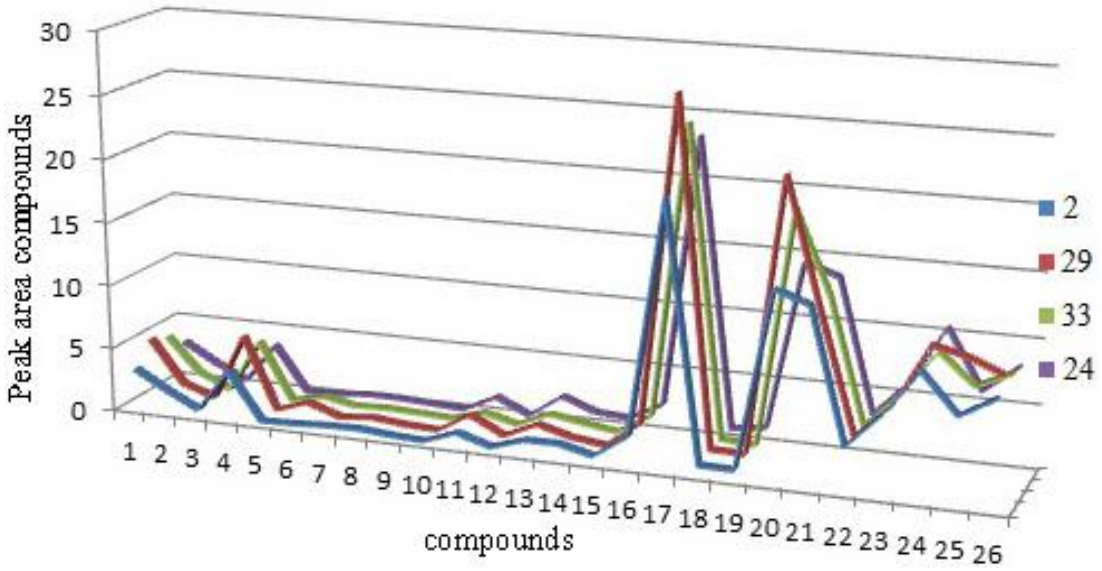

Figure 3. GC-MS graph of *A. vera* plantlets (2, 29, 33 and 24 accessions).

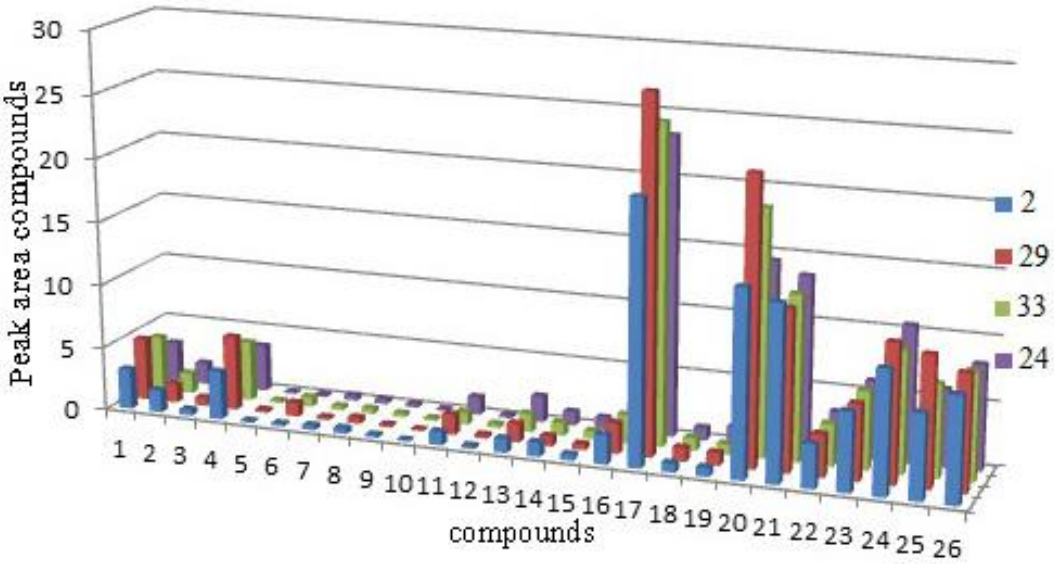

Figure 4. GC-MS graph of *A. vera* plantlet (2, 29, 33 and 24 accessions).

role in primary plant growth or development processes. These molecules are often unique in plants of a single species and are increased during times of high stress such as drought, fire and bacterial infection stresses in micropropagated plantlets.

REFERENCES

Abrie A, Staden JV (2001). Micropropagation of endangered Aloe Polyphylla. Plant G. Regu. 33(1):19-23.

Capasso F, Borrelli F, Capasso R (1998). Aloe and its therapeutics use. Phytoth. Res. 12:121-127.

Cock IE (2011). Problems of Reproducibility and Efficacy of Bioassays Using Crude Extracts, with reference to *Aloe vera*. Pharmacog. Comm. 1(1):52-62

Grace OM, Simmonds MJS, Smith GF, van Wyk AE (2009). Chemosystematic evaluation of Aloe section Pictae (Asphodelaceae). Bioch. Sys. Eco. 1:1-6.

Grindlay D, Reynolds T (1986). The *Aloe vera* phenomenon: a review of the properties and modern uses of the leaf parenchyma gel. J. Ethnopharmacol. 16:117-51.

Heggers J, Pelley R, Robson M (1993). Beneficial effects of Aloe in wound healing. Phytoth. Res. 7:S48-S52.

Henry R (1979). An updated review of *Aloe vera*. Cosmetics and Toiletries. 94:42-50.

Lakshmi PTV, Rajalakshmi P (2011). Identification of Phyto-Components and Biological Activities of *Aloe vera* Through the Gas Chromatography-Mass Spectrometry. Inter. Res. J. Pharm. 2(5):247-249.

McDaniel H, Carpenter R, Kemp M, Kahlon J, Mc Analley B (1990). Extended survival and prognostic criteria for Acemannan (ACE-M) treated HIV Patients. Antiviral Res. 1:117-125.

Meyer HJ, Staden JV (1991). Rapid in vitro propagation of *Aloe barbadensis* Mill. Plant cell Tis. and Org. Cul. 26:167-171.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays of tobacco tissue cultures. Physiol. Plantarum. 15:473-497.

Natali L, Sanchez IC, Cavallini A (1990). In vitro culture of Aloe barbadensis Mill: Micropropagation from vegetative meristems. Plant Cell Tis. Org. Cul. 20:71-74.

Nayanakantha NMC, Singh BR, Gupta AK (2010). Assessment of Genetic Diversity in Aloe Germplasm Accession from India Using RAPD and Morphological Markers. Ceylon J. Sci. Biol. Sci. 39(1):1-9.

Rajasekaran S, Sivagnanam K, Subramanian S (2006). Modulatory effects of *Aloe vera* leaf gel extract on oxidative stress in rats treated with steptozotocin. J. Pharm. Pharmacol. 57(2):241-246.

Roy SC, Sarkar A (1991). In vitro regeneration and micro propagation of *Aloe vera.* Scientia Hort. 47(1-2):107-114.

Sathyaprabha G, Kumaravel S, Ruffina D, Praveenkumar P (2010). A Comparative study on Antioxidant, Proximate analysis, Antimicrobial activity and phytochemical analysis of *Aloe vera* and *Cissus quadrangularis* by GC-MS. J. Pharm. Res. 3(12):2970-2973.

Sofowara A (1993). Medicinal plants and Traditional medicine is Africa spectrum Books LTD. Ibadan, Nigeria p.289.

Trease GE, Evans WC (1989). Pharmacognosy. 11th edn Brailliar Tiridel can Macmillian publishers.

Van Wyk BE, Yenesew A, Dagne E (1995). Chemotaxonomic survey of anthraquinones and pre-anthraquinones in roots of Aloe species. Bioch. Sys. Eco. 23:267–275.

Winter WD, Benavides R, Clouse WJ (1981). Effects of Aloe extracts on human normal and tumor cells in vitro. Econ. Bot. 35:89-95.

Yagi A, Harada N, Yamada H, Iwadare SIN (1982). Antibradykinin active material in Aloe saponaria. J. Pharm. Sci. 71:1172-1174.

Yagi A, Tsunoda M, Egusa T, Akasaki K, Tsuji H (1998). Immunochemical distinction of *Aloe vera*, *A. arborescens*, and *A. chinensis* gels [letter]. Planta Medica. 64:277-278.