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Evaluation of Aloe vera (*Aloe barbadensis Miller*) Antioxidant Activity and Some of the Morphological Characteristics in Different Vermicompost Field

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ABSTRACT: Construction and function of effective substances of medicinal plants are influenced by environmental factors such as deficiency or increased of nutrients in the soil and substrates. Therefore, a greenhouse experiment was performed in completely randomized design with four treatments and three replications. The effects of vermicompost were examined on the morphological and phytochemical features in aloe vera. Treatments consisted of four vermicompost percentages (0%, 15%, 30% and 45%) in humus soil. The considered factors were leaf weight, gel fresh weight, gel dry weight, the antioxidant capacity of the gel, glucomannan of gel, flavonoids and phenols of gel, and anthocyanins of cortex. data analysis showed that the maximum of leaf weight, gel weight, dry weight of gel and gel glucomannan was obtained in 45% of vermicompost. The maximum of gel phenol, antioxidant activity of gel and anthocyanins of cortex belonged to 30% of vermicompost and gel flavonoid in 15% of vermicompost. To achieve maximum antioxidant capacity and optimum amount of active substances, more studies and application of different field of vermicompost are required in order to increase the value of medicinal properties.

KEYWORDS: *Aloe vera*, Antioxidant capacity, Glucomannan, Phenol, Vermicompost

INTRODUCTION

The use of chemical and synthetic drugs has increased in the last half-century. However, the harmful effects that chemical and synthetic drugs have had on human life have resulted in more emphasis on herbal remedies [1]. Medicinal plants encompass a wide variety of plants, which are used for the prevention or treatment of infections. One of the most important, world-famous herbs is Aloe. This plant is an evergreen shrub that is from the Liliaceae family [46]. The most important species is *Aloe barbadensis* Miller, which is called sabre zard in Iran [41]. It has many uses in pharmacology, food, hygiene and in cosmetic industries [31-45]. The fleshy and thick leaves of this plant are due to the large

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amount of gel [9]. The gel contains 99% waterand a polysaccharide (glucomannan) at pH=4.5. The gel moisture arises from glucomannan. The gel also has numerous biological and physiological properties, including the ability to treat burns, skin wounds, anti-wrinkle, bacteria and parasite growth-inhibiting. It also increases the body's resistance against the proliferation of cancer cells and stimulates the immune system, due to the anthraquinone compounds [21-39]. Antioxidants are vital substances that protect cells from oxidative damage [19]. Natural antioxidants in plants, such as phenolic, ascorbic acid, carotenoid and anthraquinone compounds, have a strong potential for clearing free radicals in different parts of the plant [23-33]. Phenolic compounds are classified as simple phenol, phenolic acids, flavonois and anthocyanin. In medicinal plant productions, the real value is given to its quality and stability. Studies on medicinal plants in the natural ecosystems and farming indicate sustainable agricultural systems due to its compliance with natural conditions and the originality of quality of the product, which provides the best conditions for maximum effective substances [35]. Vermicompost is a kind of compost that is formed during Semi-aerobic process of decomposition of organic compounds by earthworms and soil microorganisms [7]. Organic compounds are considered as important components of soil fertility because of the beneficial effects on physical, chemical, biological and soil fertility [7-5]. It is useful in husbandry due to the hight porosity, absorption, keeping water and minerals with slow releasing. This study was performed to improve the gel quality and its medicinal properties in *Aloe barbadensis* Miller.

MATERIALS AND METHODS

Chemicals Materials

Gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin ciocalteu, quercetin were purchased from Merck and Fluka companies, and Congo Red was purchased from Suffolk England companies. All other chemicals and reagents used were of the highest commercially available purity.

Apparatus which was used should be included

Gel was homogenized by Moulinex (model DPA1).Gel was dried by an oven (Model BM120) and was measured with an digital scale (model A & D Company Limiled). Antioxidant capacity, glucomannan, flavonoid, phenol, anthocyanin measurements were conducted by a spectrophotometer (model UV-1800)

Plant material

Uniform shoots (*Aloe vera* L.) were isolated from rootstocks and transferred to pots containing the desired substrate. The experiment was done at greenhouse in a completely randomized design with four treatments, which included levels of 0%, 15%, 30% and 45% volume of vermicompost and three replications. Soil was mixed with a ratio 1:1:2:2 of garden soil, clay, leaf composts and animal manure. Crop control was done identically according to the need of pots. The factors that were examined include leaf weight, gel weight, gel dry weight, antioxidant capacity of gel, glucomannan of gel, phenol and flavonoid of gel, and anthocyanin of cortex.

Measurement of morphological characteristics

The largest leaves were isolated from near the base of each plant by a sharp knife and washed. Then, the leaf weight was calculated with a digital scale. To determine the amount of gel, aloe vera leaves were harvested between two to four hours and then washed. The top, bottom and edges of the leaves were cut. The upper epidermal tissue of leaves was removed by a sharp knife. Then, the gels were isolated and weighed by scales. To determine the gel dry weight, the samples were put in the oven (Model BM120) at 40 $^{\circ}C$ until the weight was constant. Then, the gel dry weight was measured [27-12].

Measurement of biochemical characteristics

For preparing the extraction, the fresh leaf was chopped, washed, and cut from the middle. The gel was separated by scraping it with a spoon. It was homogenized in a moulinex and then conserved at -20°C [30].

DPPH radical-scavenging activity

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used to determine the free radical-scavenging activity of the extracts. Different concentrations of each extract were equally added to methanolic solution of DPPH (100 µM). The absorption was recorded at 517 nm at room temperature, after 15 minutes of spectrophotometer (UV-1800). The experiment was repeated three times [14].

Determination of total phenol content

Total phenolic compounds were determined by the Folin-Ciocalteau method. The extract samples (20µl) were mixed with Folin Ciocalteu reagent (100µl) for 5 minutes and then the aqueous Na_2CO_3 (300 µl, 1 M) were added. The mixture was allowed to stand for 30 minutes, and the phenols were determined by colorimetric method at 765 nm. Total phenol values are expressed in terms of Gallic acid equivalent (µg/g of fresh weight). Total phenol contents were calculated as Gallic acid from a calibration curve [14].

Determination of total flavonoid content

Colorimetric aluminum chloride method was used for flavonoid determination. Briefly, 0.5 ml solution of each plant extract in methanol were mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water separately. Then, they were left for 30 minutes at room temperature. The absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). Total flavonoid contents were calculated as quercetin from a calibration curve [14].

Determination of anthocyanin

The measuring of anthocyanin was performed according to the method of Sims and Gamon [38]. In which 0.2 g of cortex of fresh leaf samples were grounded with 10 ml of 80% acetone at 4°C and centrifuged at 2500 rpm for 10 minutes at 4°C. Three milliliters aliquots of the extract were transferred to a cuvette, and the absorbance was read at 645, 663 and 537 nm with spectrophotometer.

Determination of glocomanan

This method is based on measuring changes of the congo red colored indicator by glucomannan on the specific reaction. The Eberendu and his colleagues [13] method was used for making the indicator. In summary, about 40 mg of each sample was weighed by an analytical balance and quantitatively, transferred into polypropylene conical tubes. DI water (20 mL) was added to each sample, and the mixture was placed on an orbital shaker. Then, 4.0 mL Congo red indicator was added to each tube. The mixture was left at room temperature for 20 minutes. The absorbance was record at 540 nm by spectrometer. The *aloe vera* polysaccharide was used for preparing the standard solution by method Waller and others [42]. In order to draw the calibration curve, the various standard values were used. Finally, the amount (µg/l) of gel glucomannan was calculated according to the formula [13].

STATISTICAL ANALYSIS

The data was statistically analyzed by SAS (Institute, 9.1.3) software. The significant differences were compared to the LSD test at $P<0.05$. Also, the MSTAT-C software was used for comparing the means.

RESULTS AND DISCUSION

Leaf weight

Due to the significance of vermicompost treatments on total leaf weight of *Aloe vera* in $p<0.01$ (Table 1), 45%

vermicompost increased leaf weight compare to control treatment and rest of the levels vermicompost. In this context Prabha and others [34] reported that the weight and plant growth were due to the increased absorption of mineral nutrients such as nitrogen and plant growth regulators. The Atiyeh and his colleagues [7] investigations have shown that suitable effects of vermicompost occurred due to physical, chemical, biological and microbial characteristics of changing conditions. Also, other causes included pH regulation and a significant increase in water storage capacity of the substrate. The growth increase was occurred by increasing the percent of vermicompost via physical and nutritional factors. On the other hand, the increase in plant height caused the leaf weight to increase. It is effective in increasing the amount of gel. According to Muscolo and his colleagues [26] it can stimulate hormone production such as auxin. Also, excessive microbial activity increased the concentration of nitrogen in plants [5].

Table 1. variance decomposition the effects of vermicompost on plant Aloe Vera Based on the mean-square

Sources of change	df	leaf weight(gr)	gel weight (gr)	dry gel weight (gr)	antioxidant capacity of gel(%)	glucomanan of gel (µg/l)	phenol of gel (µg/gr)	flavonoid of gel (µg/gr)	anthocyanin of cortex (mg/gr)
vermicompost	3	9020.9**	2204.23**	0.166**	6.56*	0.375**	263.07**	18.203**	0.000034*
Error	8	10.31	6.058	0.0032	1.447	0.053	2.119	0.201	0.0000074
cv	-	2.93	4.76	12.109	2.297	3.82	4.48	3.318	17.0209

ns **, *, non significant or significant at P≤0.01, P≤0.05, respectively.

Gel weight

Comparison data indicates that an increase in vermicompost causes the gel weight to increase as well, so that the maximum gel was showed in 45% vermicompost. The control treatments had the lowest amount of gel. The experiments by Cruz and others [11] and Saha and others [36] and Nematian and others [27] confirmed that the nutrient minerals, such as nitrogen and potassium, increase leaf growth and lead to a substantial amount of gel in aloe vera.

Dry weight of gel

There was a significant difference in gel dry weight between the different levels of vermicompost (Table 1). Positive influence 45% vermicompost on total leaf weight of *Aloe vera* and, indicated that this factor increased the gel weight gel dry weight. This experiment indicated a high correlation between the dry weight of gel and the fresh weight ($r = 0/94^{**}$) in $p<0.01$ (Table 3). The supply of nutrients, such as nitrogen and potassium, played a significant role in growth and primary metabolism performance. Also based on the carbon balance of minerals hypothesis and growth differentiation hypothesis, there is a characterized bilateral relationship between primary and secondary metabolism. As the availability of food for the plant increased, the amount of photosynthesis increased as well as the amount of carbon. It causes the increasing amount of carbon and the increase in carbon can be used in the synthesis of primary and secondary compounds [8-24-44-15]. Zarandi and his colleagues [47] reported that the fresh and dry weight shoots in *Ocimum basilicumplant* in all organic treatments was significantly higher than the control and chemical treatments.

The antioxidant capacity of the gel

Results of the analysis of variance table (Table 1) showed that the antioxidant capacity was significant at $p<0.01$. Hence, the maximum antioxidant capacity of the gel (53.28%) was in 30% vermicompost and soil treatment (Figure1). The control showed the lowest antioxidant activity (50.17). The significant increase in antioxidant capacity of the gel in vermicompost 30% could be due to the correlation between antioxidant capacity and phenolic compounds. The investigations of Ghasemzadeh and Jaafar [16] in *Zingiber officinale* confirmed that the increase in photosynthesis, high flavonol content and phenolic compound was associated with high antioxidant activity [29-43]. According to Atiyah and his colleagues [7] the enzyme that was produced by microorganisms in vermicompost has an effect on the physiological activity and antioxidant capacity. Also, the study of Nur and others [28] revealed the highest antioxidant capacity of cassava in the organic fertilizer vermicompost. The research of Alizadeh and others [3] showed that the antioxidant activity was increased in all treatments, but there were no significant differences between treatments on the *Satureja hotensis*. It was also shown that there is a positive correlation between total phenolics and antioxidant activity in this plant. The results indicated that 55% of antioxidant activity was due to phenolic compounds in *Satureja hortensis*. It can be proved that the antioxidant activity is not just because of phenolic compounds in plants available at this plant. It is evident that the antioxidant activity in plants is not limited to phenolic compounds. There are other secondary metabolites, such as essential oils, carotenoids and vitamins, which can cause up to 45% of antioxidant activity in Satureja hortensis. Similar results were obtained by Javanmardi and his colleagues [17].

Glucomannan of gel

The finding of this study showed that most gel glucomannan was obtained in 45% vermicompost treatment (Figure2). Also, the amount of glucomannan increased significantly in 15% and 45% vermicompost treatments compared with the control (Table 1). Since aloe vera is a CAM plant, (Krasvlas·h acid metabolism pathway) the first produced compound is malic acid, which is made of CO_2. It is used by the Calvin cycle and glycolysis for manufacturing polysaccharides [2-27]. Vermicompost increases nutrient absorption [40]. Therefore, the plants that receive more nutrients, such as nitrogen and potassium, will produce more malic acid, which increases the amount of polysaccharides [2].

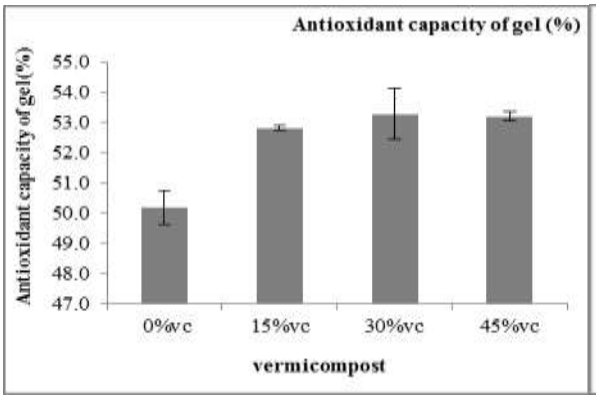

Figure1. Effects of different VC on antioxidant capacity of gel(%)in *Aloe vera*

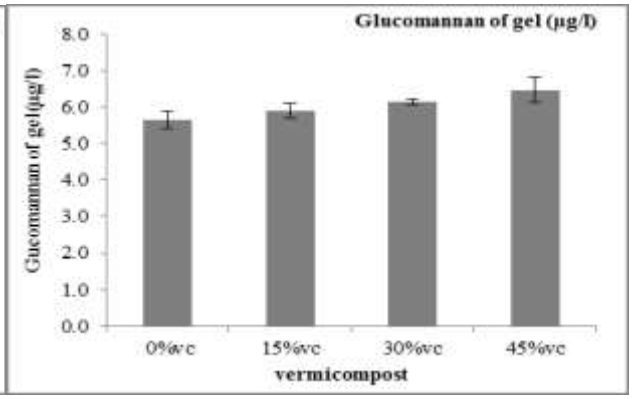

Figure2. Effects of different VC on glucomannan of gel(µg/l)in *Aloe vera*

Phenols and flavonoids gel

Vermicompost treatment was significant at p<0.01.in phenol and flavonoid content of gel. The gel Phenol was increased significantly in 30% vermicompost treatments compared with control and other vermicompost treatments (Table 1). The results also showed that the gel flavonoid significantly increased. The highest amount of flavonoid (16.28 µg/ g) was observed in 15% vermicompost and the lowest amount 10.60 µg/g in the control (Table 2). Increasing nutrient elements in the soil treated with vermicompost led to more secondary.metabolites synthesis. The increase in phenolic concentration is related to the balance between carbohydrate sources and sinks. Thus, when there are more carbohydrates, there are also more phenolic compounds [22-25]. However, excessive use of vermicompost increases a substrate's salinity, which has inhibitory effect on plant's activity [4]. This can reduce the photosynthetic rate and reduce the amount of phenolic compounds in high percentages of vermicompos.

Table 2. Results of means comparison the influence of application vermicompost on some Characteristics in Aloe vera plants

	leaf weight(gr)	gel weight (gr)	dry gel weight (gr)	antioxidant capacity of gel(%)	glucomannan of gel (µg/l)	phenol of gel (µg/gr)	flavonoid of gel (µg/gr)	anthocyanin of cortex (mg/gr)
Vermicompost 0	36.34 [d]	12.85 [d]	0.149 [d]	50.170 [b]	5.63 [c]	24.38[c]	10.60 [d]	0.0133 [b]
Vermicompost 15٪	106.297 [c]	55.054 [c]	0.448 [c]	52.818 [a]	5.89 [bc]	26.49 [c]	16.28 [a]	0.0146 [b]
Vermicompost 30٪	128.425 [b]	64.74 [b]	0.573 [b]	53.28 [a]	6.15 [ab]	45.121 [a]	12.58 [c]	0.021 [a]
Vermicompost 45٪	166.942 [a]	74.36 [a]	0.698 [a]	53.207 [a]	6.46 [a]	33.86 [b]	14.61 [b]	0.015[b]

Means within the same column followed by the same letter were not significantly different

Anthocyanin of gel epithelial tissue

The results of the variance analysis table (Table 1) showed that vermicompost increased the anthocyanin of gel epithelial tissue so that the maximum amount of anthocyanin was observed (0.021 mg/gr) in 30% vermicompost treatment and the lowest was observed in the control group. On the other hand, In addition, carbohydrates were identified as a required structure for phenolic compounds. Increasing carbohydrates resulted in more synthesis of phenolic compounds, which allocated more carbon to the shikimic acid path way [32-10-20-18]. The study conducted by Shehata and others [37] examined the effect of compost on growth, yield and chemical parameters of strawberries. It showed that the highest amount of anthocyanin was achieved in 8 tons of compost per hectare.

CONCLUSION

Overall, vermicompost not only had favorable effects on soil physical properties, but it also increased nutrient uptake, especially macro elements. Increases in absorption are variable, depending on the different characteristics. Thus, using of vermicompost mixed substrate in breeding the aloe vera has an effective role in improving quality and quantity leaves and gel antioxidant properties. The effectiveness is dependent on the functional characteristics of combination of vermicompost in substrate

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Table 3. Correlation coefficients between some Characteristics in Aloe vera plants

	leaf weight(gr)	**gel weight (gr)**	**dry gel weight (gr)**	**antioxidant capacity of gel(%)**	**glucomannan of gel (µg/l)**	**phenol of gel (µg/gr)**	**flavonoid of gel (µg/gr)**	**anthocyanin of cortex (mg/gr)**
leaf weight(gr)	1							
gel weight (gr)	0.97**	1						
dry gel weight (gr)	0.96**	0.94**	1					
antioxidant capacity of gel(%)	0.704**	0.74**	0.77**	1				
glucomannan of gel (µg/l)	0.82*	0.75**	0.82**	0.56 *	1			
phenol of gel (µg/gr)	0.56 *	0.63 *	0.62*	0.50 ns	0.47*	1		
flavonoid of gel (µg/gr)	0.60 *	0.66 *	0.55 ns	0.55 ns	0.32 ns	-0.05 ns	1	
anthocyanin of cortex (mg/gr)	0.33 ns	0.37 ns	0.46 ns	0.41 ns	0.49 ns	0.73**	-0.069 ns	1

ns **, *, non significant or significant at P≤0.01, P≤0.05, respectively.

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