Determination of Antioxidant Potential of Selected Parts of Aloe Vera Plant

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RESEARCH

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ABSTRACT

Over 360 species of Aloe are known among this Aloe barbadensis. MILLER is most used species. Aloe Vera juice is extracted from Aloe barbendesis having antioxidant properties that may provide several health benefits. Consumers are interested and willing to look at natural alternatives for health benefits. Objective of this study was to determine the antioxidant potential of Aloe vera (whole leaf, leaf skin and leaf gel) and to develop a product using Aloe vera juice. Aloe Jello was prepared using Aloe Vera juice, unflavored gelatin, Stevia (as a sweetener) and small pieces of Aloe Vera. A 5-point Hedonic scale sensory test was conducted in the sensory laboratory with 30 untrained panelists. The shelf-life experiments were conducted for attributes including pH, color and water activity. Sensory test was conducted for the product acceptability in which the overall acceptability was scored 4 or better by 50% of the panelists. The appearance of the product was liked (score 4 or better) by most (74%) of the panelists. The pH of Aloe Jello was 3.35 and the water activity (Aw) was 0.96. The L*, a*, b* color values of the product were 33.98, 1.53, and 15.57, respectively. Results suggest that Aloe Vera may have potential for use in food products and nutraceuticals to promote its consumption for the associated health benefits.

Keywords: Antioxidant, Nutraceuticals, Water activity.

LIST OF ABBREVIATIONS

TPC: Total phenolic content; TFC: Total flavonoid content; DPPH: 1,1-diphenyl-2picrylhydrazyl; TEAC: Trolox equivalent antioxidant capacity; FRAP: ferric reducing antioxidant power; ORAC: Oxygen Radical absorbance capacity; NORS: Nitric Oxide Radical Scavenging; G.A.E: Gallic Acid Equivalent; DW: Dry Weight; C.E: Catechin equivalent; T.E: Trolox equivalent; MWE: Methanol whole *Aloe vera* leaf extract; MSE: Methanol leaf skin extract; MGE: Methanol leaf gel extract; AWE: Aqueous whole *Aloe vera* leaf extract; ASE: Aqueous leaf skin extract; AGE: Aqueous leaf gel extract; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; WE: Whole *Aloe vera* leaf extract; SE: *Aloe vera* leaf skin extract; GE: *Aloe vera* leaf gel extract.

INTRODUCTION

In There are over 360 species of Aloe known, with Aloe barbadensis MILLER being the most used species. It is a xerophytic plant which belongs to the family Liliaceae. The plant is found in dry and arid regions. The plant body can be divided into two main parts based on its use for research purposes: the leaves which contain a high

concentration of anthraquinone compounds and the gel. The leaves and gel are used as food and are also known for their medicinal properties such as curing skin problems, and treating burns and wounds [1, 2]. Aloe vera is also used as a food supplement or a nutraceutical. Polysaccharides in the pulp of the plant include glucuronic acid containing polysaccharides [3, 4], mannan [5, 6, 7, 8, 9, 10, 11], galactan [12], arabinorhamnogalactan [13], arabinan [12] and others. Diabetes mellitus is an abnormality linked to connective tissues. The abnormalities can be quantitative [14] and qualitative [15, 16, 17] such as the decrease in collagen content of the skin due to acceleration in degradation of the young collagen cell or reduction in biosynthesis of collagen. This process is followed by an increase in the production of free radicals' [18, 19, 20] or by antioxidant defense dysfunctions [21, 22, 23]. In diabetic patients, these abnormalities are the main cause of defects in the wound healing capability of the body (24). The major cause for development, progression and complications related to diabetes is believed to be oxidative stress [25, 18]. An increase in number of free radicals' cause damage to cellular proteins, lipids in the membranes and nucleic acids which results in cell death. A major focus of this research will be the study of antioxidants present in Aloe vera responsible for health benefits against chronic diseases such as diabetes. The antioxidant defense system consists of various components which includes vitamins (A, C, and E), glutathione, and phase II enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and reductase) [26, 27]. The formation of free radical species is involved in etiology of several chronic illnesses, the antioxidant defense system protects the body against these adverse health effects [28].

Consumers are considering natural products for several health benefits. The average American has become more concerned about their health, from millennials all the way to the swing generation, whether it is losing or maintaining weight. A survey (Functional Foods/ Foods for Health Consumer Trending Survey) conducted by International Food Information Council showed that 80% of Americans believe that functional foods improve overall health. It also revealed that 69% of Americans believe foods and beverages containing functional ingredients may improve physical and mental performance and 68% believe that these can improve overall appearance [29]. Therefore, consumers are more interested in looking for alternatives which may help control and prevent chronic diseases such as diabetes, cancer and others. This may be the reason for an increased interest in specialized meal plans. As consumers are becoming more aware of labels, the trend is moving more towards cleaner labels, more natural, as well as chemical-free products. Another trend for 2016 includes specific foods that can be deemed “lifestyle enhancers,” or products that boost overall health. With the world becoming faster paced, “on-the-go” meals are the norm with an increase in snack consumption. There is an increase in the consumption of fortified foods because of the convenience factor [30], in addition to large number of Americans more deficiency in micronutrients. Based on this information, the idea of developing an Aloe vera product arose because of the need to provide products that are more satisfying for those health-conscious consumers.

Statement of problem

Phytochemicals are biologically active secondary metabolites that are naturally found in plants. These are not essential for sustaining life; however, research [31] has demonstrated that they may offer protection against some of the leading causes of death in the U.S. including chronic diseases such as diabetes.

Justification of Study

Type 2 diabetes mellitus is one of the major health related threats in developed and developing countries. In the past decades, drugs have been used to manage this metabolic disorder, which are expensive and have side effects which can add to the complications in the body. Hence, to solve this problem many researchers are focusing on alternative treatments which do not harm the health of the patients. In the present scenario, the interests of the public towards natural products have led scientists to research on the effects of certain plant extracts in the

treatment of this disorder. Therefore, further studies are required to identify a specific mechanism of antioxidants which may contribute to the therapeutic properties of Aloe vera.

OBJECTIVES

The study was carried out to draw comparison in the antioxidant properties of the methanol and water extracts of this plant. The objectives of this study were multifaceted and included determination of the total phenolic content, total flavonoid content and total antioxidant activity of Aloe vera, whole, skin & gel extracts evaluated by 2,2-diphenyl-1-picrylhydrazyl radical solution (DPPH), Ferric reducing antioxidant potential (FRAP), oxygen radical absorbance capacity (ORAC), Trolox equivalent antioxidant capacity (TEAC), and nitric oxide radical scavenging activity (NORS). Second, to determine the inhibitory effect of Aloe vera extracts on α-amylase, α-glucosidase, and lipase enzymes. Third, to develop a product using Aloe vera (Aloe jello).

MATERIALS AND METHODS

Chemical analysis was conducted to evaluate the antioxidative properties of Aloe vera. Then, a product was developed using Aloe vera gel.

Sample preparation

Fresh Aloe vera leaves were purchased from Garden Cove (Huntsville, AL). The leaves were divided into two sections. Sections of the leaves were cut into small pieces (size about 3mm) and weighed. The other Aloe vera leaves were used for extraction of gel. For extraction of gel, the skin was removed from one side, and the gel removed, and weighed. The skin was also cut into pieces, stored in an airtight bag and weighed. Following freeze drying (VirTis Genesis 35L SpScientific, Warminster, PA), methanol and water extracts were prepared. These extracts were further used for antioxidant, and chemical assays.

Chemical analysis

Chemical analysis of Aloe vera as whole, gel and skin were carried out on water and methanol extracts. The chemical assays included tests for phenolic, flavonoid components and antioxidant properties.

Total phenolic content

The Folin-Ciocalteau method [32] with slight modifications was followed to determine the total polyphenols.

Total flavonoid content

The colorimetric assay proposed by Marinova, Ribarova, & Atanassova [33] was used to determine the flavonoid content using catechin as a standard.

Antioxidant assays

The antioxidant activity of Aloe vera extracts was tested using selected assays such as FRAP, TEAC, nitric oxide, and ORAC.

Ferric Reducing Antioxidant Potential

FRAP (Ferric Reducing Antioxidative Potential) assay was conducted which measures the potential of antioxidant to reduce a ferrous ion to Ferric ion using the method developed by Benzie & Strain [34] with slight modifications using the ferrous sulfate (FeSO4.7H2O) standard.

Oxygen Radical Absorbance Capacity (ORAC)

ORAC assay was determined using the method developed by Huang et al [35] with slight modifications. Samples were compared against a diluted Trolox standard.

Trolox Equivalent Antioxidant Capacity (TEAC)

TEAC of extracts was determined using the protocol suggested by Miller et al., [36] with slight modifications.

Nitric oxide radical scavenging activity (NORS)

The nitric oxide radical scavenging activity was determined using modified Griess Illosvoy reagent [37].

RESULTS & DISCUSSION

Table 1 shows the total phenolic content in the aqueous and methanol extracts of whole leaf, skin and gel of Aloe vera. The methanol whole extract (MWE) had significantly higher polyphenols as compared to methanol skin and gel extracts (MSE & MGE). Similar trend was observed in aqueous extracts with aqueous whole extract having the higher phenolic content then skin and gel extract. The phenolic content was significantly ($p \leq 0.05$) higher in AWE (aqueous whole extracts) (58.4 mg GAE/100g DW) compared to ASE (aqueous skin extract) (14.7 mg GAE/100g DW) and AGE (aqueous gel extract) (13.7 mg GAE/100g DW). However, phenolic content in ASE and AGE were not significantly different. Methanol skin extract (MSE) (11.2 mg GAE/ 100 g DW) and Methanol gel extract (MGE) (7.9 mg GAE/ 100g DW) had significantly ($p \leq 0.05$) lower polyphenol compounds compared to MWE (20.1 mg GAE/100g DW). The phenolic content in AWE was 2.9 times higher compared to MWE. No significant differences were observed in phenolic content between ASE (14.7 mg GAE/ 100g DW) and MSE (11.2 mg GAE/ 100 g DW). Similarly, no significant differences were seen in the phenolic content present in AGE (13.7 mg GAE/ 100g DW) and MGE (7.9 mg GAE/ 100g DW). Overall, WE had significantly ($p \leq 0.05$) higher phenolic content compared to SE and GE (Aqueous & Methanol extracts).

Research has shown diet in particular functional foods, to play a major role in prevention of chronic diseases. Functional foods are foods containing antioxidants, minerals, vitamins, omega-3 fatty acids, or even dietary fiber are known to be beneficial in reducing the risk of chronic diseases. These functional ingredients can be found in plants, and oils from marine animals [38, 39]. Antioxidants present in plants are tocopherols, carotenoids, ascorbic acid, polyphenols, and flavonoids, which may provide protective effects against degenerative diseases [40]. Studies [41, 42] have demonstrated several biological effects related to the consumption of foods rich in phytochemicals. These compounds may be effective in reducing the risk associated with chronic diseases such as diabetes and cancer [43]. The factors such as nature of plants, temperature, and polarity of the solvent along with the extraction method used may affect the extraction of phenolic compounds in plant foods. For example, methanol due to its lower polarity compared to water can extract most polyphenols in plant materials [44, 45]. In the present study three different parts of the Aloe vera leaf were used with two different solvents to quantify the total phenolic, flavonoid and antioxidant capacity of Aloe vera. Phenolic compounds act as antioxidants and play a crucial role in the neutralization of free radicals based on their redox reaction potential [46]. Phenolic compounds reduced the FC reagent resulting in the formation of a blue color, which was measured at 750 nm against a Gallic acid standard. Total phenolic content of AWE (5.84 mg/100g) was significantly ($p \leq 0.05$) higher compared to ASE and AGE. Likewise, MWE (2.01 mg/100g) had significantly ($p \leq 0.05$) higher phenolic content compared to MSE and MGE ($p \leq 0.05$). The total phenolic composition is consistent in this study compared to previous research conducted [47, 48].

Total flavonoid content of the aqueous and methanol Aloe vera extracts for the whole leaf, skin and gel was determined, and the results are presented in Table 2. Total flavonoids in AWE were 1.6 to 1.9-fold higher compared to ASE and AGE. However, there were no significant differences in the flavonoid content in ASE (2.7 mg CE/ 100g DW) and AGE (2.3 mg/100g DW). Significantly ($p \leq 0.05$) higher total flavonoids were seen in MWE (6.1 mg CE/100g DW) as compared to MSE (0.24 mg CE/100g DW) and MGE (1.6 mg CE/100g DW). However, no significant differences were observed in flavonoid content in MSE and MGE. There was a significant ($p \leq 0.05$) difference in flavonoid content in MWE (6.1 mg CE/100g DW) and AWE (4.4 mg CE/100g DW). Whereas there were no significant differences in flavonoid content in ASE, MSE and AGE and MGE. Flavonoid content was over 2.5-fold higher in MWE

compared to MSE and MGE. Similarly, flavonoid content was about 1.5-fold higher in AWE compared to ASE & AGE.

Flavonoids are large secondary metabolites in plants. In the present study, flavonoid content was determined using the aluminum chloride (AlCl3) colorimetric method. AlCl3 forms stable acid complexes with the C-4 keto or hydroxyl group of the flavones and flavanols along with the dihydroxyl groups in the A and B ring of flavonoids. Flavonoid content of the Aloe vera extracts showed a similar trend as the total phenolic content because flavonoids are a major part of the phenolic compounds [49]. Flavonoid content of AWE (0.44 mg/100g) was significantly (p≤0.05) higher compared to ASE and AGE. Likewise, MWE (0.61 mg/100g) had significantly (p≤0.05) higher phenolic content compared to MSE and MGE. The whole extract exhibited higher flavonoid content, this may be due to the flavonoids in both skin and gel extract as whole extract has both parts of Aloe vera.

Antioxidant activity of Aloe vera Extracts

Table 3 shows the ferric reducing antioxidant power (FRAP) of MWE, MSE, MGE, AWE, ASE, and AGE. AGE (13.26 mM Fe (II)/100g DW) had a significantly (p≤0.05) higher FRAP compared to ASE (11.79 mM Fe (II)/100g DW), and AWE (6.00 mM Fe (II)/100g DW). Whereas, ASE had a 1.96-fold higher FRAP compared to AWE. There were no significant differences in FRAP of MWE (15.6 mM Fe (II)/100g DW) and MGE (15.76 mM Fe (II)/100g DW). However, FRAP in MSE (8.36 mM Fe (II)/100g DW) was significantly (p≤0.05) lower compared to MWE and MGE. A significantly (p≤0.05) higher FRAP was also observed in MWE as compared to AWE. Similarly, a 1.2-fold higher FRAP was seen in MGE compared to AGE and 1.4-fold higher in ASE compared to MSE. Aloe vera gel (AGE and MGE) had higher FRAP compared to whole & skin (AWE, MWE, ASE and MSE).

In the presence of antioxidants, the reducing power of Aloe vera extract was determined based on the transformation of ferric ion to ferrous ions imparting blue color. In this study, MWE showed potent reducing power. However, FRAP was significantly (p≤0.05) lower in AWE compared to ASE and AGE. This may be due to the difference in the solvents used. Methanol due to its lower polarity compared to water can extract most polyphenols in plant materials [44, 45].

Trolox equivalence antioxidant capacity (TEAC) of MWE, MSE, MGE, WWE, WSE, and WGE is shown in Table 4. TEAC of MSE (167.59 mM T.E/100g DW) was significantly (p≤0.05) higher compared to MWE (23.11mM T.E/100g DW) and MGE (43.25 mM T.E/100g DW). Whereas no significant difference was seen in TEAC between MWE and MGE. ASE (42.99 mM T.E/100g DW) had significantly (p≤0.05) lower TEAC compared to AWE (98.93mM T.E/100g DW), and AGE (97.78 mM T.E/100g DW). However, there was no significant difference in TEAC of AWE and ASE. A significantly (p≤0.05) higher TEAC was seen in AWE compared to MWE. Similarly, TEAC in MSE was 4.23-fold higher compared to ASE and it was seen to be 3.87-fold higher in AGE compared to MGE.

Nitric oxide radical scavenging activity (NORS) of AWE, ASE, AGE, MWE, MSE, MGE is shown in Table 5. AWE (435.94 mM NO/100g DW) had significantly (p≤0.05) higher NORS activity compared to AGE (327.07 mM NO/100g DW) and ASE (224 mM NO/100g DW). NORS in ASE was 1.95-fold lower compared to AWE and 1.46-fold lower compared to AGE. Whereas NORS of AWE was 1.33-fold higher compared to AGE. MGE (116 mM NO/100g DW) had significantly (p≤0.05) higher NORS activity compared to MSE (48 mM NO/100g DW), and MWE (41.83 mM NO/100g DW). While there was no significant difference in NORS in MWE and MSE. AWE had 14.88-fold higher NORS compared to MWE. Likewise, ASE had a 3.8-fold greater NORS compared to MSE. NORS activity in AGE was observed to be 2.98-fold lower compared to MGE.

Oxygen radical scavenging activity of AWE, ASE, AGE, MWE, MSE, MGE is shown in Table 6. AGE had significantly (p≤0.05) higher (898.268 mM T.E/100g DW) ORAC compared to ASE (293.188 mM T.E/100g DW), and AWE (289.977 mM T.E/100g DW). MWE (226.8 mM T.E/100g DW) had a significantly (p≤0.05) higher ORAC compared to MGE (182.917 mM T.E/100g DW) and MSE (105.439 mM T.E/100g DW). ORAC was 3.1-fold higher in

AGE compared to ASE and AWE. However, there was no significant difference in the ORAC activity of ASE and AWE. MWE had a 1.24-fold higher ORAC compared to MGE and 2.15-fold higher ORAC compared to MSE. Whereas, MGE had a 1.73-fold higher ORAC compared to MSE. ORAC of AWE was significantly ($p \leq 0.05$) higher compared MWE. Similarly, ORAC was significantly ($p \leq 0.05$) higher in ASE compared to MSE and AGE was higher compared to MGE.

Significant differences were observed in the scavenging and antioxidant capacity among different Aloe vera extracts (AWE, ASE, AGE, MWE, MSE, and MGE) estimated based on FRAP, TEAC, NORS and ORAC. The ABTS radical is generated by potassium persulfate, to determine the antioxidant activity of hydrogen donating antioxidants. Higher TEAC of AGE may be due to the ability to donate hydrogen and terminate the oxidation process by converting free radicals into their stable forms [50]. However, lower TEAC was seen in MSE compared to MWE and MGE.

The ORAC method evaluates the absorbance capacity of a sample by taking the oxidation reaction induced by peroxyl radical to completion through hydrogen atom transfer [51]. ORAC activity of AGE was higher compared to ASE and AWE. Sodium nitroprusside generates the nitric oxide radical with an optimum pH, which further interacts with the oxygen to form nitrite ions. The nitric oxide scavengers compete with oxygen leading to lower production of nitrite ions [52]. The antioxidant potential is related to the phenolic and flavonoid content of the extracts [47, 53, 54, 55, 48]. The remarkably higher antioxidant and scavenging capacity of Aloe vera is probably due to antioxidant compounds like phenolic, flavonoid, vitamins A, C & E and so on.

Product Development

Sensory Evaluation of Aloe jello

A 5-point Hedonic scale was used (30 panelists) to determine the sensory attributes and to check the overall acceptability of the product. Panelists were asked questions about their consumption habits, product attributes and their willingness to purchase the product.

Demographics: Females were 60% of the total panelists participating in the sensory analysis of the Aloe jello. Whereas only 40% of the panelists were male. The participants belonged to different ethnic groups, 77% of them were African Americans, 10% others, 10% Asians, and 3% Caucasians. Most (67%) of the participants were from the ages of 17 to 25 years. Thirty-four percent of the individuals on the sensory panel were from the age group of 26 to 45 years.

Consumption Habits: Questions were based on the consumption of Aloe vera and its products or gelatinized products. All the panelists consumed Aloe vera once a month or never indicating the underutilization of Aloe vera in the U.S. When asked about the consumption of fruits 60% of the panelists indicated that they consumed fresh fruits every day or three times a week. Whereas rest (40%) of the panelists consumed fresh fruits three times a week, once a week or once a month. About 63% of the panelists consumed sweetened gelatin products once a month or never. While 34% consumed gelatin products every two weeks or once a week. Only 3% of the panelists consumed gelatin products three times a week. with no daily consumers. Consumption of Aloe vera juice was very low among panelists. Seventy percent of panelists never consumed Aloe vera juice, while 20% consumed it once a month and only 10% consumed it every two weeks. However, none of the panelists consumed Aloe vera juice on a regular basis or often. Ninety percent of the panelists were willing to purchase sweetened gelatin products with added health benefits, 3% were not sure and 7% were not interested before sampling the product. Sixty-nine percent of panelists were interested in purchasing a sweetened gelatin product containing Aloe vera however, 17% were not. Whereas, 14% were not sure about purchasing a sweetened gelatin product containing Aloe vera

Product attributes: The attributes (Figure 1) described for the Aloe jello were color, appearance, taste, texture, aroma, sweetness and overall acceptability. All attributes were ranked on a 5-point hedonic scale and results are shown in. Seventy-three percent of the panelists liked the color, appearance and texture of the product and

gave a score of 4 or better on a 5-point hedonic scale. Whereas, taste, aroma and overall acceptability were scored 4 or more on a 5-point hedonic scale by ≥ 50% of the panelists. Product appearance (Figure 2) was evaluated using the following product appearance attributes enticing, moist, not appealing, sticky, and dry. Mouth feel attributes (Figure 3) were described as dry, gummy, chunky, chewy and smooth. Fifty-three percent of the panelists rated the product as having a smooth mouth feel while, 47% perceived it as gummy.

Physiochemical characteristics

The physiochemical characteristics such as water activity, pH, texture and color of Aloe vera product were measured. Figure 4 shows water activity of the Aloe jello. No significant difference was seen in the water activity of Aloe jello in week 0 (0.961), week 1 (0.962) and week 2 (0.968). Figure 5 shows the pH of Aloe jello. There was no significant difference observed in the pH of Aloe jello over the period of two weeks. The pH for week 0 was 3.45, week 1- 3.41 and week 2- 3.40. Texture of the Aloe vera product (Aloe jello) was observed to have no significant difference over a time of two weeks as depicted in Figure 6. Table 7 color value L*, a*, b* for Aloe jello over a storage period of two weeks. The negative color value a* is most likely due to the green color of the product and no significant difference was seen over the storage time. The L* value for week 1 (19.93), week 2 (19.52), week 2 (19.41) were not observed to be significantly different. Similarly, the b* value also did not show any significant difference over the storage period of two weeks.

CONCLUSION

Aloe vera is an annual succulent plant which is used for various medicinal, cosmetic and nutraceutical purposes. Aloe vera plant and its components have many biological activities such as anti-viral, anti-bacterial, laxative, protection against radiations, anti-inflammatory and stimulation of immune system [56, 57]. Antioxidant, anti-diabetic and anti-inflammatory effects of Aloe vera were determined in this study due to its myriad of health benefits. Our study concluded that all the Aloe vera extracts (whole, skin and gel) regardless of extraction solvents showed antioxidant potential.

To evaluate the health benefits particularly, the anti-diabetic potential of Aloe vera, a study needs to be performed to observe the protective effects of its extracts against glucose induced cytotoxicity in pancreatic (HIT-T15) cells. Aloe vera juice was used in the Aloe jello developed in this study. Aloe jello was bitter as per the comments given by the sensory panel, which might be due to use of stevia and Aloe juice. Further research needs to be conducted to overcome the bitter after taste by utilizing different sweetening agents.

LIMITATIONS

The antioxidant properties of Aloe Vera might vary based on the environmental growth conditions, maturity and the cropping cycle of the plant. Other issues include the extraction of phytochemicals specially from the whole leaves and the jell as well.

REFERENCES

1. Grindlay, D., & Reynolds, T. (1986). The Aloe vera phenomenon: a review of the properties and modern uses of the leaf parenchyma gel. Journal of ethnopharmacology, 16(2-3), 117-151.
2. Joshi, S. P. (1998). Chemical constituents and biological activity of Aloe barbadensis: a review. J Med Aromatic Plant Sci, 20, 768-773.
3. Hranisavljevic-Jakovljevic, M., & Miljkovic-Stojanovic, J. (1981). Structural study of an acidic polysaccharide isolated from Aloe arborescens Mill. I. Periodate oxidation and partial acid hydrolysis. Glas Hem Drus Beogr, 46, 269-73.
4. Wozniewski, T., Blaschek, W., & Franz, G. (1990). Isolation and structure analysis of a glucomannan from the leaves of Aloe arborescens var. Miller. Carbohydrate research, 198(2), 387-391.
5. Segal, A., Taylor, J. A., & Eoff, J. C. (1968, January). A RE-INVESTIGATION OF POLYSACCHARIDE MATERIAL FROM ALOE VERA MUCILAGE. In Lloydia (Vol. 31, No. 4, p. 423).

6. Yagi, A., Marino, K., Nishioka, I., & Kuchino, Y. (1977). Aloe mannan, polysaccharide, from Aloe arborescens var. natalensis. Planta medica, 31(01), 17-20.
7. Yagi, A., Machii, K., Nishimura, H., Shida, T., & Nishioka, I. (1985). Effect of aloe lectin on deoxyribonucleic acid synthesis in baby hamster kidney cells. Experientia, 41(5), 669-671.
8. Waller, G. R. (1978, February). A chemical investigation of Aloe barbadensis Miller. In Proceedings of the Oklahoma Academy of science (Vol. 58, pp. 69-76).
9. Anjaneyalu, Y. V., & Gowda, D. C. (1979). Structural studies of an acidic polysaccharide from Ocimum basilicum seeds. Carbohydrate Research, 75, 251-256.
10. Radjabi-Nassab, F., Ramiliarison, C., Monneret, C., & Vilkas, E. (1984). Further studies of the glucomannan from Aloe vahombe (liliaceae). II. Partial hydrolyses and NMR 13C studies. Biochimie, 66(7-8), 563-567.
11. t'Hart, L. A., Nibbering, P. H., Van Den Barselaar, M. T., Van Dijk, H., Van Den Berg, A. J., & Labadie, R. P. (1989). Effects of low molecular constituents from Aloe vera gel on oxidative metabolism and cytotoxic and bactericidal activities of human neutrophils. International journal of immunopharmacology, 12(4), 427-434.
12. Mandal, G., & Das, A. (1980). Structure of the glucomannan isolated from the leaves of Aloe barbadensis Miller. Carbohydrate research, 87(2), 249-256.
13. Mabusela, W. T., Stephen, A. M., & Botha, M. C. (1990). Carbohydrate polymers from Aloe ferox leaves. Phytochemistry, 29(11), 3555-3558.
14. Schneider, S. H., Fineberg, S. E., & Blackburn, G. L. (1981). The acute metabolic effects of glucagon and its interactions with insulin in forearm tissue. Diabetologia, 20(6), 616-624.
15. Spanheimer, R. G., Umpierrez, G. E., & Stumpf, V. (1988). Decreased collagen production in diabetic rats. Diabetes, 37(4), 371-376.
16. Spanheimer, R. G. (1988). Direct inhibition of collagen production in vitro by diabetic rat serum. Metabolism, 37(5), 479-485.
17. Spanheimer, R. G. (1992). Correlation between decreased collagen production in diabetic animals and in cells exposed to diabetic serum: response to insulin. Matrix, 12(2), 101-107.
18. Baynes, J. W., & Thorpe, S. R. (1999). Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes, 48(1), 1-9.
19. D'agnillo, F., & Chang, T. M. (1993). Cross-linked hemoglobin-superoxide dismutase-catalase scavenges oxygen-derived free radicals and prevents methemoglobin formation and iron release. Biomaterials, Artificial Cells and Immobilization Biotechnology, 21(5), 609-621.
20. Young, I. S., Tate, S., Lightbody, J. H., McMaster, D., & Trimble, E. R. (1995). The effects of desferrioxamine and ascorbate on oxidative stress in the streptozotocin diabetic rat. Free Radical Biology and Medicine, 18(5), 833-840.
21. Saxena, A. K., Srivastava, P., Kale, R. K., & Baquer, N. Z. (1993). Impaired antioxidant status in diabetic rat liver: Effect of vanadate. Biochemical pharmacology, 45(3), 539-542.
22. Halliwell, B. (1990). How to characterize a biological antioxidant? Free radical research communications, 9(1), 1-32.
23. McLennan, S. V., Heffernan, S., Wright, L., Rae, C., Fisher, E., Yue, D. K., & Turtle, J. R. (1991). Changes in hepatic glutathione metabolism in diabetes. Diabetes, 40(3), 344-348.
24. Goodson, W. H. (1979). Wound healing and the diabetic patient. Surg Gynecol Obstet, 149, 600-608.
25. Ceriello, A. (2000). Oxidative stress and glycemic regulation. Metabolism, 49(2), 27-29.
26. Fehér, J., Csomós, G., & Vereckei, A. (1987). Control of free radical reactions in biological systems. In Free radical reactions in medicine (pp. 11-17). Springer, Berlin, Heidelberg.
27. Polidori, M. C., Stahl, W., Eichler, O., Niestroj, I., & Sies, H. (2001). Profiles of antioxidants in human plasma. Free Radical Biology and Medicine, 30(5), 456-462.
28. Halliwell, B. (1994). Free radicals and antioxidants: a personal view. Nutrition reviews, 52(8), 253-265.
29. International Food Information Council. (2012). 2012 Food & health survey: Consumer attitudes toward food safety, nutrition & health. Retrieved from https://www.foodinsight.org/articles/2012-food-and-health-survey

30. Sloan, E., & Adams Hutt, C. (2012). Beverage trends in 2012 and beyond. Agro Food Industry Hi Tech, 23(4), 8.
31. Bloch, A., & Thomson, C. A. (1995). Position of the American Dietetic Association: Phytochemicals and functional foods. Journal of the American Dietetic Association, 95(4), 493-496.
32. Shackelford, L., Mentreddy, S. R., & Cedric, S. (2009). Determination of total phenolics, flavonoids and antioxidant and chemopreventive potential of basil (Ocimum basilicum L. and Ocimum tenuiflorum L.). International Journal of Cancer Research, 5(4), 130-143
33. Marinova, D., Ribarova, F., & Atanassova, M. (2005). Total phenolic and total flavonoids in Bulgarian fruits and vegetables. Journal of the university of chemical technology and metallurgy, 40(3), 255-260.
34. Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry, 239(1), 70-76.
35. Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-wells format. Journal of agricultural and food chemistry, 50(16), 4437-4444.
36. Miller, N. J., Sampson, J., Candeias, L. P., Bramley, P. M., & Rice-Evans, C. A. (1996). Antioxidant activities of carotenes and xanthophylls. FEBS letters, 384(3), 240-242.
37. Juvekar, A., Sakat, S., Wankhede, S., Juvekar, M., & Gambhire, M. (2009). Evaluation of antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata. Planta Medica, 75(09), PJ178.
38. Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews, 4(8), 118.
39. Shahidi, F. (2004). Functional foods: their role in health promotion and disease prevention. Journal of Food Science, 69(5), R146-R149.
40. Morton, J. F. (1987). Fruits of warm climates. JF Morton.
41. Arts, I. C., & Hollman, P. C. (2005). Polyphenols and disease risk in epidemiologic studies–. The American Journal of Clinical Nutrition, 81(1), 317S-325S.
42. Winston, C., & Beck, L. (1999). Phytochemicals: Health protective effects. Canadian Journal of Dietetic Practice and Research, 60(2), 78.
43. Sabu, M. C., & Kuttan, R. (2002). Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. Journal of Ethnopharmacology, 81(2), 155-160.
44. Cai, Y., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences, 74(17), 2157-2184.
45. Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. Journal of Chromatography A, 1054(1-2), 95-111.
46. Bhaskar, H., & Balakrishnan, N. (2009). In vitro antioxidant property of laticiferous plant species from western ghats Tamilnadu, India. International Journal of Health Research, 2(2).
47. Hu, Y., Xu, J., & Hu, Q. (2003). Evaluation of antioxidant potential of Aloe vera (Aloe barbadensis Miller) extracts. Journal of agricultural and food chemistry, 51(26), 7788-7791.
48. Moniruzzaman, M., Rokeya, B., Ahmed, S., Bhowmik, A., Khalil, M. I., & Gan, S. H. (2012). In vitro antioxidant effects of Aloe barbadensis Miller extracts and the potential role of these extracts as antidiabetic and antilipidemic agents on streptozotocin-induced type 2 diabetic model rats. Molecules, 17(11), 12851-12867.
49. Ismail, H. I., Chan, K. W., Mariod, A. A., & Ismail, M. (2010). Phenolic content and antioxidant activity of cantaloupe (Cucumis melo) methanolic extracts. Food chemistry, 119(2), 643-647.
50. Tachakittirungrod, S., Okonogi, S., & Chowwanapoonpohn, S. (2007). Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. Food chemistry, 103(2), 381-388.
51. Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. Journal of agricultural and food chemistry, 49(10), 4619-4626.
52. Ebrahimzadeh, M. A., Nabavi, S. M., Nabavi, S. F., Bahramian, F., & Bekhradnia, A. R. (2010). Antioxidant and free radical scavenging activity of H. officinalis L. var.

angustifolius, V. odorata, B. hyrcana and C. speciosum. Pak J Pharm Sci, 23(1), 29-34.

53. Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules, 14(6), 2167-2180.
54. Beretta, G., Granata, P., Ferrero, M., Orioli, M., & Facino, R. M. (2005). Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Analytica Chimica Acta, 533(2), 185-191.
55. Saritha, V. (2010). Antioxidant and antibacterial activity of Aloe vera gel extracts. International Journal of Pharmaceutical & Biological Archive, 1(4).
56. Zhang, L., & Tizard, I. R. (1996). Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from Aloe vera gel. Immunopharmacology-New York, 35, 119-128.
57. Ramamoorthy, L., Kemp, M. C., & Tizard, I. R. (1996). Acemannan, a beta-(1, 4)-acetylated mannan, induces nitric oxide production in macrophage cell line RAW 264.7. Molecular Pharmacology, 50(4), 878-884.

PEER REVIEW

Not commissioned. Externally peer reviewed.

FIGURES

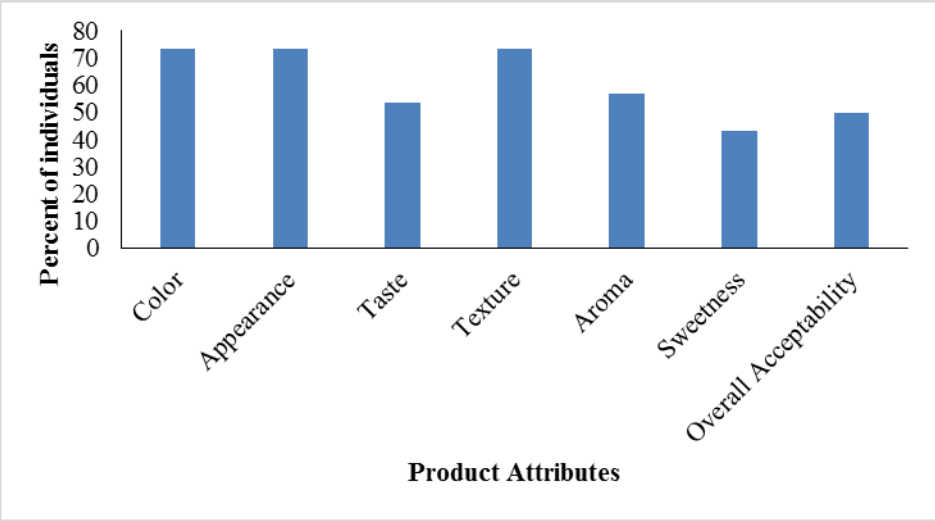

Figure 1. Product attributes for the Aloe vera product (Aloe jello). n=30.

Values are presented as the percent of total panelists. Ranked 4 or higher on 5-point hedonic scale

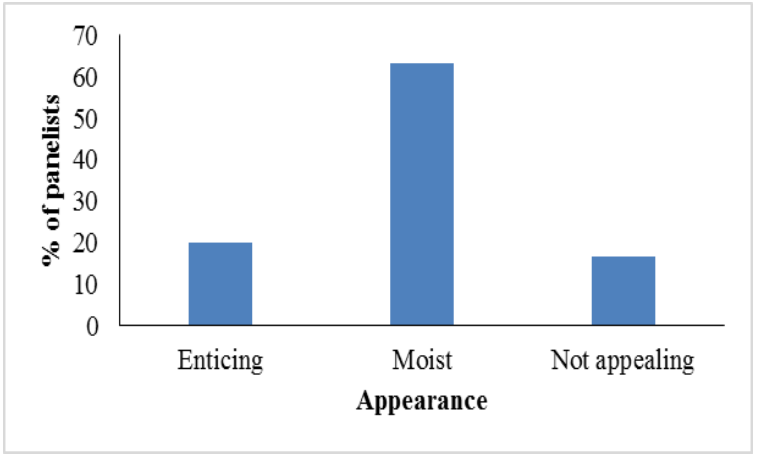

Figure 2. Product appearance described by the panelists. n=30. Values show percent of panelists

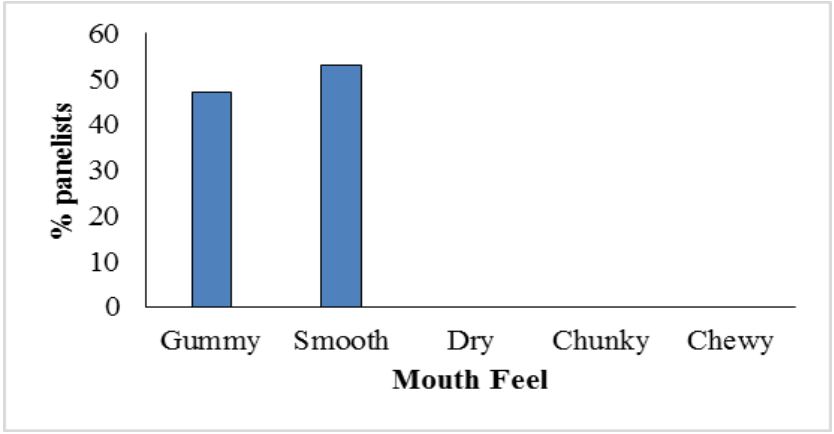

Figure 3. Mouth feel descriptions given by the panelists for the Aloe vera product (Aloe jello). n=30

Values are given as percent of total panelists.

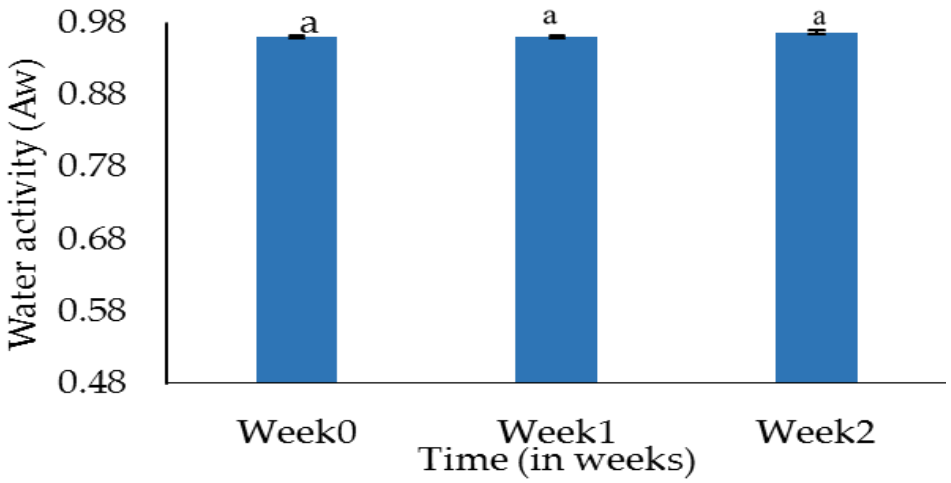

Figure 4. Water activity of Aloe jello.

Values are means ± SEM, error bars without a common letter (abc) differ ($p \leq 0.05$).

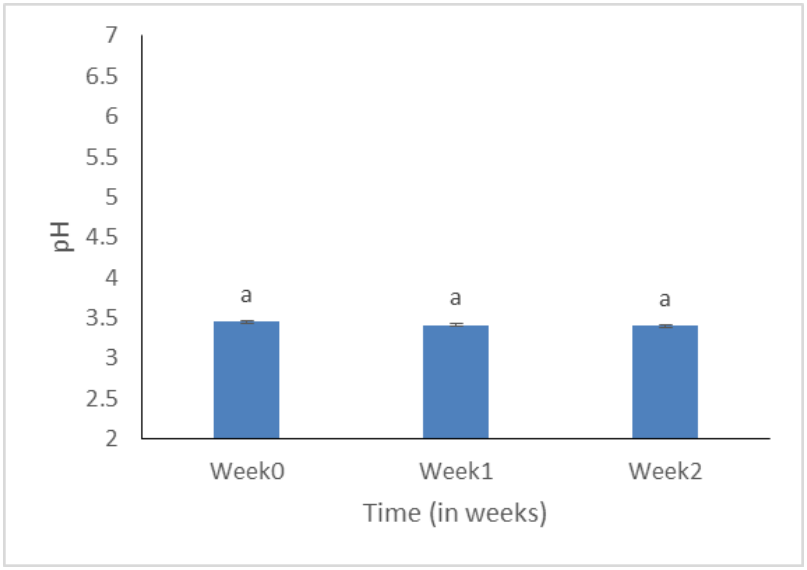

Figure 5. pH of Aloe jello over a period of two weeks.

Values are means ± SEM, error bars without a common letter (abc) differ ($p \leq 0.05$).

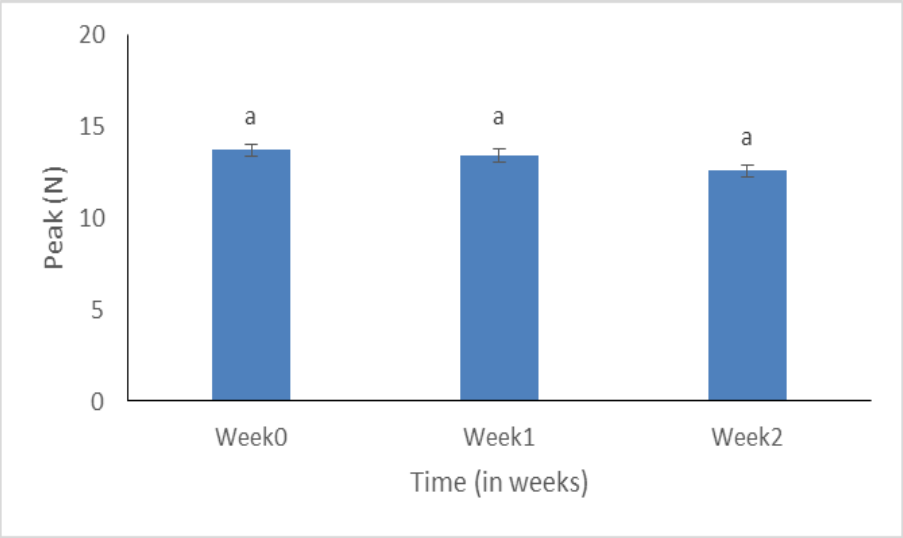

Figure 6. Texture of Aloe jello.

Values are means ±SEM, error bars without a common letter (abc) differ ($p \leq 0.05$).

TABLES

Table 1. Total polyphenol content in Aloe vera extracts.

Treatments	Aqueous Extracts (mg GAE/100g DW)	Methanol Extracts (mg GAE/100g DW)
WE	5.84 ±0.34 ^{ax}	2.01±0.05 ^{bx}
SE	1.47±0.04 ^{ay}	1.12±0.03 ^{ay}
GE	1.37±0.16 ^{ay}	0.79±0.03 ^{az}

Values (n=3) are means ± SEM; Means in the column with superscript (^{xyz}) without a common letter differ (p≤0.05). Means in rows with superscripts (^{ab}) without a common letter differ (p≤0.05).

Abbreviations: WE- whole Aloe vera leaf extract, SE- Aloe vera leaf skin extract, GE- Aloe vera leaf gel extract, GAE- Gallic acid equivalent, DW- dry weight.

Table 2. Total Flavonoid content in Aloe vera extracts.

Treatments	Aqueous Extracts (mg CE/100g DW)	Methanol Extracts (mg CE/100g DW)
WE	0.44 ±0.02 ^{bx}	0.61±0.04 ^{ax}
SE	0.27±0.007 ^{ay}	0.24±0.02 ^{ay}
GE	0.23±0.004 ^{ay}	0.16±0.01 ^{ay}

Values (n=3) are means ± SEM; Means in the column with superscript (^{xyz}) without a common letter differ p≤0.05. Means in the rows with superscripts (^{ab}) without a common letter differ (p≤0.05).

Abbreviations: WE- whole Aloe vera leaf extract, SE- Aloe vera Leaf Skin extract, GE- Aloe vera leaf Gel extract, CE- Catechin equivalent, DW- dry weight.

Table 3. Ferric reducing antioxidant power of Aloe vera extracts.

Treatments	Aqueous Extracts (mM Fe(II)/100g DW)	Methanol Extracts (mM Fe(II)/100g DW)
WE	6.00 ±0.02 ^{bz}	15.6±0.04 ^{ax}
SE	11.79±0.007 ^{ay}	8.36±0.02 ^{by}
GE	13.26±0.004 ^{ax}	15.76±0.01 ^{bx}

Values (n=3) are means ± SEM; Means in the column with subscript (^{xyz}) without a common letter differ p≤0.05. Rows with superscripts (^{ab}) without a common letter differ.

Abbreviations: WE- whole Aloe vera leaf extract, SE- Aloe vera Leaf Skin extract, GE- Aloe vera leaf Gel extract, CE- Catechin equivalent, DW- dry weight.

Table 4. Trolox equivalent antioxidant capacity of Aloe vera extracts

Treatments	Aqueous Extracts (mM TE/100g DW)	Methanol Extracts (mM TE(II)/100g DW)
WE	98.93 ±6.29 ^{ax}	23.11±0.14 ^{by}
SE	42.99±0.11 ^{ay}	167.58±1.71 ^{bx}
GE	97.78±4.2 ^{ax}	43.25±1.08 ^{by}

Values (n=3) are means ± SEM; Means in the column with subscript (^{xyz}) without a common letter differ (p≤0.05). Rows with superscripts (^{ab}) without a common letter differ (p≤0.05) with in the Aloe sections (whole, skin & gel).

Abbreviations: WE- whole Aloe vera leaf extract, SE- Aloe vera Leaf Skin extract, GE- Aloe vera leaf Gel extract, TE- Trolox equivalent, DW- dry weight.

Table 5. Nitric Oxide radical scavenging activity of Aloe vera extracts.

Treatments	Aqueous Extracts (mM NO/100g DW)	Methanol Extracts (mM NO/100g DW)
WE	435.94 ±11.67 ^{ax}	41.83±1.67 ^{by}
SE	224.00±21.39 ^{ay}	48.00±1.25 ^{by}
GE	327.07±11 ^{az}	116.00±0.83 ^{bx}

Values (n=3) are means ± SEM; Means in the column with superscript (^{xyz}) without a common letter differ p≤0.05. Means in the rows with superscripts (^{ab}) without a common letter differ p≤0.05.

Abbreviations: WE- whole Aloe vera leaf extract, SE- Aloe vera Leaf Skin extract, GE- Aloe vera leaf Gel extract, CE- Catechin equivalent, DW- dry weight.

Table 6. Oxygen Radical Absorbance Capacity of Aloe vera extracts

Treatments	Aqueous Extracts (mM TE/100g DW)	Methanol Extracts (mM TE/100g DW)
WE	289.98 ±1.06 ^{ay}	226.80±1.93 ^{bx}
SE	293.19±0.64 ^{ay}	105.44±0.56 ^{bz}
GE	898.27±1.37 ^{ax}	182.92±1.09 ^{by}

Values (n=3) are means ± SEM; Means in the column with superscript (^{xyz}) without a common letter differ (p≤0.05), comparing among Aloe vera parts. Means in the rows with superscripts (^{ab}) without a common letter differ (p≤0.05), comparing between solvents.

Abbreviations: WE- whole Aloe vera leaf extract, SE- Aloe vera Leaf Skin extract, GE- Aloe vera leaf Gel extract, TE- Trolox equivalent, DW- dry weight.

Table 7. Color values for top of Aloe jello.

Time (in weeks)	L*	a*	b*
Week0	19.93±1.29^{a}	-3.22±0.20^{a}	18.40±2.25^{a}
Week1	19.52±0.59^{a}	-3.33±0.29^{a}	18.16±2.9^{a}
Week2	19.41±1.02^{a}	-3.47±0.16^{a}	18.02±2.09^{a}

Values are means ±SEM, means in the columns with superscripts (^{abc}) without a common letter differ (p≤0.05).