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Profiling of Phenolic Composition and Antioxidant Activity of *Aloe vera* Seedlings from Smart farm

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ABSTRACT
Phenolic composition of different extracts of Aloe vera seedlings from smart farm and their antioxidant activity was determined for the first time. Phenolic compounds were identified using a reversed phase high performance liquid chromatography with diode array detection (HPLC-DAD) method. Results showed the identification of five phenolic compounds in water extract namely aloesin and its derivative, hydroxyl anthracene and aloeresin derivatives, and aloin. There were nine phenolic compounds identified in methanol–water extract, which are aloin, two emodinderivatives, anthranols, isobarbaloin, derivatives of aloetic acid and cinnamicacid. The amounts of aloesin, aloinand aloeresinwere higher among all phenolic compounds. Total phenolic compounds and radical scavenging activity were higher in water and methanol–water extract than their corresponding methanol extracts. In conclusion, Aloe vera seedlings are a good source of natural antioxidants with significant biological functions and may serve as food ingredients and as an enhancer for maintaining the immune function of the human body.

Keywords: *Aloe vera seedlings; HPLC-DAD; phenolic composition, antioxidant activity; smart farm*

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I. Introduction

Aloe vera (L.) Webb. (*Aloe barbadensis* Mill.) belongs to the family *Liliaceae*and is a green and has triangular, fleshy leavesforming a rosette with serrated edges.Aloe is a widely used leaf from the Asia to the US and EU market. The leaves are filled with brown or yellowish milky juice that contains most bioactive compounds and thus a highly cultivated plant.

The aloe is rich in important bioactive compounds, such as flavonoids, terpenoids, lectins [1, 2], fattyacids, anthraquinones [3], mono- and polysaccharides (pectins, hemicelluloses, glucomannan), tannins, sterols(campesterol, β-sitosterol), enzymes, salicylic acid, minerals (calcium, chromium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium and zinc) and vitamins (A, C, E, β-carotene, B1, B2, B3, B6, choline, B12, folic acid) [4-6].The rich chemical composition of the plant depends on a large number of factors: the type and conditions of cultivation, harvest time, climate, the position of leaves on the stem, aloe species and the method used for harvesting leaves [7]. The optimal time to harvest aloe leaves is after three years of the plant's growth, because then it has the highest content of polysaccharides (6.55 g/kg) and flavonoids (4.70 g/kg) [8, 9].

The bioactive components of aloe have anti-inflammatory effect and aid the treatment of gastrointestinal diseases, i.e., inflammations, gastric, duodenal and intestinal ulcers. They aid lipid and carbohydrate metabolism, which helps to maintain normal blood sugar and cholesterol levels as well as normal body weight. Due to aloin, the daily intake of aloe juice should not exceed 30–40 mL, because excessive consumption may not only have a strong laxative effect but also toxic effects. When aloe is applied externally, it helps to regenerate burnt or frostbitten skin [3, 4, 10, 11].

There is, however, a lack of information regarding the phenolic composition of aloe seedlings from smart farm and their antioxidant activity. This paper reported for the first time important phenolic compositions of different extracts of aloe seedling using high performance liquid chromatography with diode array detection (HPLC-DAD) method and their antioxidant potentials.

II. Materials and Methods

2.1. Materials

Fresh aloe seedlings were collected from the Aloe Smart-Farm (GIST &KimJeongMoon Aloe Ltd, Korea), and its adventitious roots were removed. Aloesin, aloin,cinnanmicacid and aloeresin were from TCI (TCI, Tokyo, Japan). isobarbaloin and all other chemicals were of HPLC grade and were obtained from Sigma-Aldrich (Steinheim, Germany), or as otherwise mentioned.

2.2. Sample Collection and Preparation

The aloe seedlings were removed from the whole plant. The aloe seedlings were washed with distilled water and were completely dried. The dried aloe seedlings were crushed, grinded and converted into finely divided powder. The samples for analysis were prepared from the powdered materials.

2.3. Preparation of Extracts

Extracts were prepared in methanol and water (1:1, v/v). Briefly, 1 g of powdered sample was mixed with 20 mL of methanol–water mixture. The mixture was placed on a water bath at 30 ◦ C for one hour. Then, the samples were centrifuged at 4000 rpm for 10 min. After that, 2 mL from each sample was filtered using Agilent membrane polytetrafluorethylene (PTFE) (Agilent Technologies, Waldbronn, Germany) with pore size of 0.45 µm and transferred into 2 mL Agilent HPLC vials. Similarly, two more extracts in 100% methanolic and 100% pure deionized water were also prepared using similar procedures. One part of the above extracts were used for thedetermination of totalphenolic contents (TPC) using Folin–Ciocaltaeu Reagent. The results of TPC for both methods of analyses were compared.

2.4. HPLC-DAD Analyses of Phenolic Compounds

The phenolic profile of the aloe seedlings was determined using the Agilent Infinity Better 1260 HPLC system (Agilent, Waldbronn, Germany) having a quaternary pump, degasser, auto-sampler and DAD. The separation of the phenolic compounds was achieved using an Agilent ZorbaxEclipse C18 (406 × 250 mm, 5 µm) column. The binary gradient system consists of solvent A(methanol:aceticacid:deionized water, 10:2:88, v/v/v) and solvent B (methanol: acetic acid: deionized water, 90:2:8, v/v/v). The gradient program used was started from 100% A to 85% A at 5 min, and then 50% A at 20 min, 30% A at 25 min, and 100% B from 30 to 40 min [126]. The elution was achieved at 25 min due to the elution of all compounds present. The DAD was set to 280 nm for analysis of phenolic compounds. The spectra were recorded from 190 to 500 nm. The identification and quantification of phenolic compound was carried out using available standards, retention times, and their UV spectra. In cases where an authentic standard was not available, compounds were identified by comparing UV spectra with reported literature and quantified using the available relevant standard phenolic compounds based on the similar chromatographic response as given earlier [10].

2.5. Total Phenolic Contents

Total phenolic contents (TPC) were determined using the Folin–Ciocalteu reagent. The reagent was freshly prepared in the lab. Briefly, a sample (0.5 mL) was mixed with 1.5 mL of FC reagent and reacted in the presence of salt for 1 h. Absorbance of the resultant mixture was recorded at 765 nm. An aloesin standard calibration curve was prepared in the concentration range of 5–100 mg. The TPC measured was expressed as mg of GAE/100 g.

2.6. Radical Scavenging Activity

Radical scavenging activity (RSA) was measured using DPPH radicals. DPPH solution of 0.1 mM was prepared in methanol. DPPH solution of 1.90 mL was mixed with 10 µL of methanolic extracts of the aloe seedlings. The mixture was kept in the dark for 30 min and the absorbance was measured at 515 nm using Pharmaspec 1700 spectrophotometer (Shimadzu, Tokyo, Japan). The percent RSA of the samples were calculated using the equation: %RSA = (Ac − As)/Ac, where Ac is the absorbance of control and as is the absorbance of the test sample.

2.7. Data Analysis

All samples were measured in triplicate. Data were analyzed for variation by a one-way analysis of variance (ANOVA) and Holm–Sidak method at $\alpha = 0.05$ using Graph Pad Prism 5 for Windows version 5.03 (Graph Pad Software Inc., La Jolla, CA, USA).

III. Results and Discussion

3.1. Phenolic Composition

Phenolic compounds were identified using the UV absorption spectra and retention times of the authentic standard compounds or by comparing spectra with those reported in the literature.Only those peaks were selected for quantification where the spectral purity was higher than 96%. The identified compounds were quantified using the standard calibration curves (Aloesin, Aloin, isobarbaloin, aloeresin and cinnamic acid) and expressed as mg/100 g of the sample. Figure 1 shows the separation of phenolic compounds in water and methanol–water mixer. Each peak number is designated for the specific compound as shown in Table 1. The chemical structures of the major phenolic compounds are shown in Figure 2. Only five compounds were identified in the water fraction (Figure 1A). Compound 1 was eluted at 1.8 min and identified asaloesin (6.667 mg/100 g). Kolayli et al. [13] also identified aloesin in different kinds of aloe seedlings. This shows that aloesin may be one of the phenolic markers in aloe seedlings. Compound 2 was tentatively identified as analoesinderivative with λmax of 270 nm from the work of Santos et al. [12].

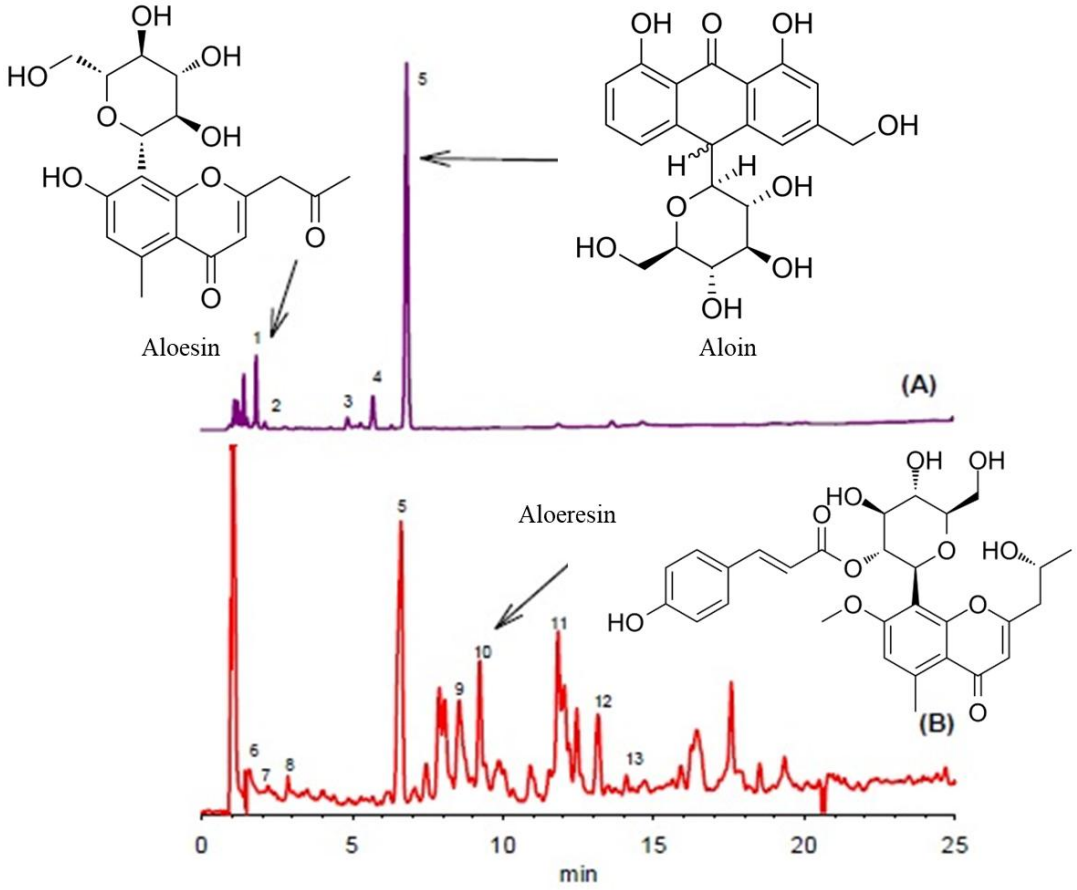

Figure 1. Representative high performance liquid chromatography with diode array detection (HPLC-DAD) chromatograms of the separation of phenolic compounds at 360 nm. (A) Water extract of aloe seedlings, and (B) methanol–water extract of aloe seedlings.

This compound was present in a very small amount (0.982 mg/100 g). Compound 3 eluted at 4.8 min and was found to be hydroxyl anthracene with λmax of 261 and 278 nm with a concentration of 2.18 mg/100 g. Previous studies [13] showed hydroxyl anthracenewere only identified in standard aloe seedlings, and was not detected in other aloe varieties. These authors reported a high amount of anthracenein aloe seedlings. Similarly, compound 4 was tentatively identified as a aloeresin derivative with λmax of 280 nm (5.42 mg/100 g). Peak 5 was identified as aloin by comparing the retention time and absorption spectra with authentic standard aloin.

Table 1. Identification and quantification of phenolic compounds in aloe seedlings using reversed phase HPLC-DAD. Peaks 1–5 were identified in water extract and peaks 5–13 in water-methanol extract.

Peak	Retention Time (min)	Identity	HPLC-DAD λmax (nm)	Amount (mg/100g)	Reference
1	1.8	Aloesin	271	6.667	Standard
2	2.1	Aloesin derivative	270	0.982	Santos et al. [12]
3	4.8	Hydroxyl anthracene	261,278	2.18	Santos et al. [12]
4	5.7	Aloeresin derivative	280	5.42	Santos et al. [12]
5	6.8	Aloin	298, 323	46.3	Standard
5	6.8	Aloin	298, 323	66.0	Standard
6	1.4	Emodin derivative	227, 258	1.02	Santos et al. [12]
7	1.6	Emodin derivative	226, 259	0.455	Santos et al. [12]
8	2.8	Anthranols	232, 270	0.433	Santos et al. [12]
9	8.5	Isobarbaloin	254, 355	3.91	Standard
10	9.2	Aloeresin	280	4.33	Standard
11	11.8	Aloetic acid	284	8.65	Santos et al. [12]
12	13.5	Cinnamic acid derivative	286, 320	1.24	Santos et al. [12]
13	14.3	Cinnamic acid	254, 368	6.52	Standard
		Total Amounts	Water	81.249	
			Mixture	72.858	
			Methanol	12.456	

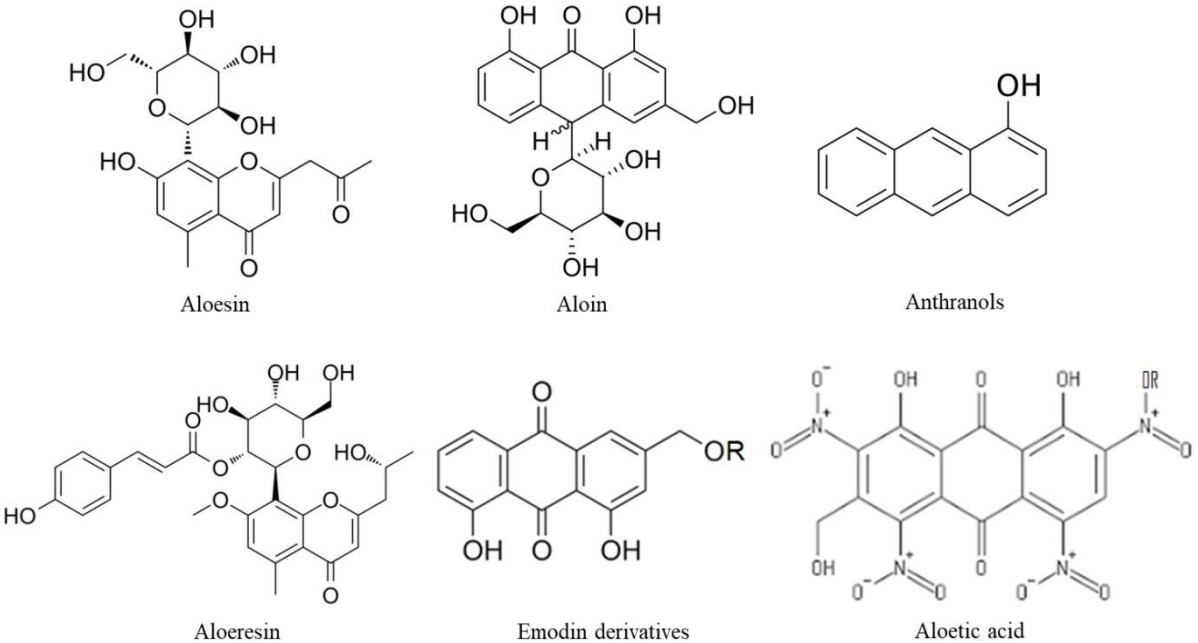

Figure 2. Structure of the major identified phenolic compounds. The R group represents the possible derivative of identified phenolic moiety.

The highest amount of aloin was found in the aqueous fraction of aloe seedlings (46.3 mg/100 g) among the identified phenolic compounds. Aloin was reported to be present in a very small amount in aloe leaves of different varieties [13]. Aloin has several biological roles, of which antioxidant activity is widely known [14]. The HPLC-DAD chromatogram of the methanolic extract of the seeds shows only aloesin and their derivatives with no other identifiable phenolic compound and was thus excluded to aloe in Figure 1. However,

the total phenolic compounds present in the methanolic fractions were quantified to be 12.456 mg/100 g, much lower than other fractions.Figure 1B shows the separation of phenolic compounds in the methanol–water mixed extract. Nine phenolic compounds were identified in mixed extract. A relatively large amount of cafaloin was found in this extract with concentration of 66.0 mg/100 g. Compounds 6 and 7 were found to be emodin derivatives as identified from the work of Santos et al. [12]. Emodinwas previously reported in different kinds of aloe leaves by Kolayli et al. [13]. Similarly, compound 8 was tentatively identified as anthranols with λmax of 232 and 270 nm (0.433 mg/100 g). Compound 9 was identified as isobarbaloin with λmax of 355 and 254 nm and a concentration of 3.91 mg/100 g. This compound was identified by comparing the retention time and absorption maxima of the authentic standard compound. The results of the human studies showed that isobarbaloinis extensively metabolized to 3-hydroxyphenylacetic acid (36%), 3-methoxy-4-hydroxyphenylacetic acid (8%) and 3,4-dihydroxyphenylacetic acid (5%) in humans [15]. The peak 10 was identified as aloeresin with a concentration of 4.33 mg/100 g, which was found to elute at 9.2 min. Aloeresin was not reported in different kinds of aloe leaves, while a small amount of epialoeresin was quantified in a standard variety of aloe [13]. Similarly, compound 11 was tentatively identified as analoetic acid derivative with a concentration of 8.65 mg/100 g. Aloeticacid was previously identified inthe standard aloe variety [13], which suggests that this phenolic compound may be metabolized further to form its derivatives in aloe seedlings. Compounds 12 and 13 were tentatively identified as cinnamic acid derivative (1.24 mg/100 g) and cinnamic acid (6.52 mg/100 g). Cinnamic acid was identified by comparing the UV absorption spectra and retention time of the standard cinnamic acid. Cinnamic acid acts as a strong antioxidant against human low density lipoproteins (LDL) oxidation [16] and has *in vitro* anti-proliferative and apoptotic properties. Aloe seedlings have a small quantity of cinnamic acidand may thus serve as a good source of these natural polyphenolic compounds.

3.2. Total Phenolic Contents

A standard aloesin calibration curve was prepared in the concentration of 5–100 mg/mL. The calibration equation was $y = 0.0094x + 0.0086$ with R^2 of 0.9976. Figure 3 shows the total phenolic contents (TPC) of the water, methanolic and methanol–water mixer using FolinCiocalteu (FC) reagent and HPLC. It was observed that the water extract has significantly higher TPC than its corresponding methanolic extract, while the methanol–water mixer has significantly higher TPC than the methanolic extract and lower than the water extract. The results are in agreement with Ismail et al. [17], who determined that the seedlings of the aloe have the lowest TPC as compared to the methanolic extracts of general aloe leaves. There was no significant difference ($p < 0.05$) in the TPC values analyzed with FC reagent and HPLC for water and water–methanol mixture. However, the amount of TPC was significantly lower than the values obtained using FC reagent. This might be due to the lower sensitivity of the detector for the methanolic extract of the aloe seedlings. The TPC of aloe seedlings reported here is significantly higher than the values reported by Chun et al. [18]. This suggests that aloe seedlings are richer in TPC than their corresponding leaves or whole plant parts.

3.3. Antioxidant Activity

Antioxidant activity was determined using diphenyl picryl hydrazine (DPPH) radicals' scavenging potential as shown in Figure 4. It was observed that radical scavenging activity of the methanol–water extract was higher than its corresponding pure water and methanolic extracts. The pure methanolic extract has a lower activity than the water extract. Previous work [18] showed that the radical scavenging activity of the aloe leaves was 17.5%, which is lower than the values reported here for water and methanol–water extract, and is relatively similar to the methanolic extract of aloe seedlings. Ismail et al. [17] showed that the methanolic extract of the aloe seedlings has the highest DPPH radical scavenging activity than methanolic extracts of aloe leavesor whole plant parts. The high antioxidant activity of the water and methanol–water extract may be due to the high amounts of aloin present in these extracts. The antioxidant potential of other reported phenolic compounds cannot be ruled out, which may also contribute potentially.

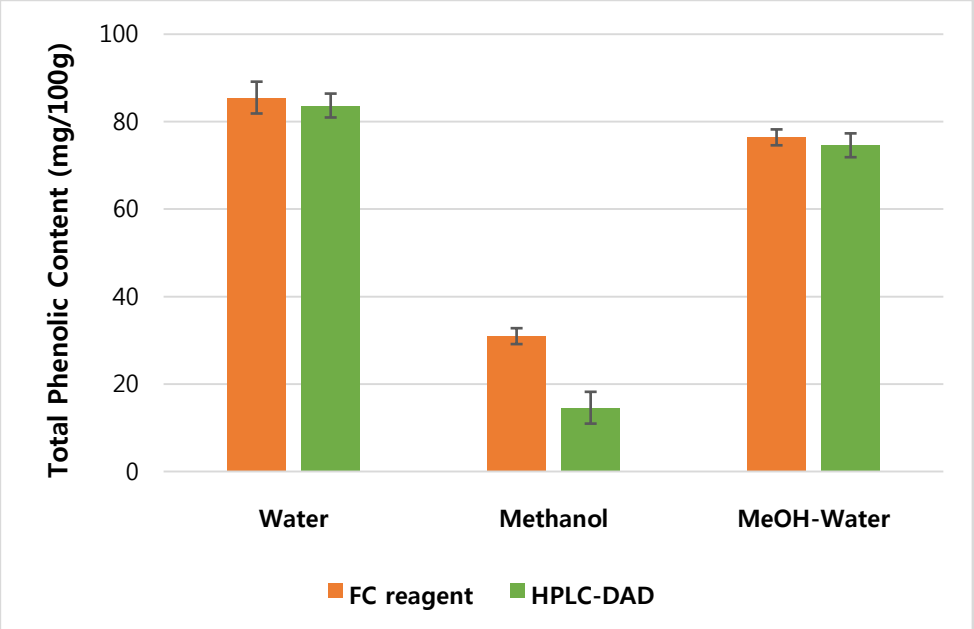

Figure 3. Total phenolic contents (TPC) of the different extracts of aloe seedlings. Values are mean of triplicate readings. Different letters (a–c) in the same procedure represent significant at $p < 0.05$ (Holm–Sidak method).

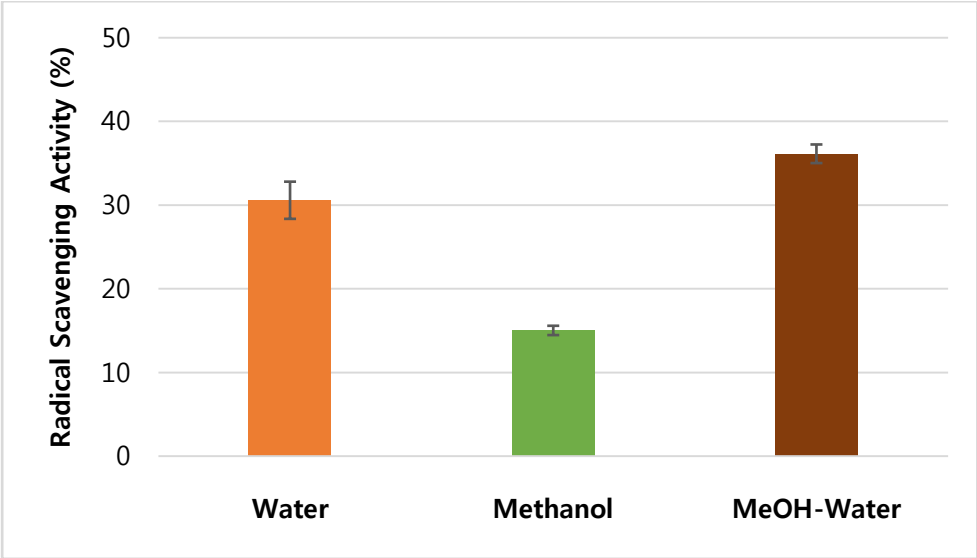

Figure 4. Radical scavenging activity (RSA) of the different extracts of melon seeds. Values are the mean of triplicate readings. Different letters (a–c) represent significant at $p < 0.01$ (Holm–Sidak method).

IV. Conclusions

In conclusion, different extracts of aloe seedlings from smart farm were analyzed for phenolic composition, total phenolic contents and antioxidant activity. The HPLC-DAD results revealed the identification of five phenolic compounds in water extracts, namely aloesin and its derivative, hydroxyl anthracene and aloeresin derivatives, and aloin. The methanol–water extract has revealed nine phenolic compounds, which include aloin, two emodinderivatives,anthranols,isobarbaloin,derivatives of aloetic acid and cinnamicacid.The amounts of aloeresin, aloin, and aloeresin were higher among all phenolic compounds. Total phenolic compounds and radical scavenging activity were higher in water extract and methanol–water extract than its corresponding methanol extracts. These results concluded that aloe seedlings are a good source of natural antioxidants and may serve as food ingredients and as an enhancer for maintaining the immune function of the human body.

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Abbreviations

HPLC-DAD: high performance liquid chromatography with diode array detection; TPC: total phenoliccontents; RSA: radical scavenging activity; AE: aloesin equivalent; DPPH: diphenylpicryl hydrazine.

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