Pop RM *et al.* (2022)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 50, Issue 2, Article number 12732
DOI:10.15835/nbha50212732
Research Article

Pot *Aloe vera* gel – a natural source of antioxidants

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Abstract

Aloe vera (*Aloe barbadensis* Miller) is widely spread around the world and it is used in the food and cosmetic industry and traditional medicine due to its high content in bioactive compounds. Thus, this article investigated the phenolic compounds of Romanian *Aloe vera* gel and its antioxidant capacity. The gel was extracted using 4 different solvents: methanol, ethanol, water, and acetone. Total phenolics were measured by the Folin-Ciocalteu method, total flavonoids by aluminium chloride reaction, and the antioxidant capacity by DPPH radical-scavenging activity. The profile of the phenolic compound was determined using Fourier transform infrared spectroscopy and Liquid Chromatography-Diode Array Detection–Electro-Spray Ionization Mass Spectrometry. The methanolic extract had a significantly higher antioxidant activity, followed by ethanolic, water, and acetone extracts. The methanolic extract had also the highest total polyphenol content, while ethanolic extract had the highest total flavonoid content. The extracts contained 14 compounds identified as 7 chromones (Aloesin, Neoaloesin, Aloinoside A, Aloinoside E, Aloe-emodin-glucoside, Isoaloeresin D, Methoxycoumaroyl-aloresin) 2 flavones (Luteolin-glucoside, Apigenin-glucoside), one hydroxycinnamic acid (Caffeic acid) and 4 to anthrones (Aloin A, Aloin B, Emodin, Aloe-emodin). The LC-MS results showed important quantities of Aloesin, Neoaloesin, Aloeresin E, and Aloe-emodin-glucoside while FTIR analysis showed the presence of polysaccharides, pectins, anthraquinones, and saponins.

Keywords: antioxidant activity; phenolic compounds; infrared spectroscopy; liquid chromatography-mass spectrometry

Received: 00 Xxx 2021. Received in revised form: 03 June 2022. Accepted: 23 Jun 2022. Published online: 30 Jun 2022.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Aloe vera (*Aloe barbadensis* Miller) belongs to Asphodelaceae (Liliaceae) family that comprises around 548 species (Puia A *et al.*, 2021). It is a perennial shrubby or arborescent plant with shallow roots, xerophytic, succulent, of green colour (Surjushe, 2008; Puia A *et al.*, 2021). Because of its xerophyte character, the *Aloe vera* plant is adapted to live in low water areas, and therefore, they possess large water storage tissue (99-99.5% water content), from where the succulent characteristic (Radha and Laxmipriya, 2015). Thus, *Aloe vera* is mainly grown in the dry regions found in Africa, Asia, Europe, and America (Surjushe, 2008). *Aloe vera* met a large distribution within Europe and around the entire world (Radha and Laxmipriya, 2015).

Its large spread around the world can be explained by its large and wide use in traditional medicine, in the food industry mainly as a functional food ingredient, or in the cosmetic industry (Bunea *et al.*, 2020; Hamman, 2008). Thus, literature data suggest that *Aloe vera* was intensively used in the topic treatment of various skin disorders (Svitina *et al.*, 2019), especially wounds, irritations, and burns healing (Maenthaisong *et al.*, 2007), specifically for its anti-inflammatory, antioxidant, and antibacterial properties (Yagi *et al.*, 2002; Hęś *et al.*, 2019). Its oral administration was used to treat constipation, ulcers, diabetes, cough, and headaches (Ashafa *et al.*, 2011; Ali Kamel Mohamed, 2011; Goudarzi *et al.*, 2015; Quispe *et al.*, 2018). The gel or the whole-leaf preparations were also used as an adjuvant treatment to chemotherapy (Foster, 2011). Still, the use of *Aloe vera* without a specific diagnosis should maintain awareness of the risk of cancer, especially in diseases associated with a higher incidence of cancer (Puia and Puia, 2013; Harris *et al.*, 2020).

Generally, the gel is commonly used in the therapeutic approach (Yagi *et al.*, 2002; Sumi *et al.*, 2019). Even though *Aloe vera* gel has around 98.5-99.5% water, the remaining dry constituents have more than 200 different phytochemicals, with polysaccharides as major compounds (Hamman, 2008). Besides polysaccharides, compounds like glycoproteins, phenolic compounds (anthraquinones, flavonoids, flavonols), minerals, enzymes, amino acids, vitamins, sterols, and saponins have also been identified (Sumi *et al.*, 2019). Among these, polysaccharides and polyphenols, are commonly associated with the health benefits of *Aloe vera* gel (Du, 2007; Pérez *et al.*, 2012; Kaithwas, 2014; Kang *et al.*, 2014).

Because its complex chemical composition highly depends on the geographic area, soil composition, climate conditions, growth and harvesting periods, and also postharvest treatments it is very important to determine their exact composition since bioactive compounds are directly influencing the biological properties (Ray and Aswatha, 2013; Ray and Ghosh, 2018; Martínez-Sánchez *et al.*, 2020). Accordingly, it is known that phenolic compounds, are natural exogenous antioxidants that can inhibit enzymes activity and by this, the inhibition of reactive oxygen species (ROS), can chelate ions of metals, thus affecting the reactions cascade that leads to lipids peroxidation, and can synergistically act with other antioxidants (Kurek-Górecka *et al.*, 2013). Nevertheless, a change in concentration and profile of these bioactive compounds will directly affect the antioxidant capacity and all the claimed health pharmacological properties.

Thus, this article aimed to identify the phenolic compounds of a Romanian *Aloe vera* gel extracted from an easily available source of an aloe pot plant and to check its antioxidant capacity.

Materials and Methods

Plant material and extraction procedure

Aloe vera (*Aloe barbadensis* Miller) was pot grown in Cluj-Napoca, Romania. Specimens were further identified at the University of Agricultural Science and Veterinary Medicine in Cluj-Napoca, Romania. Fresh *Aloe vera* leaves, with lengths between 40 and 60 cm were removed from a three-year-old plant. Before gel extraction, leaves were cleaned by rinsing them with distilled water. The gel was obtained as described by Munos

et al. (2015). Accordingly, the cortex was separated from the parenchyma with a knife, obtaining the fresh *Aloe vera* gel (AVG) filets. Immediately, the gel fillets (5 g) were separately homogenized using a homogenizer HG-15D (Witeg, Germany) at 2700 rpm for 1 min with 10 mL of methanol to obtain the methanolic extract (AVGM), with 10 ml ethanol to obtain the ethanolic extract (AVGE), with 10 ml of water to obtain the water extract (AVGW) and 10 ml of 70% acetone to obtain the acetone extract (AVGA). The mixtures were further sonicated for 40 min at room temperature. Next, they were stored for another 24 h at 4 °C, in dark conditions. Before centrifugation (5000 g for 10 min) and filtration (0.22 μm nylon syringe filters), they were sonicated again for 30 min. The extraction protocol is described in Figure 1.

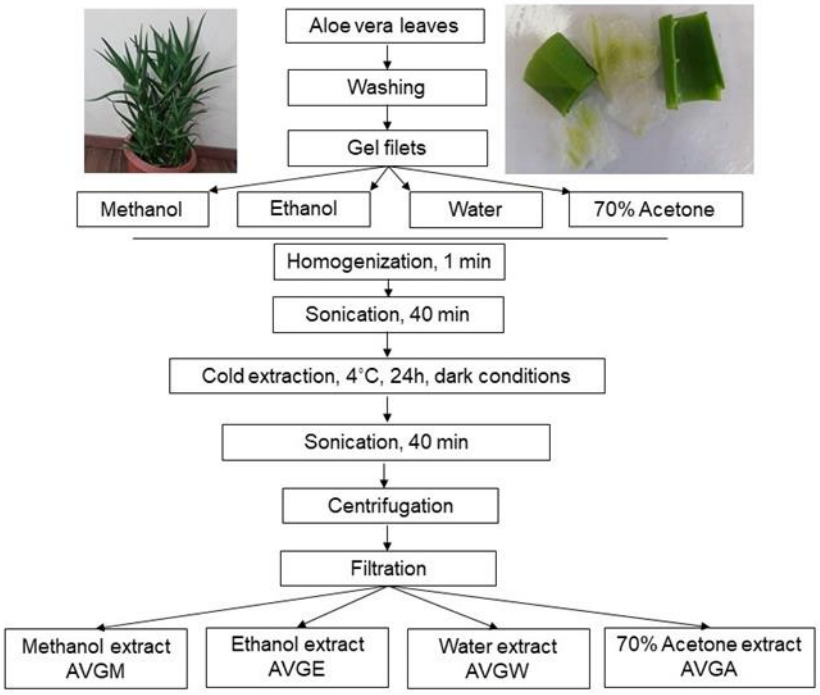

Figure 1. *Aloe vera* gel extraction protocol

Total polyphenol content

Total polyphenols (TPC) were determined by the Folin-Ciocalteu method as described in Folin and Ciocalteu (1927) and modified as described by Pop *et al.* (2018). Accordingly, 25 μL of each *Aloe vera* extract was mixed with 125 μL, 0.2 N Folin-Ciocalteu reagent and with 100 μL sodium carbonate solution (7.5% w/v). The mixture was further homogenized and incubated (2h, at 25 °C, in the dark). The absorbance at 760 nm was recorded using a Synergy HT Multi-Detection Microplate Reader (BioTek Instruments, Inc., Winooski, VT, U.S.A.). Gallic acid was used for the calibration curve (r2=0.9945). Results were expressed in mg gallic acid equivalents (GAE)/g fresh weight of *Aloe vera* gel. The experiments were performed in triplicate. Mean values and standard deviations were used to express the results.

Total flavonoids

Total flavonoids (TF) content was determined according to the method described in Christ and Müller (1960). Accordingly, 1 mL of each *Aloe vera* extract was mixed with 0,3 mL of 5% sodium nitrite solution, with 0,3 mL of 10% aluminium chloride solution, 2 mL of 1M sodium hydroxide solution, and with distilled water to adjust the final volume of the mixture to 10 mL. Further, the mixtures were incubated for 15 min. Afterward, the absorption was recorded at 510 nm using a spectrophotometer (Jasco v530). The calibration curve was performed based on the quercetin standard (r2=0.9914). The results were expressed as mg quercetin

(QE) per 1g fresh weight *Aloe vera* gel. The experiments were performed in triplicate. Mean values and standard deviations were used to express the results.

Antioxidant activity

The DPPH radical-scavenging activity of each *Aloe vera* gel extract was evaluated as described in Pop *et al.* (2018). Accordingly, 250 µL of the sample was combined with 1750 µL DPPH solution (0.02 mg/mL in methanol). The mixture was incubated for 30 min at room temperature. The absorbance was read at 517 nm using a Synergy HT Multi-Detection Microplate Reader (BioTek Instruments, Inc., Winooski, VT, U.S.A.). The calibration curve was performed using Trolox (r2=0.9985). The results were expressed as Trolox equivalents per 1 g fresh weight *Aloe vera* gel.

Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR spectra of *Aloe vera* gel extracts were performed using a Shimadzu IR Prestige- 21 FTIR spectrometer, equipped with attenuated total reflectance (ATR) and internal reflection accessory (Zinc Selenide Composite -ZnSe). The extracts were pipetted directly on the surface of the ZnSe ATR crystal. The spectra were measured from 4000-650 cm^{-1}.

Liquid Chromatography-Diode Array Detection–Electro-Spray Ionization Mass Spectrometry (HPLC-DAD-ESI MS)

The separation and quantification of individual phenolic extract were performed as described in Pop *et al.* (2018). The compounds identification was done using an Agilent 1200 HPLC with a DAD detector coupled with an Agilent 6110 single quadrupole MS. Compounds separation was done using an Eclipse XDB C18 column (4.6 × 150 mm, particle size 5 µm, Agilent Technologies, U.S.A.) at room temperature. Two mobile phases were used. Mobile phase (A) consisted of 0.1% acetic acid/acetonitrile (99:1) in distilled water (v/v) while mobile phase (B) consisted in 0.1% acetic acid in acetonitrile (v/ v). The separation was performed using a gradient program as follows: 5% B (0-2 min) followed by an increase from 5-40% B (2-18 min) then 40-90% B (18-20 min) and 90% B (20-24 min). After 24 min the percentage of B was decreased from 90% to 5% (24-25 min). The absorbance spectrum was recorded between 200 and 600 nm. A flow rate of 0.5 mL/min was used. The MS compounds fragmentation was performed using the ESI source in the (+) mode. A capillary voltage of 3000 V, a temperature of 350 °C, and a nitrogen flow of 8 L/min were used. Compounds were scanned in the range of 100-1000 m/z. The phenolic acids were recorded at 280 nm and the flavonols at 340 nm. Agilent ChemStation Software (Rev B.04.02 SP1, Palo Alto, California, U.S.A.) was used for data analysis. Analysis was performed in triplicate. Compounds identification was performed considering the UV–visible spectra, the retention time, and mass spectra.

Statistical analysis

All results were expressed as mean ± standard deviation (SD). Comparisons between compounds' concentrations of the different extracts were performed using the one-way ANOVA with the Tuckey test. The p-value < 0.05 was considered significant. The Pearson coefficient method was used to exhibit a correlation between total polyphenol content, total flavonoid content, and antioxidant activity of the extracts. The correlation was considered significant at the 0.05 level (2-tailed). To analyse the data, IBM SPSS Statistics software, version 20 (SPSS Inc. Chicago, IL, USA) was used.

Targeted chemometric analysis was applied to discriminate between *Aloe vera* gel extracts using phenolic compounds composition and concentrations with principal component analysis (PCA). The used software was UnscramblerX 10.1 Software, version 10.1 (CAMO Software AS, Norway).

Results

Total polyphenol content, total flavonoids, and antioxidant activity

Total polyphenol content (mg GAE/ g *Aloe vera* gel), total flavonoids content (mg QE/ g *Aloe vera* gel), and the antioxidant activity, measured by the DPPH test (mM TE/ g aloe vera gel), of *Aloe vera* gel methanol (AVGM), ethanol (AVGE), water (AVGW) and acetone (AVGA) extracts are presented in Table 1.

Table 1. Total polyphenol content (TPC), total flavonoids content (TF), and the antioxidant action measured by the DPPH test of *Aloe vera* gel methanol (AVGM), ethanol (AVGE), water (AVGW), and acetone (AVGA) extracts

Aloe vera gel extract type	TPC (mg GAE / g *Aloe vera* gel)	TF (mg QE/ g *Aloe vera* gel)	DPPH (mM TE/ g *Aloe vera* gel)
AVGM	0.077±0.007[a]	0.084±0.002[a]	1.015±0.003[a]
AVGE	0.045±0.010[b]	0.095±0.005[a]	0.574±0.007[b]
AVGW	0.051±0.004[b]	0.071±0.007[b]	0.325±0.030[c]
AVGA	0.058±0.005[b]	0.084±0.002[a]	0.223±0.008[d]

*Notes (legend): Values are expressed as mean ± SD (n=3).
a,b, c, d Different letters between extracts on a column denote significant differences (Tuckey test, $p < 0.05$).

AVGM exhibited the significantly highest antioxidant activity, followed by AVGE, AVGW, and AVGA, the difference between all the extracts being significant. Regarding the total polyphenol content, AVGM had also the significantly highest content, followed by AVGA, AVGW, and AVGE extracts, the differences between the last three being non-significant. When total flavonoid content was analysed, AVGE had the highest content, followed by AVGM and AVGA with the same content and AVGW with significantly lower content. Total polyphenol content was correlated with the DPPH antioxidant activity ($p=0.03$).

FTIR analysis

Figure 2 shows the FTIR spectra of *Aloe vera* gel's different extracts. Generally, spectra in the range of 3600-3200 cm^{-1} indicate the presence of the hydroxyl groups (-OH). All sample extracts exhibited a broad absorption band at 3298 cm^{-1}, which suggests the presence of phenolic groups like aloin and emodin, which contain hydroxyl groups (−OH) that are bonded directly to the aromatic hydrocarbon group (Torres-Giner *et al.*, 2017). The absorption bands in the range of 2960-2850 cm^{-1} can be attributed to functional CH_2 methylene compounds (Bele and Khale, 2016). Further, the absorption band at 2927 cm^{-1} can be assigned to the aliphatic –CH and $–CH_2$ groups, symmetric and asymmetric C–H stretching (Lim and Cheong, 2015). The absorption band at 1728 cm^{-1} can be attributed to the C=O stretching from the carboxylic acid RCOOH from *Aloe vera* extracts (Kumalaningsih *et al.*, 2012). The absorption peak at 1631 cm^{-1} is associated with the asymmetrical and symmetrical –COO- stretching of the carboxylate compounds (Lim and Cheong, 2015). Also, the C=O stretching bond can be assigned to anthraquinones, saponins, and polysaccharides (Bele and Khale, 2016). Moreover, the absorptions band at 1631 cm^{-1} and 1076 cm^{-1} can also be attributed to acemannan, a mucopolysaccharide characteristic of *Aloe vera* leaves (Ray and Ghosh, 2018). Also, the absorption band at 1076 cm^{-1} can be assigned to C–O stretching from pectins (Lim and Cheong, 2015). Bands between 1350 and 1470 can be assigned to the non-symmetric scissoring and bending of CH_3 from aliphatic compounds (Ray and Ghosh 2018), while peaks from 1260 to 1000 cm^{-1} could be assigned to the C-O-C glycosidic symmetric stretching vibration from acetylated polysaccharides (Ray and Ghosh, 2018). The peak 1238 cm^{-1} can indicate the C-O-C stretching vibration from the -COCH3 methyl acyl groups, suggesting the presence of o-acetyl ester (Torres-Giner *et al.*, 2017; Nejatzadeh-Barandozi and Enferadi, 2012). The band at 1043 cm^{-1} can be assigned to the C−O and C−OH bonds in the glucan units of polysaccharides (Torres-Giner *et al.*, 2017). Finally, the

low absorption band between 900 and 800 cm^{-1} can be ascribed to the C–H out-of-plane deformation in the pyranoside ring and mannose (Torres-Giner *et al.*, 2017).

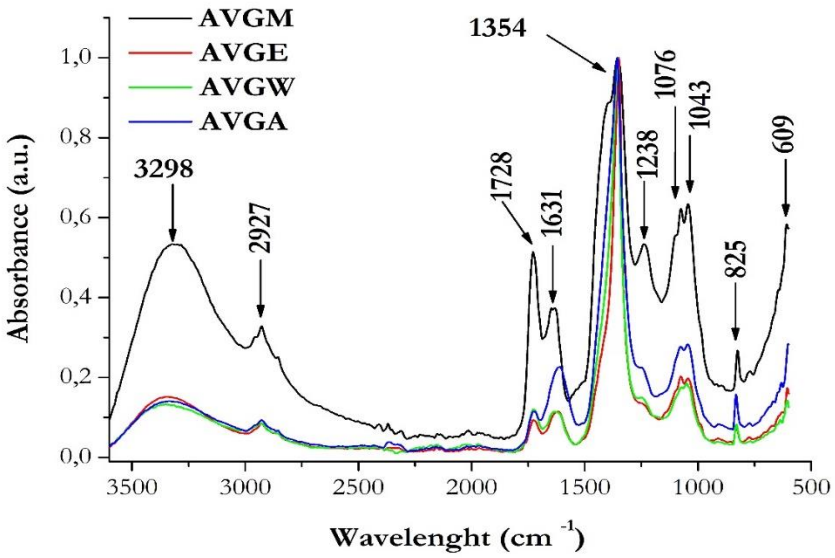

Figure 2. FTIR fingerprint of *Aloe vera* gel methanolic extract (AVGM), *Aloe vera* gel ethanolic extract (AVGE), *Aloe vera* gel water extract (AVGW), and *Aloe vera* gel acetone extract (AVGA)

Liquid Chromatography-Diode Array Detection–Electro-Spray Ionization Mass Spectrometry (HPLC-DAD-ESI MS) analysis

In total 14 compounds were tentatively identified as presented in Figure 3 and Table 2. Among them, 7 compounds belonged to the chromones subclass, 2 flavones, one hydroxycinnamic acid subclass, and 4 to the anthrone class (Table 2). The compound identification was performed according to their retention time, their UV absorption maxima, their protonated molecules $[M+H]^+$ as eluted in the Total Ion Chromatogram (TIC) and literature data (Table 2).

The number of compounds identified in the three types of extracts contained approximately the same number as follows: 14 for AVGM and AVGE, 12 for AVGW, and 11 for AVGA.

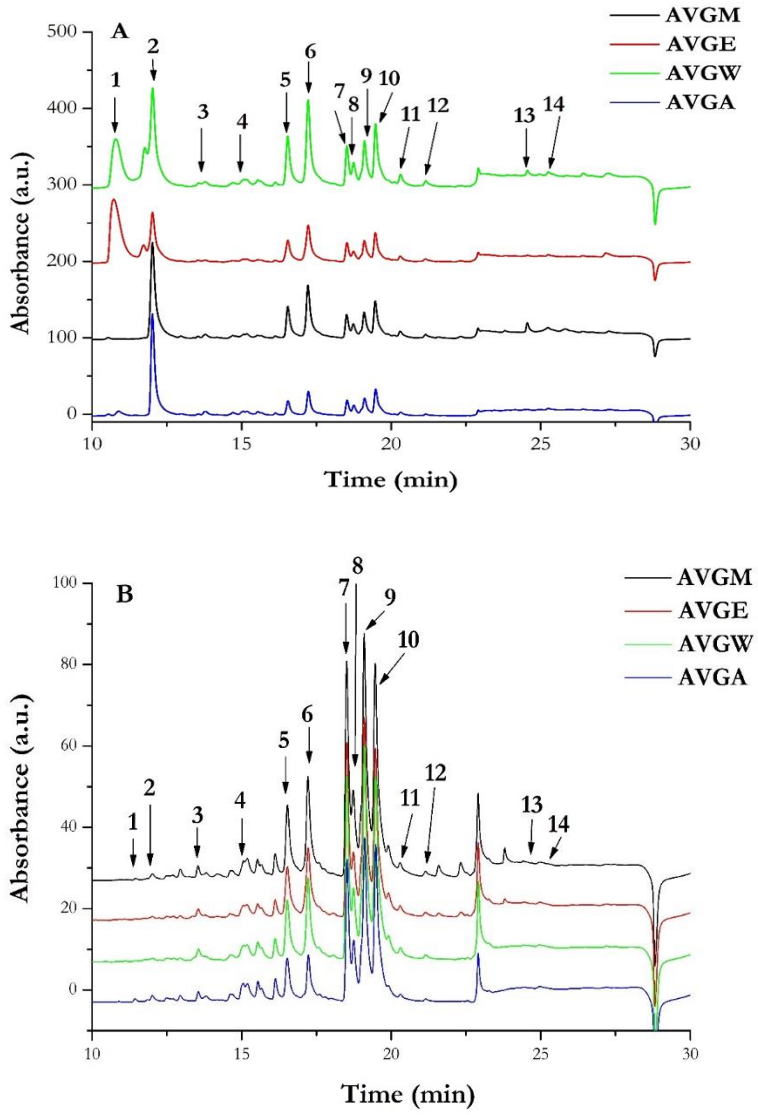

Figure 3. HPLC chromatogram at 280 nm (A) and 340 nm (B) of *Aloe vera* gel methanolic extract (AVGM), *Aloe vera* gel ethanolic extract (AVGE), *Aloe vera* gel water extract (AVGW), and *Aloe vera* gel acetone extract (AVGA)

Table 2. *Aloe vera* gel methanolic extract (AVGM), *Aloe vera* gel ethanolic extract (AVGE), *Aloe vera* gel water extract (AVGW), and *Aloe vera* gel acetone extract (AVGA) phenolic compounds identification by LC-ESI (+) MS

No	Retention time	UV λmax (nm)	[M+H]+ (m/z)	Tentative identification	Subclass	References
1	10.72	260	395	Aloesin	Chromone	(Quispe *et al.*, 2018)
2	12.01	299	395	Neoaloesin	Chromone	(Park and Kwon, 2006)
3	13.54	317	181	Caffeic acid	Hydroxycinnamic acid	(Quispe *et al.*, 2018)
4	15.10	235, 299, 345	449	Luteolin-glucoside	Flavone	(Quispe *et al.*, 2018)
5	16.54	231,239,250sh ,299	565	Aloinoside A	Chromone	(Park and Kwon, 2006)
6	17.22	239, 251sh, 299	541	Aloeresin E	Chromone	(Park and Kwon, 2006)
7	18.51	259, 299, 352	419	Aloin A	Anthracene compound (anthrone)	(Quispe *et al.*, 2018)
8	18.73	259, 299, 349	433	Apigenin-glucoside	Flavone	(Añibarro-Ortega *et al.*, 2019)
9	19.10	259, 299, 352	419	Aloin B	Anthracene compound (anthrone)	(Quispe *et al.*, 2018)
10	19.46	259, 299, 349	433	Aloe-emodin-glucoside	Chromone	(Quispe *et al.*, 2018)
11	20.30	239, 251sh, 299	557	Isoaloeresin D	Chromone	(Quispe *et al.*, 2018)
12	21.14	239, 299	555	Methoxycoumaroyl-aloresin	Chromone	(Añibarro-Ortega *et al.*, 2019)
13	24.54	255, 299sh	271	Emodin	Anthracene compound (anthrone)	(Quispe *et al.*, 2018)
14	25.25	239, 254	271	Aloe-emodin	Anthracene compound (anthrone)	(Park and Kwon, 2006)

The quantities of the phenolic compounds identified in the different extraction solvents are shown in Table 3. Aloesin, Aloinoside A, E, Aloe-emodin-glucoside, Isoaloeresin D, and 7-Methyl-aloeresin A were statistically significant among all extracts.

Table 3. *Aloe vera* gel methanolic extract (AVGM), *Aloe vera* gel ethanolic extract (AVGE), *Aloe vera* gel water extract (AVGW), and *Aloe vera* gel acetone extract (AVGA) phenolic compounds concentration

Compound/ extraction solvent	Concentration (µg/ml gallic acid equivalents)			
	AVGM	AVGE	AVGW	AVGA
Aloesin	2.322±0.1^{a}	54.337±0.8^{b}	20.405±0.1^{c}	3.993±0.2^{d}
Neoaloesin	43.943±1.5^{a}	33.452±0.4^{b}	34.681±0.9^{b}	38.145±1.8^{c}
Caffeic acid	2.543±0.0^{a}	2.297±0.1^{a}	2.690±0.2^{ab}	2.199±0.1^{ac}
Luteolin-glucoside	3.452±0.2^{a}	3.157±0.1^{ab}	3.624±0.1^{ac}	3.870±0.1^{ad}
Aloinoside A	12.936±0.2^{a}	10.037±0.1^{b}	10.725±0.1^{c}	6.327±0.1^{d}
Aloeresin E	23.206±0.0^{a}	17.752±0.1^{b}	19.128±0.0^{c}	9.791±0.2^{d}
Aloin A	7.604±0.2^{a}	6.474±0.2^{bc}	6.720±0.1^{bc}	5.442±0.1^{d}
Apigenin-glucoside	6.057±0.0^{a}	4.975±0.1^{bcd}	5.319±0.1^{bc}	4.730±0.2^{bd}
Aloin B	11.192±0.1^{a}	9.668±0.1^{bc}	10.012±0.0^{bc}	8.145±0.1^{d}
Aloe-emodin-glucoside	16.720±0.1^{a}	13.378±0.2^{b}	14.140±0.1^{c}	11.978±0.1^{d}
Isoaloeresin D	4.558±0.1^{a}	3.943±0.0^{b}	2.617±0.1^{c}	2.936±0.0^{d}
7-Methyl-aloeresin A	3.329±0.2^{a}	2.936±0.0^{b}	2.445±0.0^{c}	0.000^{d}
Emodin	4.263±0.0^{a}	2.052±0.0^{b}	0.000^{cd}	0.000^{cd}
Aloe-emodin	5.442±0.2^{a}	2.396±0.0^{b}	0.000^{cd}	0.000^{cd}

*Notes (legend): Values are expressed as mean ± SD (n=3).
a,b, c, d Different letters between extracts on a line denote significant differences (Tuckey test, p <0.05)

To further analyze a specific extraction pattern of the different solvents, PCA was used to discriminate between samples. For this purpose, the concentrations of the different compounds identified and quantified by HPLC-MS were used. As observed in the PCA plot (Figure 4), each sample was projected along the first two principal components axes in relation to the highest concentrations of different compounds among different extracts. The score plot, generated from comparisons of the first two PCs explained 90% and 9% of the total variance for PC1 and PC2, respectively. Since PC1 and PC2 explained 99 % of compounds concentrations among extracts, this plot offers enough information to analyze the overall variations. PC1 was correlated positively with Aloesin E and negatively correlated with Neoaloesin. Accordingly, AVGE extract has the highest concentration of Aloesin E and the lowest of Neoaloesin (as observed in the score loadings, data not shown). PC2 was positively correlated with Aloeresin E and negatively correlated with Luteolin-glucoside. Accordingly, AVGM has the highest concentration in Aloeresin E while AVGA has the highest concentration of Luteolin-glucoside.

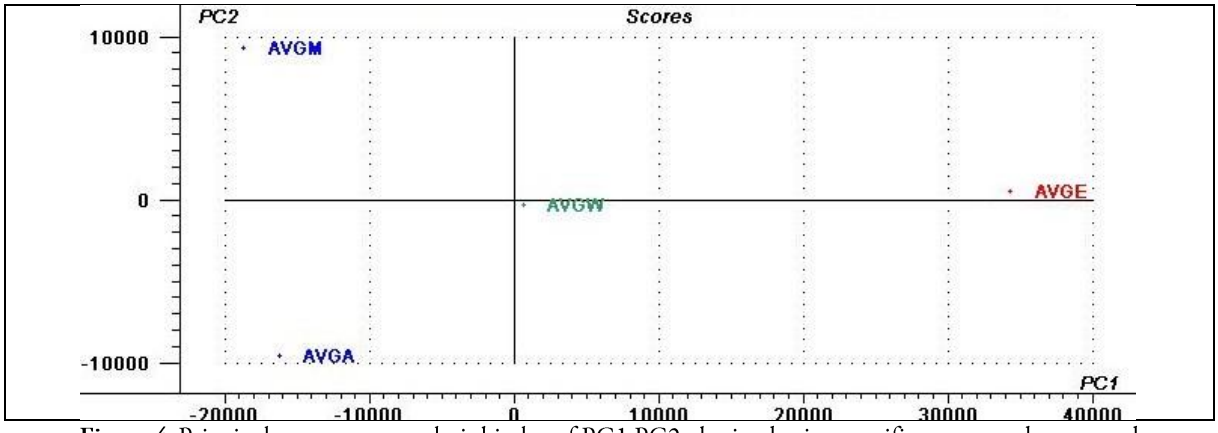

Figure 4. Principal component analysis bi-plot of PC1-PC2 obtained using specific compounds extracted in the different extraction solvents as follows: *Aloe vera* gel methanolic extract (AVGM), *Aloe vera* gel ethanolic extract (AVGE), *Aloe vera* gel water extract (AVGW) and of *Aloe vera* gel acetone extract (AVGA)

Discussion

It is already known that the chemical composition of plants is influenced by multiple factors like geographical area, precipitation, soil quality, temperature, solar radiation, species, or genetic factors (Enachi, 2020). Also, taking into account that *Aloe vera* knew a large distribution around the globe being able to adapt to a variety of climates like deserts, grasslands, and coastal or alpine locations it can also be concluded that a large variation between its phytochemical composition will be encountered. Because the *Aloe vera* industry has known an important development, *Aloe vera* gel is the most used by-product, being used on a large scale such as fresh gel, juice, or as an ingredient in multiple food supplements for health, medicinal or cosmetic purpose (Ahlawat and Khatkar, 2011), it is very important to establish the exact chemical composition.

Even though the *Aloe vera* leaf gel has 98% water, the remaining solid contains mainly polysaccharides (55%), sugars (17%), minerals (16%), proteins (7%), lipids (4%), and phenolic compounds (1%) (Hamman, 2008)(Ahlawat and Khatkar, 2011), compounds that induce the pharmacological and therapeutic activities reported so far in the literature for the aloe gel products (Talmadge *et al.*, 2004; Hamman, 2008). Most of the studies are focused on *A. vera* polysaccharides, especially acemannan which is considered the main responsible for inducing the anti-inflammatory, osteogenic and antibacterial effects with a positive influence in accelerating the healing of lesions (Kanama *et al.*, 2015).

Besides polysaccharides, the phenolic content was linked with Aloe gel anti-oxidant potential and its UV absorbing activity (Ray and Ghosh, 2018). Their antioxidant effects have been intensively studied and they refer to their scavenging of free radicals (Yagi *et al.*, 2002; Sumi *et al.*, 2019), prevention of lipid oxidation (Ramachaiahgari *et al.*, 2012; Tafi *et al.*, 2020), reduction in hydroperoxide formation (Xia *et al.*, 2010; Cesar *et al.*, 2018; Ceravolo *et al.*, 2021). Phenolic compounds are therefore responsible for the gel antioxidant activities.

The results suggest that *Aloe vera* gel was rich in TPC and TF, but with lower quantities than detected for other Aloe plants. For example, TPC found in the gel extracted from *Aloe vera* leaves grown in a garden in Bangladesh ranged from 0.99 to 4.64 mg GAE/g while TF content ranged from 17.55 to 36.91 mg Rutine equivalents/g d.w. (Uddin *et al.*, 2020). Regarding the quantities of TPC and TF of other Aloe spp grown in Sarajevo, it was observed that TPC (2.8 mg GAE/g) and TP (3.37 mg QE/g) were lower than the one grown in South Asia but higher than the one obtained for the pot cultivated species in Romania. When compared to the antioxidant DPPH activity, the Romanian methanol gel extract showed higher values than the ones obtained for the *Aloe vera* gel grown in Chile (0.34 mMTE/g FM)(Quispe *et al.*, 2018), suggesting that TPC was higher in the Romanian variety. The AWGM antioxidant capacity was in the same range as the antioxidant capacity exhibited by the flowers (1.25 mM ET/g FM) of the same Chilean *Aloe vera* plant. This great variability among aloe species grown in different areas of the globe confirms that plants are reacting to the various abiotic and biotic stress conditions via phenolic compounds accumulation (Chowdhary *et al.*, 2021). Therefore, it is reasonable to assume that pot plants are exposed to fewer stressor factors, thus the quantity of polyphenols is lower when compared to outdoor-grown species.

Regarding the profile of phenolic compounds of *Aloe vera* gel, several studies are comparatively assessing the composition of the peel, flowers, gel, and roots (Añibarro-Ortega *et al.*, 2019; Keyhanian and Stahl-Biskup, 2007; Quispe *et al.*, 2018). For example, the profile of phenolic compounds in the different parts of *Aloe vera* grown in Chile was assessed by UHPLC-PDA-QOT/ MS (ultrahigh-performance liquid chromatography photodiode array quadrupole Orbitrap mass spectrometry). Among the twenty-five compounds detected overall, only nine compounds were detected in the gel of *Aloe vera* (Quispe *et al.*, 2018). Among them, eight phenolic compounds were identified both in the gels of the Romanian pot *Aloe vera* and of the Chilean Pica Oasis *Aloe vera*, namely: Aloesin, Caffeic acid, Luteolin-glucoside, Aloin A, Aloin B, Aloe-emodin-glucoside, Isoaloeresin D and Emodin. *A. grandidentata, A. perfoliata* (El Sayed *et al.*, 2016), and *A. ferox Miller* (El Sayed

et al., 2016) were also found to contain the chromone Aloesin. The LC-MS characterization of a phenolic compound extracted from the gel of *Aloe barbadensis* Mill grown in Yemen, Western Asia showed a richer spectrum in anthrone compounds. While in the Romanian species were identified 14 compounds with 4 belonging to the anthrone class (Aloe-emodin, Emodin, Aloin B, and Aloin A), in the species grown in Yemen there were identified 8 compounds, all from the anthrone class (Homonataloin B, Homonataloin A Aloinoside B, Aloinoside A, Microdontin B, Microdontin A)(Aldayel *et al.*, 2020). Aloin A and Aloin B were the only two compounds found in both species.

Phenolic compounds composition was also confirmed by the FTIR analysis, mainly by the presence of the phenolic -OH absorption band identified in the extracts. When compared with the gel obtained from Indian *Aloe vera* harvested in different growth periods, the presence of polysaccharides, acetylated polysaccharides (acemannan), pectins, anthraquinones, and saponins were identified in both, Romanian and Indian species (Ray and Ghosh, 2018). The detection of acemannan, one of the principal bioactive polysaccharides identified in *Aloe vera* (Liu *et al.*, 2019) was found in high quantities in Romanian species too, as derived from its high-intensity absorption bands. The polysaccharides content, especially acemannan was also confirmed in the gel obtained from A vera grown in Thailand (Chokboribal *et al.*, 2015), and *Aloe vera* grown in Mexico (Miramon-Ortíz *et al.*, 2019) or the ones grown in India (Ray and Aswatha, 2013; Kiran and Rao, 2016).

Most of the literature data present the characterization of *Aloe vera* composition of plants grown in natural conditions or controlled conditions like botanical gardens, while there is little information regarding the plants grown in pots. One of the existing studies focused on the pot plants is referring to the effect of different stress conditions (like salt stress) and its effect on minerals content, proline, total protein, and specific enzyme (Murillo-Amador *et al.*, 2014). Other existing studies which involve Romanian *Aloe vera* grown under greenhouses conditions or in commercially available pots are focused on leaves extracts antioxidant and antimicrobial properties (Fiț *et al.*, 2013; Carac *et al.*, 2016), thus we have no records regarding the chemical composition of such pot grown plants. The bioactive compounds of seven different Aloe species grown in the Botanical Garden of Jibou in Romania analysed the bioactive compounds in the Aloe species leaves in terms of carotenoids, lipids, and Vitamin C (Bunea *et al.*, 2020) with no reference to polyphenol composition. Thus, the analysis of Romanian pot *Aloe vera* gel utilizing two complementary analyses (HPLC-MS and FTIR) offers, for the first time to our knowledge, information regarding gel composition.

Conclusions

The investigations carried out in this study showed that the composition of aloe gel depends on the extraction solvent, the most suitable solvent extraction being methanol and ethanol. The LC-MS results showed important quantities of phenolic compounds like Aloesin, Neoaloesin, Aloeresin E, and Aloe-emodin-glucoside while FTIR analysis highlighted the presence of polysaccharides, pectins, anthraquinones, and saponins in the *Aloe vera* gel extracts.

Authors' Contributions

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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