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ORIGINAL ARTICLE

LC-MS characterization of bioactive metabolites from two Yemeni Aloe spp. with antioxidant and antidiabetic properties

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Abstract Two Yemeni Aloe*(s)* have been investigated; the resin from *A. perry* Baker (APR, Socotran Aloe), and the gel from *A. vera* (AVG, Saber Yamaniis). LC-MS for APR identified aloin B, aloinoside B/A, homonataloin B and microdontin B/A as the major components, constituting 67.7% w/w of the extract. AVG showed the same pattern of anthrones (19.5% w/w), in addition to the chromones aloesin, aloeresin A, aloeresin D and aloeresin E. Dihydro-isocoumarin glucoside was identified in both Aloe species. Aloe extracts showed high antioxidant activity: DPPH (0.09 & 0.05 mM/g TE), ABTS (0.06 & 0.03 mM/g TE), and FRAP (20.5 & 15.5 mM Fe^{+2}E), for APR & AVG, respectively. The antidiabetic properties was evaluated through inhibition of α-glucosidase enzyme. APR showed inhibitory activity with IC_{50} 0.76 μg/mL higher than AVG (IC_{50} 0.76 mg/mL). Aloin A showed the highest inhibitory activity with IC_{50} 0.34 mg/mL that was higher than acarbose (0.54 mg/mL) the positive control, indicating that the activity of Aloe extract is linked to the aloin and other anthrone compounds. These findings highlight the phytochemical profile,

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antioxidant and potential antidiabetic activity of the Yemeni Aloe species and draw attention to their potential application in food, medicine and cosmetic products.

1. Introduction

Nowadays, searching for natural bioactive compounds has been the goal for food, pharmaceutical and cosmetic industries, due to their therapeutic and commercializable properties. The literature describes many plants with pharmaceutical activities, but Aloe plants, specifically *A. vera* is the most widely applied medicinal plant worldwide. Aloe plants have long been used in traditional medicine for their curative and therapeutic properties. Recent reviews have shown that Aloe extracts have many biological effects, including anti-tumor, antimicrobial, anti-inflammatory, antioxidant, antidiabetic, and wound-healing properties (Kumar et al., 2019). In addition to the biological effects on human body, the antioxidant activity of Aloe makes it a safe natural ingredient in food processing to increase the shelf life and nutritional value of food (Hęś et al., 2019).

A. barbadensis Miller L. (trivially labeled as *A. vera*) is the most extensively cultivated genotype in the world (Liao et al., 2006) and the most commonly used source of Aloe gel. Taxonomically, Aloe belongs to the family Aloeaceae that is widely growing in tropical and subtropical regions, mostly in the African continent. There are more than 500 species of Aloe found in Africa, and on the Indian and the Arabian Peninsula, including *A. vera* and *A. perryi* Baker, the subjects of this study (Kumar et al., 2019). *A. perryi*, also known as Perry's Aloe is endemic to the island of Socotra in Yemen. One of the key differences between the two species is the fact that *A. perryi* has a red flower, whereas *A. vera* has yellow flowers (Al-Fatimi et al., 2007; Al-Oqail et al., 2016). Aloe species in general and most commonly *A. perryi* originating from the Arabian Peninsula have been extensively used in traditional medicine (Al-Fatimi et al., 2005). Several studies indicated the role of *A. perryi* in the treatment of eye (Al-Fatimi et al., 2005), stomach ailments, constipation, and malaria (Al-Oqail et al., 2016; Mothana et al., 2012), and for its antimicrobial effects (Ali et al., 2001). Aloe leaves contain several classes of bioactive compounds including anthraquinones, anthrones, chromones, flavonoids, amino acids, lipids, carbohydrates, vitamins and minerals. The elements Al, B, Ba, Ca, Fe, Mg, Na, P, Si etc. have also been reported to be present in *A. vera* (Kumar et al., 2019). Anthrones, chromones and phenyl pyrones are the main phenolic metabolites described in Aloe species and possess various biological activities. The C-glycosylated chromones are known to be unique components of Aloe, and not reported in other plants (Franz and Grün, 1983).

During normal metabolic processes, there are many reactions that produce reactive oxygen species (ROS), for example hydroxyl radicals, superoxide, hydrogen peroxide, and free radicals, all of which can play physiological roles in cell signaling. However, the increased generation of free radicals can cause tissue injury and cell death, which are involved in many chronic diseases such as cancer, diabetes and other inflammatory diseases (Kumar et al., 2019). The serious imbalance between ROS production and a reduced antioxidant defense potential is called "oxidative stress". Plant-derived foods contain many phytochemicals, including polyphenols and flavonoids, which can help protect against some of the ROS-related chronic diseases as they can scavenge the excess free radicals (Rahman et al., 2006).

Diabetes mellitus (DM) is one of the major chronic diseases worldwide; it is a complex metabolic disease that has a very significant impact on health and quality of life, as well as on economic and health care systems. There are several types of anti-diabetic agents used to treat type II DM, namely, acarbose, miglitol and voglibose, which act by inhibiting α-amylase and α-glucosidase activities (Van de Laar, Floris A et al., 2005). While they are efficient in attenuating the rise in blood glucose levels in many patients, the continuous use of these drugs is often associated with undesirable side effects, such as liver toxicity and adverse gastrointestinal symptoms (Etxeberria et al., 2012). It is for this reason there is a need for natural α-glucosidase and α-amylase inhibitors which have no adverse or unwanted side effects.

The objective of this study was to investigate two Aloe products with long history of ethnobotanical use in the Arabian Peninsula; *A. perryi* resin and *A. vera* gel obtained from Yemen. HPLC and LC-MS were used for comprehensive phytochemical determination of bioactive compounds. The antioxidant property and the potential antidiabetic activity were evaluated. This detailed information will help in future design and formulation of nutraceutical preparations, and quality control of these two products.

2. Materials and methods

2.1. Chemicals

4-Dimethylaminocinnamaldehyde (DMAC), Folin Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), phosphate buffer, iron(III) chloride hexahydrate, and catechin were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Procyanidin B1 reference compounds were obtained from Chromadex (Irvine, CA, USA). Aloin A was purchased from MCE (MedChemExpress, NJ, USA). All organic solvents were of HPLC or LC-MS grades and obtained from VWR International (Suwanee, GA, USA).

2.2. Aloe samples

The resin obtained from *Aloe perryi* Baker (APR, local name Saber Socotri), and the gel obtained from *Aloe barbadensis* Mill (AVG, known as *Aloe vera* L gel, local name Saber

Yamaniis) were obtained from local markets in Hadibu and Sanaa, respectively, Yemen, in 2017.

2.3. Preparation of Aloe extracts

APR and AVG dried powders were extracted with 100% methanol. Briefly, 5 g of the powdered plant material was added to 50 mL methanol, and the mixture was sonicated for 20 min at room temperature. The mixture was then centrifuged at 5000 rpm for 10 min (Sorvall RC-6 plus, Asheville, NC, USA). The plant residue was further extracted 4 more times, each with 50 mL methanol, and the combined supernatants was evaporated to dryness using rotary evaporator. The obtained extract was dissolved in methanol and filtered using 0.22 PTFE syringe filter (Fisher Scientific, Pittsburg, PA) before analysis for phenolics, HPLC, LC-MS, and spectrophotometric assays. Three replicate from each sample were tested.

2.4. LC-MS profiling of Aloe extracts

LC-MS was performed using the Shimadzu LC-IT-TOF-MS (Shimadzu, Tokyo, Japan) with a Shim-pack XR-ODS column (50 mm × 3.0 mm, 2.2 μm). Solvent gradient consisted of 0.1% formic acid in H_2O (A) and methanol (B). Compounds were eluted into the ESI ion source at the flow rate of 0.45 mL/min with a step gradient of 5–60% B (40 min), isocratic at 60% B (2 min), 5% B (2 min), then the column was re-equilibrated for 5 min at 5% B. Column was maintained at 40 °C during the run. The MS was programed to carry out a full scan over a range of m/z 100–1500 (MS1) and m/z 100–700 (MS2) in both positive and negative modes. The heat block and curved desolvation line (CDL) temperature was maintained at 200 °C; nitrogen was used as the nebulizing gas at a flow rate of 1.5 L/min, and as the drying gas at 75 kPa; the interface voltage was (+), 4.5 kV; (−), −3.5 kV; and the detector voltage was 1.8 kV. Compounds were characterized by their MS, MS/MS spectra, UV spectra, and in comparison with literature.

2.5. Quantitative analysis of major components by HPLC-PDA

HPLC was conducted on an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA) with a photodiode array (PDA) detector, and an autosampler with Chemstation software. The HPLC was outfitted with a reversed phase Phenomenex Synergi 4 μm hydro-RP 80A column (250 mm × 4.6 mm × 5 μm, Torrance, CA.) equipped with a guard column (Phenomenex security guard cartridge, AQC 18.4 × 3.00 mm). The mobile phase consisted of 2% acetic acid in H_2O (A) and 0.5% acetic acid in acetonitrile-water 50:50 (B). The operating conditions were: autosampler tray at 10 °C; column oven at 30 °C; constant elution flow rate of 1 mL/min. Separation of Aloe compounds started with 20% B with a linear gradient to 40% B (0–10 min), 60%B (20 min), 65% B (25 min), 90% B (40 min), 90% B (42 min), and a post-run of 8 min with 20% B. Aloins and other anthrones were monitored at a wavelength of 355 nm, whereas chromones were monitored at 295 nm. A standard curve was constructed with aloin A reference standard, and anthrones were quantified as aloin A equivalent.

2.6. Total phenolics content

The total polyphenol concentrations were measured spectrophotometrically using a microplate-adapted Folin–Ciocalteu assay (Grace et al., 2016) at a wavelength of 765 nm. Results were standardized against a gallic acid standard curve, and results were expressed as gallic acid equivalent (mg GAE per g Aloe extract). All determinations were carried out in triplicate.

2.7. Total flavonoid content

The content of total flavonoid in the Aloe extracts was determined by using a colorimetric method that depends on the formation of flavonoid-aluminium complex (Roshanak et al., 2016). The color intensity was read at 510 nm using the spectrophotometer (Shimadzu UV–VIS 2450, Japan). The flavonoid content was calculated from a calibration curve constructed with catechin, and results were expressed as mg/g catechin equivalent. Determinations were carried out in triplicate.

2.8. Total proanthocyanidin content

Total proanthocyanidins were quantified colorimetrically using the DMAC method, in a 96-well plate against a standard curve constructed with procyanidin B1 reference standard, as previously described (Prior et al., 2010). Concentrations were calculated as procyanidin B1 equivalents (mg /g extract). Determinations were carried out in triplicate.

2.9. DPPH radical scavenging assay

The radical scavenging activity was measured using the stable DPPH and Trolox as reference substances according to a modified method (Grace et al., 2016). An aliquot (20 μL) of each sample was added to 180 μL of DPPH solution (150 μM in 95% ethanol) to initiate the reaction. After a reaction time of 3 h at ambient temperature, the reaction had reached completion. The decrease in absorbance of DPPH free radicals was read at 515 nm against ethanol as a blank using a Molecular Devices M3 microplate reader. Trolox (0, 100, 200, 300, 400, and 500 μM) was used as a standard antioxidant compound. Analysis was performed in triplicate for each sample and each concentration of standard. The antioxidant activity was reported in mmole of Trolox equivalents per gram extract (mM TE/g).

2.10. ABTS radical scavenging assay

The ABTS assay was performed as described earlier (Re et al., 1999), and is based on the reduction of $ABTS^{\bullet+}$ radicals by antioxidants in the Aloe extracts. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS solution (7 mM) with 2.4 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For the assay, the $ABTS^{\bullet+}$ solution was diluted in deionized water or ethanol to an absorbance of 0.7 (±0.02) at 734 nm. After the addition of 9 μL of the extract solutions to 271 μL of $ABTS^{\bullet+}$ solution, the

absorbance reading was taken at 30 °C 10 min after initial mixing. All determinations were carried out in triplicate. The antioxidant activity was reported in mmole of Trolox equivalents per g extract (mM TE/g).

2.11. Ferric reducing antioxidant power (FRAP) assay.

The antioxidant capacity of Aloe extracts was determined using the FRAP assay. The reduction of the colorless ferric complex (Fe^{3+} tripyridyltriazine) to a blue-colored ferrous complex (Fe^{2+}−tripyridyltriazine) performed by the action of electron-donating antioxidants. The assay was performed in a 96-well microplate according the standard method (Grace et al., 2016), with minor modifications. The working FRAP reagent was freshly prepared by mixing 10 volumes of 30 mM acetate buffer, pH 3.6, with 1 vol of 10 mM TPTZ in 40 mM hydrochloric acid and with one volume of 20 mM $FeCl_3$. A standard curve was constructed using various concentrations of $FeSO_4{\cdot}7H_2O$. FRAP solution (175 µL), warmed at 37 °C was added to three replicates of the sample (25 µL), whereas the same volume of acetate buffer was added to the fourth one (blank). The reaction mixture was incubated for 30 min at 37 °C, and then the absorbance was measured at 593 nm. The absorbance of the blank was subtracted from the absorbance of the samples. In this assay, the reducing capacity of the extracts tested was calculated with reference to the reaction signal given by a Fe^{2+} solution. FRAP values were expressed as mmole Fe^{2+} per g extract (mM Fe^{+2}/g).

2.12. α-Glucosidase inhibitory activity

The inhibition of α-glucosidase enzyme activity was determined using the standard method (Lordan et al., 2013). Briefly, one mg of α-glucosidase from *Saccharomyces cerevisiae* (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 100 mL of 100 mM phosphate buffer (PB, pH 6.8). The Aloe extracts were prepared in 80% methanol (serial concentrations 10–0.08 mg/mL). To each well of a 96-well plate, 10 uL of Aloe extract or solvent (control) was mixed with 100 uL α-glucosidase working solution, and incubated at 30 °C for 5 min. 100 µL of the substrate, p-nitrophrnyl-α-D-glucopyrano side (PNPG; 1 mL in PB), was charged to each well, and absorbance was measured at 405 nm for 30 min using Molecular Devices M3 microplate reader at 30 °C (Molecular Devices Inc., Sunnyvale, CA, USA). Blank wells (sample + PB + P NPG) were subtracted from sample wells (sample + enzyme + PNPG), and results were compared to no inhibition control (enzyme + PNPG). Acarbose was used as a positive control. For each sample, the percentage inhibitory activity was calculated using the following equation:

$$\%\,\text{inhibitory activity} = \left[A_{\text{control}} - \left(A_{\text{sample}} - A_{\text{blank}}\right)/A_{\text{control}}\right] \times 100$$

where A_{control} is the absorbance of the uninhibited enzyme, A_{sample} is the absorbance of the enzyme treated with samples, and A_{blank} is the absorbance of the extract with no enzyme. Concentration of inhibitor that inhibited 50% of the enzyme activity (IC_{50}) was determined from the curve constructed with log concentration of sample against the percentage inhibition.

Half maximal inhibitory concentrations (IC_{50}) data was calculated after logarithmic transformation and expressed as the geometric mean with 95% confidence intervals .using GraphPad Prism V8 (GraphPAD Software, Inc., La Jolla, CA).

3. Results and discussion

3.1. Total phenolics, flavonoids and proanthocyanidins

The beneficial health-promoting properties present in Aloe leaves are attributed to their phytochemical composition and in particular to characteristic polyphenols, which are one of the major groups of compounds acting as free radical terminators or primary antioxidants (Miliauskas et al., 2004).

The Aloe leaves produce two exudates; the first one is the resin or latex which comes from the cells in the outer green rind of the leaf, and the second is the transparent sap/mucilage coming from the inner parenchyma zone of the leaf, which is called the gel. Polyphenols accumulate in high amounts in the leaf rind, while polysaccharides are the major components in the leaf gel (Joseph and Raj, 2010).

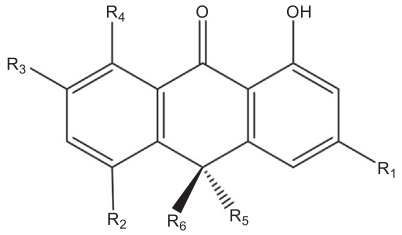
The methanol extracts from APR and AVG accounted for 87.5% and 80%w/w of the dried powders, respectively. Results presented in Table 1 indicate that APR extract contained total phenolics of 205 ± 14.2 mg/g GAE (20.5 mg/100 g). The obtained value is much higher than all Aloe species investigated recently (Cardarelli et al., 2017), where the highest value reported was 14.35 g/100 g. Total flavonoids measured 24.02 ± 0.97 mg/g (2.4 mg/100 g) as catechin equivalent, and the proanthocyanidin group was the lowest phenolic class measuring only 0.95 ± 0.05 (0.09 mg/100 g) as procyanidin B1 equivalent.

The methanol extract from the Yemeni AVG contained total phenolics of 97.2 ± 12.0 mg/g; total flavonoids 9.27 ± 1.07 mg/g, and total proanthocyanidins 0.55 ± 0.005 mg/g. Several factors can affect the quality and quantity of a particular phytochemical. Phytochemical composition of plants is influenced

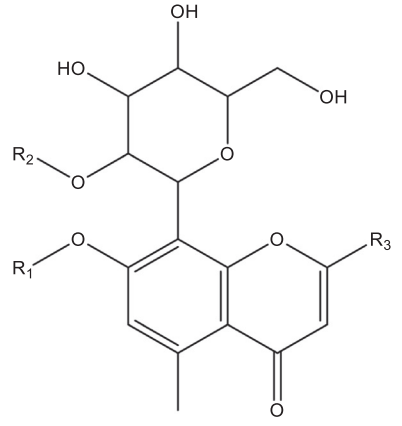
Table 1 Total phenolics, flavonoids, proanthocyanidins, and antioxidant activities in the Yemeni Aloe species.

Assay	*Aloe perryi* resin	*Aloe vera* gel
Total phenolics (mg/g gallic acid E)	292 ± 7.34	97.5 ± 12.0
Total flavonoids (mg/g catechin E)	24.0 ± 0.97	9.27 ± 1.07
Total proanthocyanidins (mg/g procyanidin B1 E)	0.95 ± 0.05	0.55 ± 0.005
DPPH assay (mM/g TE)	0.09 ± 0.002	0.05 ± 0.002
ABTS assay (mM/g TE)	0.06 ± 0.013	0.03 ± 0.001
FRAP assay (mM/g Fe^{+2} E)	20.5 ± 0.30	15.5 ± 0.29

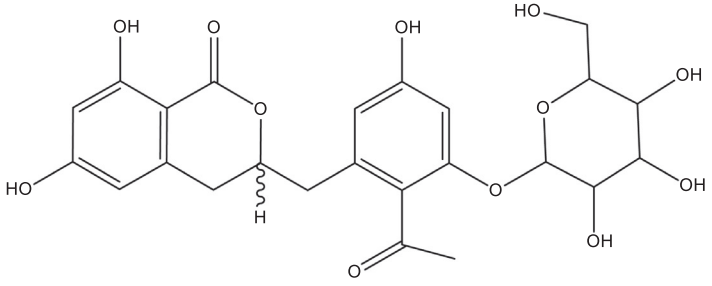
DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2-azinobis(3-ethyl-benzothiazoleine-6-sulfonic acid, FRAP ferric reducing antioxidant power assay (FRAP), E: equivalent. Each value is expressed as mean ± standard Error of Mean (n = 3).



Anthrones	R1	R2	R3	R4	R5	R6
Aloin B (**12**)	CH_2OH	H	H	OH	C-glc	H
Aloin A (**13**)	CH_2OH	H	H	OH	H	C-gluc
Homonataloin (**14**)	CH_3	H	OH	OCH_3	H	C-glc
Homonataloin (**16**)	CH_3	H	OH	OCH_3	C-glc	H
Aloinoside B (**17**)	CH_2O-glc	H	H	OH	C-glc	H
Aloinoside A (**18**)	CH_2O-glc	H	H	OH	H	C-glc
Microdontin B (**19**)	CH_2OH	H	H	H	C-(6′-*O*-p-coumaroyl)-glc	H
Microdontin A (**20**)	CH_2OH	H	H	H	H	C-(6′-*O*- p-coumaroyl)-glc



Chromones	R_1	R_2	R_3
Aloesin (**2**)	H	H	CH_2COCH_3
Aloeresin A (**6**)	CH_3	p-coumaroyl	CH_2COCH_3
Isoaloeresin D (**11**)	CH_3	p-coumaroyl	$CH_2C(H,OH)CH_3$
Aloeresin E (**15**)	CH_3	cinnamoyl	$CH_2C(H,OH)CH_3$



Dihydroisocoumarin glucoside [**7**,**8**]

Fig. 1 Structures of the main components identified in *Aloe perryi* resin and *A. vera* gel.

by a variety of environmental factors including the geography, climate, soil type, sun exposure, grazing stress, seasonal changes, age of the plant, etc. (Nduche and Otaka, 2019).

3.2. HPLC quantification of anthrones

Anthrones could be distinguished from other compounds based on their UV spectra, which were further confirmed by LC-MS. HPLC of Aloe extracts was conducted at three different wavelengths, 355, 295 and 280 nm, to select the best profile showing all peaks. The HPLC profile recorded at 355 nm revealed only the anthrone peaks, while the 295 nm showed both the anthrones and chromone compounds (see Fig. 1 for chemical structures, Fig. 2 for HPLC). This enabled the distinction between anthrones from other phenolics. Quantification was performed based on peak area measurement against

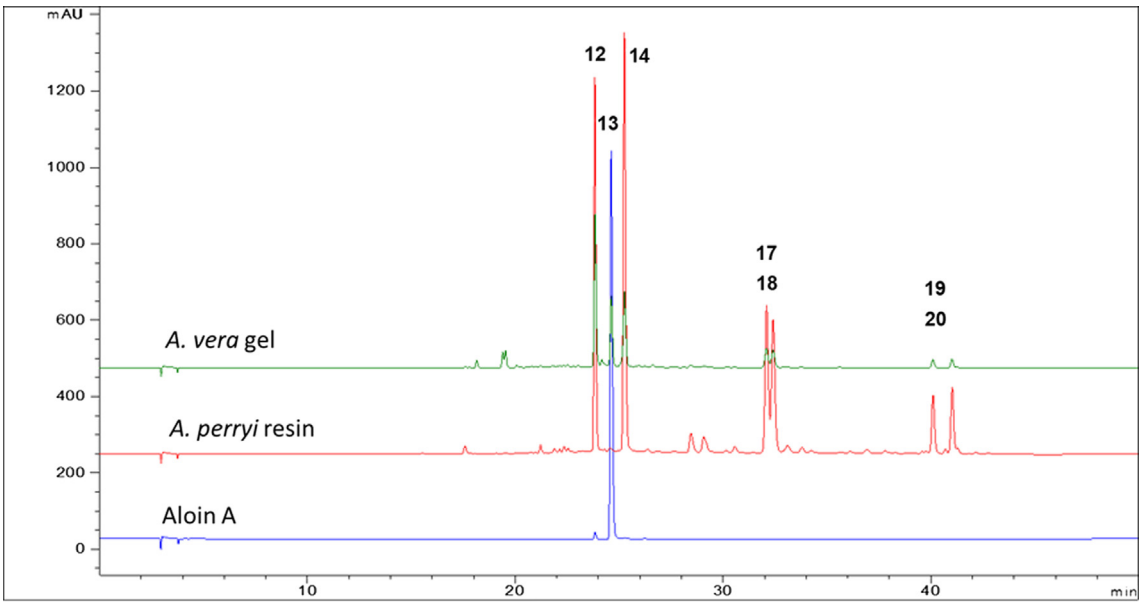


Fig. 2 HPLC profiles of the methanol extracts from the *Aloe* perryi Baker resin and A. vera L. gel, recorded at 355 nm.

a standard curve constructed with the commercially available aloin A, and results were expressed as mg/g aloin A equivalent (Table 2). Total anthrones measured 677 mg/g (67.7% w/w) of APR extract. Major metabolites were the aloinosides B & A (317 mg/g), microdontins B & A (150 mg/g), homonataloin B (120 mg/g) and aloin B (88.6 mg/g). AVG extract contained total anthrones 194.9 mg/g (19.5% w/w). Aloins B & A, homonataloins B &A, aloinosides B & A, and microdontins B & A measured 64.7, 59.2, 48.7, and 23.3 mg/g, respectively (Table 2).

3.3. Liquid chromatography-mass spectrometry profiling

The extracts from APR and AVG were subjected to untargeted screening by liquid chromatography-ion trap-top of flight-mass spectrometer (LC-IT-TOF-MS). All compounds were detected in the positive ion mode as $[M + H]^+$. A typical total ion chromatograms (TIC) of the two Aloe extracts is shown in Fig. 3. MS^2 studies of the molecular ion of each compound were performed for compound characterization (see Supplimentary data). Compounds were identified based on their accurate masses, fragment masses, UV spectra, elution sequence and with the aid of literature. A total of 20 compounds with their MS, MS^2 and UV spectral data are presented in Table 3, listed according to their elution order.

Anthrones were the main group of phenolics in the investigated Aloe products. The preliminary identification of anthrones was their characteristic UV maximum absorption at 270, 300 and 355 nm. A characteristic pattern of aloin (often referred to as barbaloin) (**12**, **13**), aloinosides (**17**, **18**), and microdontoside (**19**, **20**) was found in the two Aloe species with varying concentrations (see Table 2 for quantitative analysis). The presence of aloin, as a major bioactive ingredient, is known for *A. barbadensis* (*A. vera*) and is considered a taxonomic marker for this species (Zhong et al., 2015). The diglucosides aloinosides B & A, and microdontins B & A were the major metabolites in APR. Aloin B (**12**) was also present in APR but not aloin A (**13**), while both isomers were present in AVG. The occurrence of this taxonomic distribution in Aloe was found in 95% of the Aloe plants (36 species) screened in North-East Africa (Viljoen et al., 2001). This could provide a link between a species in south Yemen and its counterparts from the in African continent. The same pattern of anthrones was also identified in the latex of the Ethiopian *A. sinana* Reynolds (Minale et al., 2014), that supports the link between the

Table 2 Anthrone content in *Aloe perryi* resin and *A. vera* gel extracts.

Rt min		*Aloe perryi* resin extract (mg/g)[a]	*Aloe vera* gel extract (mg/g)[a]
23.52	Aloin B	88.6 ± 3.2	43.3 ± 2.8
24.26	Aloin A	–	21.4 ± 2.0
24.81	Homonataloin B	120 ± 4.3	47.3 ± 2.0
25.49	Homonataloin A	–	11.9 ± 0.9
31.15	Aloinoside B	160 ± 5.8	24.0 ± 1.2
31.44	Aloinoside A	157 ± 5.6	23.7 ± 0.9
39.16	Microdontin B	73.5 ± 3.7	11.2 ± 0.8
40.08	Microdontin A	76.7 ± 3.0	12.1 ± 1.0
	Total anthrones	677 ± 25.6	195 ± 11.6

[a] Compounds were measured by HPLC-UV at 355 nm and quantified as aloin A equivalent (n = 3).

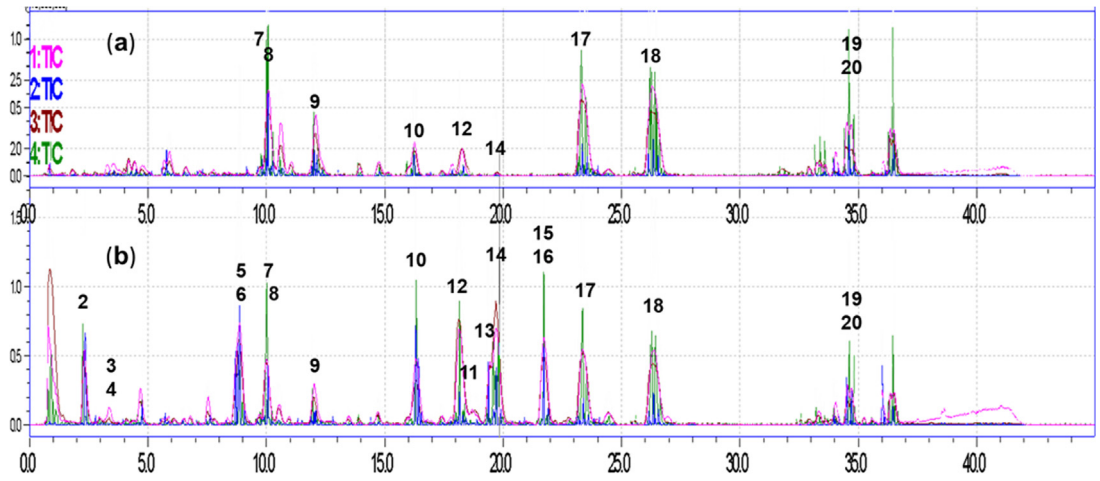


Fig. 3 Total Ion Chromatograms (TIC) of LC-MS of *Aloe perryi* resin (a) and *A. vera* gel (b). Refer to Table 3 for compound identification.

Ethiopian Aloe species with *A. perryi* and *A. vera* from Yemen. Homonataloins B & A are another major anthrone compounds characterised and quantified in Aloe species. These two isomers were detected in only few species indigenous to south Yemen and east Africa (Viljoen et al., 2001). Dihydroisocoumarin glucoside was detected as twin peak in the extracted ion chromatogram of *m/z* 507 $[M + H]^+$ (**7**, **8**). It was characterized in both APR and AVG extracts. The same compound had been previously reported in *A. hildebrandtii*, native to north Somalia (Viljoen et al., 2001). The absolute configuration at C-3 of the compound has not been determined. In our analysis, the two conformational structures were present in AVG. The structure is closely related to the corresponding aglycone feralolide that was reported in *A. vera* (Lucini et al., 2015). It appears that this compound is a useful taxonomic marker among Aloe species.

Another group of phenolic compounds characteristic for Aloe is the chromones (1,4-benzopyrone derivatives, Fig. 1). Chromones were reported as the main phenolics in *A. barbadences* (Joseph and Raj, 2010). Chromones were present at very low levels in APR, while they were prominent in AVG extract. The most abundant chromones were aloresin A (***6***), isoaloresin D (**11**), and aloresin E (**15**). These results agree with the reported literature on *A. vera* (*A. barbadenses*) (Joseph and Raj, 2010).

3.4. Antioxidant activity

Aloe has developed a high reputation as a health promoting dietary component, with most activity ascribed to its antioxidant activity. Antioxidant activity was measured by DPPH, ABTS and FRAP assays. The DPPH and ABTS methods evaluate the free radical scavenging capacity of a sample, while the FRAP method evaluates the content of electron-donating species with a certain redox potential (Prior, 2015). The results of the three assays are shown in Table 1. The radical scavenging activity measured 0.09 ± 0.002 mM, and 0.06 ± 0.01 mM Trolox equivalent (APR), 0.05 mM and 0.03 mM TE (AVG), for DPPH and ABTS assays, respectively. The FRAP assay results were 20.5 ± 0.3 and 15.5 ± 0.29 mM/g $FeSO_4$ equivalent for APR and AVG, respectively. The antioxidant results agree with the reported literature for *A. vera* (Kim et al., 2014). It was previously reported that *A. perryi* methanolic extract had 94% radical scavenging activity when tested at 1 mg/mL, which was comparable to the activity ascorbic acid in the same experiment (Mothana et al., 2009). The antioxidant activity of anthrone compound (200 ppm) was found to inhibit the peroxidation of linoleic acid with comparable activity to the known synthetic compound BHA (butylated hydroxyanisole) as indicated previously (Yen et al., 2000).

3.5. Potential antidiabetic activity (inhibition of α-glucosidase activity)

The α-glucosidase has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia, which is the earliest metabolic symptom occurring in type II diabetes. The enzyme cleaves glycosidic bonds in complex carbohydrates yielding absorbable monosaccharides. Hence, the inhibition of α-glucosidase can slow down the glucose liberation from dietary carbohydrates and delay its absorption resulting in reduced postprandial plasma glucose levels, and suppression of postprandial hyperglycemia (Kumar et al., 2011). Numerous studies have reported on the antidiabetic properties of various plant extracts through inhibition of carbohydrate-hydrolysing enzymes, as evaluated in this study (Alam et al., 2019).

The inhibitory effect of Aloe extracts against α-glucosidase was determined using p-nitrophenyl-α-D-glucopyranoside (PNPG) as a colorimetric indicators. Results indicated that the Aloin A standard reference compound showed the highest inhibitory activity (IC_{50} 0.34 ± 0.05 mg/mL), and was higher than the positive control acarbose (IC_{50} 0.54 ± 0.14 mg/mL), with no significant differences ($P < 0.05$). APR extracts showed also high inhibitory activity with IC_{50} 0.67 ± 0.25 mg/mL, whereas, AVG was less active measuring at IC_{50} 1.09 ± 0.14 mg/mL (Table 4 and Fig. 4). Aloin has been reported as a potent inhibitor of α-glucosidase enzyme (Ghamari et al., 2013). Therefore, the α-glucosidase inhibitory activity APR and AVG can be referred to the presence of large concentrations of aloin and its glycosides in the extracts. Moreover, the *in vivo* hypoglycemic property of *A. perryi* was investigated

Table 3 Mass spectral and UV data of the characterized compounds from *Aloe perryi* Baker resin and *A. vera* gel extracts.

Peak #	Rt (min)	MS1 (*m*/*z*)	MS2 (*m*/*z*)	UV λ_{max} (nm)	Molecular formula	Compound identification	*A perryi* resin	*A vera gel*
1	2.152	411.1344 [M + H]+	261	293	$C_{19}H_{22}O_{10}$	Aloenin	—	+
2	2.357	395.1337 $[M + H]^+$	377, 233	245, 297	$C_{19}H_{22}O_9$	Aloesin	—	+
3	3.03	397.1524 $[M + H]^+$	287, 257, 233, 203	229, 252, 293	$C_{19}H_{24}O_9$	8-*C*-glucosyl-*R*-aloesol	—	+
4	3.48	409.1518 [M + H]+	247, 259	240, 293	$C_{20}H_{24}O_9$	7-*O*-methyl aloesin	—	+
5	8.96	449.1439 [M + H]+	431, 289, 269	228, 296, 336	$C_{22}H_{24}O_{10}$	8-*O*-methyl-7-hydroxyaloin	—	+
6	8.80	541.1752 [M + H]+	523, 421, 275, 233	237, 253, 299	$C_{28}H_{28}O_{11}$	Aloeresin A	—	+
7	10.07	507.1530 [M + H]+	458, 398, 351	218, 265, 302	$C_{24}H_{26}O_{12}$	Dihydroisocoumarin glucoside	+	+
8	10.63							
9	12.17	529.1340 [M + H]+	485, 367, 323	230, 268, 295	$C_{26}H_{24}O_{12}$	8-*C*-(2′-*O*-coumaroylglucosyl)-7-hydroxy-5-methyl-chromone-2-carboxylic acid	+	+
10	16.46	555.1861 [M + H]+	435, 417, 375, 289, 247	245, 299	$C_{29}H_{30}O_{11}$	7-*O*-methyl aloeresin A	+	+
11	18.23	557.2059 [M + H]+	395, 233	237, 269, 299	$C_{29}H_{32}O_{11}$	Isoaloeresin D	—	+
12	18.19, 19.78	419.1337 [M + H]+	401, 239, 211	268, 297, 354	$C_{21}H_{22}O_9$	Aloin B	+	+
13						Aloin A	+	+
14	19.50	433.1521 [M + H]+	271, 256	236, 245, 299	$C_{22}H_{24}O_9$	Homonataloin B	+	+
15	21.74	541.1707 [M + H]+	523, 421, 275, 233	252, 301	$C_{29}H_{32}O_{10}$	Aloeresin E	—	+
16	21.81	433.1521 [M + H]+	271, 261	236, 245, 299	$C_{22}H_{24}O_9$	Homonataloin A	—	+
17	23.44	565.1750 [M + H]+	419, 239, 211	268, 297, 357	$C_{27}H_{32}O_{13}$	Aloinoside B	+	+
18	26.35					Aloinoside A	+	+
19	34.48	565.1759 [M + H]+	419, 239, 211	228, 299	$C_{30}H_{28}O_{11}$	Microdontin B	+	+
20	34.68					Microdontin A	+	+

Table 4 IC_{50} values for Aloe extracts and aloin A inhibitory effects on α-glucosidase enzyme activity.

Sample	IC_{50} (μg/mL as final concentration)
A. perryi resin	0.67 ± 0.25^{abc}
A. vera gel	1.09 ± 0.14^{a}
Aloin A	0.34 ± 0.05^{bc}
Acarbose	0.54 ± 0.14^{bc}

Data represents the mean ± SEM ($n \geq 3$); means with different superscript letters (a, b, c) within the same column are significantly different ($P < 0.05$). Data were analyzed by one-way ANOVA with treatment as a factor. Post hoc analyses of differences between individual experimental groups were made using the Dunnett's multiple comparison tests.

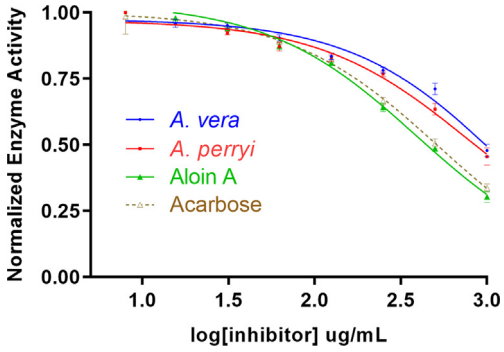


Fig. 4 Inhibitory activity of *Aloe perryi resin*, *A. vera* gel extracts, and aloin reference standard vs acarbose as a positive control. Samples were evaluated at final concentration range of 0.8–1000 μg/mL.

on diabetic albino rats through intraperitoneal injection of Aloe extract at 2 mg/Kg, which resulted in 22.5% reduction of the fasting glucose level after 54 h (Ibegbulem and Chikezie, 2013). It was also found that polysaccharides play a role in the antidiabetic activities of Aloe species by increasing the insulin level and hence showing hypoglycemic properties (Minjares-Fuentes and Femenia, 2019). Polysaccharides isolated from eight Aloe species growing in Egypt, including *A. vera* had been tested for their α-glucosidase inhibitory activity. Their results indicated that polysaccharides had moderate α-glucosidase inhibitory activity (El Sayed et al., 2016).

4. Conclusion

This is the first comprehensive phytochemical analysis, antioxidant and α-glucosidase inhibitory activity on *A. perryi* Baker growing wild in Socatra Island, Yemen. The resin (green rind exudate) was highly rich in phenolic content. HPLC-UV and LC-MS showed anthrones as the major phenolic constituent, represented by aloin B, homonataloin B aloinosides B & A, and microdontins B & A. *A. vera* gel is another commercial product that has been screened in this study. It is known that the gel is the sap produced from parynchyma cells in the most inner zone of the leaf. It is composed mainly of polysaccharies, therefore it was expected to have low values of phenolic content. The results obtained here indicated moderate levels of phenolics, moderate antioxidant and α-glucosidase activity. The HPLC-UV and LC-MS showed anthrones, and chromones as main components. The results of this study indicate the great potential of *A. perryi and A. vera* plants of the Yemen ethnomedicine for production of bioactive compounds, and these results will help in promoting them for use in food, nutraceutical and cosmetic preparations.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2020.02.003.

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