

Determination of total phenolic content and antioxidant activity of ethanol extracts from *Aloe* spp.

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Abstract: The ethanol extracts of leaf peel and gel of *Aloe* spp. were analyzed for their total phenolic and flavonoid profiles and screened for their antioxidant activity. The total phenolic content of the three different plant extracts and one commercial product of *Aloe vera* was determined by Folin-Ciocalteu method and flavonoid content was assessed by AlCl₃ method. Peel extract had the highest total phenolic content (7.99 mg gallic acid equivalents (GAE)/g extract) and flavonoid content (9.17 mg quercetin equivalents (QE)/g extract). The lowest content of phenolic and flavonoid compounds was observed in Soxhlet extract of *Aloe* gel. The *in vitro* antioxidant activity determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assays revealed that all extracts exhibited low antioxidant activity compared to quercetine and thymoquinone as standards. The best antioxidant activity had the peel extract what is in correlation with content of phenolic and flavonoid compounds.

INTRODUCTION

There are over 360 species in the genus *Aloe*. The *Aloe* genus has been used for thousands of years in the treatment of burns, wounds, skin irritations and constipation. *Aloe* has broad range of pharmacological properties, including antiinflammatory, antiviral, antioxidative actions, antibacterial, immunostimulant, antifungal, analgesic, antitumor, antidiabetic and inhibition of tumor cells activation and proliferation (Kammoun, Miladi, Ali, et.al, 2011; Nejat-zadeh-Barandozi, 2013, Ray, Gupta, Ghosh, 2013).

Aloes have been used therapeutically, certainly since Roman times and perhaps long before, different properties being ascribed to the inner, colourless, leaf gel and to the exudates from the outer layers (Reynolds and Dweck, 1999). The plant is made of turgid green leaves joined at the stem in a rosette pattern. Each leaf consists of two parts: an outer green rind (skin) and an inner clear pulp (gel). *Aloe* products have long been used in health foods and for medical and cosmetic purposes. These products range from aloe drink to aloe gels, powders, capsules, creams etc. for both internal and external uses for a wide variety of indications. *Aloe vera* L. (syn.: *Aloe*

barbadensis Miller) is a perennial succulent plant belonging to the Aloaceae family. *Aloe vera* is most widely accepted and used for various medical and cosmetic purposes (Miladi and Damak, 2008).

The different species of *Aloe* have different chemical compositions. In 1851, it was discovered that potency of *Aloe* was result of aloin, a bitter juice that dried to yellow powder (Shelton, 1991). Anthraquinones derivatives in *Aloe vera* gel play an important role in the treatment of tumors, diabetes, ulcer and cancer. Keeping this fact in view, the resent study was undertaken to isolate the phenolic anthroquinones from *Aloe vera* leaf gel (Rajendran, Narayanan, Gnanavel, 2007, Ravi, Kabilar, Velmurugan, et. al, 2011). It also has a high content of 1,8-dihydroxyanthraquinone derivatives (aloe emodin) and their glycosides (aloin), which are used as cathartic. Phytochemical analysis revealed the presence of alkaloid, carbohydrate, tannin, steroid, triterpenoid in *Aloe vera* extracts by HPTLC method (Patel, Patel, Dhanabal, 2012). Sugar analysis of the polysaccharides after gel permeation chromatography revealed that glucose and galactose were the most abundant monosaccharide in the

neutral polysaccharides from the *Aloe vera* gel juice and skin juice, respectively. The acidic polysaccharides from the two juices consisted of glucuronic acid, galactose, glucose, mannose, and xylose with variable proportions (Nejatzadeh-Barandozi and Enferadi, 2012).

The use of reversed phase high performance liquid chromatography (RP-HPLC) allowed the identification of 18 phenolic constituents. Leaf skin extracts were characterized by the abundance of catechin, sinapic acid and quercitrin. Gentisic acid, epicatechin and quercitrin were the most prominent phenolic compounds of the flowers, (López, de Tangil, Vega-Orellana, et. al, 2013).

Phenolic acids, *p*-coumaric, ferulic, homoprotocatechuic, β -phenyllactic, protocatechuic, caffeic, *p*-hydroxybenzoic, *p*-hydroxyphenylacetic, syringic, *o*-hydroxyphenylacetic, trans-cinnamic and vanillic acid were identified in leaves of several *Aloe* species (Nowak, Cybul, Starek, 2009).

Total phenolic content of the four different extracts of *Aloe vera* leaf skin were determined by Folin-Ciocalteu method (Miladi and Damak, 2008), and the highest content of total phenolic and best antioxidant activity were in chloroform-ethanol fraction.

There are some findings that indicate that *Aloe* gel is an effective radioprotective agent (Saini and Saini, 2011). It can be very useful in increasing the tolerance dose of radiation in cancer patients and in preventing the diarrhea which occurs during radiotherapy of pelvic and abdominal cancer as gastrointestinal tract represents one of the major dose limiting organs in radiotherapy. Many of the health benefits associated with *Aloe vera* have been attributed to the polysaccharides contained in the gel of the leaves (Hamman, 2008).

Effect of the ethanolic extract of *Aloe vera* gel on tissue antioxidants is due to reduction in blood glucose level in diabetic rats, which prevents excessive formation of free radicals through various biochemical pathways and also reduces the potential glycation of the enzymes (Rajasekaran, Sivagnanam, Subramanian, 2005).

Free radical scavenging activity of *Aloe vera* extracts get by supercritical carbon dioxide extraction (Hu, Q., Hu, Y., Xu, 2005) were compared to BHT and α -tocopherol as a positive probe, and the results showed that extract are stronger antioxidants.

There are a wide range of research from all over the world based upon different species of *Aloe* for antimicrobial activity (Taiwo, Olukunle, Ozor, et. al., 2005; Alemdar and Agaoglu, 2009; Lawrence, Tripathi, Jeyakumar, 2009; Khaing, 2011; Karpagam and Devaraj, 2011) and antioxidant activity (Beppu, Koike, Shimpō, et. al, 2003; Botes, Van der Westhuizen, Loots, 2008; Kumalainsih and Wijana, 2013).

Aloe vera products have long been used in health foods for medicinal and preservative purposes. Potential use of aloe products often involves some type of processing, e.g. heating, dehydration and grinding. Processing may cause irreversible modifications to the polysaccharides, affecting their original structure which may promote important changes in the proposed physiological and pharmaceutical properties of these constituents (Ramachandra and Rao, 2008). All the investigation found that commercial soap and commercial gel samples extremely increase aloin and phenolic compounds (Ravi, et. al, 2011).

The objective of our research work was to investigate the total phenolic and flavonoid content and the antioxidant properties of the ethanol extract of peel and gel from *Aloe* spp. by radical scavenging methods including, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS).

EXPERIMENTAL

All used reagents were of the highest purity grade available and purchased from the Sigma-Aldrich Chemical Company (Germany).

Plant material

The plant *Aloe* spp. was bought at flower market. The leaves were washed with distilled water and peel was separated from the gel.

Isolation of extracts by Soxhlet extraction using ethanol as solvent was done from peel (76.0 g) S-A.p and gel (44.4 g) S-A.g, while ultrasound extraction was used for extraction of gel (28.5 g) U-A.g. Beside these samples, one commercial product of *Aloe vera* was used to compare results with crude plant extracts.

Total phenolic content

Total phenolic content of the examined extracts was determined by a slight modification of the method by Singleton and Rossi 1965. A 100 μ L of sample solution, prepared in ethanol, in various concentrations was diluted with 5 mL of distilled water was mixed with 500 μ L of Folin-Ciocalteu reagent, previously diluted two-fold.. After 10 minutes, 1.5 mL of 20% solution of sodium carbonate was added, and the solution obtained was diluted to 10 mL. Prepared samples were kept for 2 h at room temperature, and the absorbance was measured at 765 nm. The data were calculated according to a standard curve of gallic acid (0.5–10 μ g/mL), and they were expressed as gallic acid equivalents (GAE) per gram of extracts.

Total flavonoid content

Total flavonoids in the plant extracts examined were determined by using a slight modification of the method given by Meda, Lamien, Romito, et. al, 2005. The principle of method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminium chloride forms acid labile complexes with the *ortho*-dihydroxyl groups in the A- or B-ring of flavonoids. A 0.5 mL of diluted extract solution was mixed with 0.5 mL of aluminium chloride (2%). After incubation at room temperature for 20 min, the absorbance of the reaction mixture was measured at 415 nm. A blank sample contained 0.5 mL of sample and 0.5 mL of distilled water. A 0.5 mL sample of aluminium chloride mixed with 0.5 mL of distilled water was used to zero the spectrophotometer. The data were calculated according to a standard curve of quercetin (3–20 μ g/mL), and they were expressed as quercetin equivalents (QE) per gram of extracts.

1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity (DPPH)

The ability of the extract components to donate hydrogen atom or electron and scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by the slightly modified method of Brand-Williams, Cuvelier, Berset, (1995). A portion of sample solution (100 μ L) was mixed with 1.0 mL of 5.25×10^{-5} mol/L DPPH• in ethanol. Decreasing of absorbance of tested mixtures was monitored every 1 min for 30 min at 517 nm using Perkin-Elmer Lambda 25 UV/Vis spectrophotometer. Ethanol was used as blank, and quercetine and thymoquinone were used as a positive probe. The DPPH• solution was freshly prepared daily, stored in a flask covered with aluminum foil, and kept in the dark at 4°C before measurements. The radical-scavenging activity of the tested samples, expressed as percentage inhibition of DPPH, was calculated according to the formula

$$(\%)AA = [(A_0 - A_t)/A_0] \times 100$$

where A_t is the absorbance value of the tested sample and A_0 is the absorbance value of DPPH, in particular time. Percent inhibition after 30 min was plotted against concentration, and the equation for the line was used to obtain the IC_{50} value. A lower IC_{50} value indicates greater antioxidant activity.

2,2-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical-scavenging activity (ABTS)

The ABTS method is based on the reduction of the green ABTS radical cation (7.00 mmol/L) that was obtained by its oxidation with equal volume of potassium persulphate (2.45 mmol/L), (Katalinic, Milos, Kulisic, et. al, 2006) for 12–16 h at 4°C in the dark. On the day of analysis, the $ABTS^{+}$ solution was diluted with methanol to absorbance of 1.00 (± 0.02) at 734 nm. After the addition of 100 μ L of sample solution to 1.0 mL of $ABTS^{+}$ solution, decrease of absorbance was monitored every 1 min for 10 min at 734 nm using a Perkin-Elmer Lambda 25 UV/Vis spectrophotometer. Methanol was used as a blank, and quercetine and thymoquinone were used as positive probe. The radical-scavenging activity of the tested samples, expressed as percentage inhibition of $ABTS^{+}$, were calculated according to the formula

$$(\%)AA = [(A_0 - A_t)/A_0] \times 100$$

where A_0 and A_t are the absorbance values of the ABTS and the test sample, at particular times, respectively. Percent inhibition after 10 min was plotted against concentration, and the equation for the line was used to obtain the IC_{50} value. A lower IC_{50} value indicates greater antioxidant activity.

RESULTS AND DISCUSSION

The yield of gel extracts were different for Soxhlet and ultrasound extraction and were 5.2% and 3.5%, respectively. Soxhlet extract of peel gave a yield of 2.4%.

Total phenolics

The total phenolic content was measured by Folin-Ciocalteu assay and expressed as mg gallic acid equivalents per gram of extract.

Table 1: Total phenolic, and total flavonoid content

Sample	Yield (%)	Total phenolic mg(GAE)/g	Total flavonoid mg(QE)/g
U-g	3.5	2.80 \pm 0.07	3.37 \pm 0.20
S-g	5.2	2.06 \pm 0.25	0.29 \pm 0.03
S-p	2.4	7.99 \pm 0.26	9.17 \pm 0.19
Av-prod	-	0.11 \pm 0.01	0.005 \pm 0.0003

U-g – Ultrasound extract of gel, S-g – Soxhlet extract of gel, S-p – Soxhlet extract of peel

Peel extract had the highest total phenolic content (7.99 \pm 0.26 mg(GA)/g), while gel extracts had almost three times lower content for Soxhlet and four times lower for ultrasound extract (Table 1). From these results we can conclude that method of extraction had very important role, because during Soxhlet extraction degradation of thermosensitive compounds could occur. Previous results (Miladi and Damak, 2008, Kammoun, et. al, 2011) showed that content of phenolics is low in water extract (2mg(GAE)/g), while in chloroform-ethanol extract content of phenolic compounds was about 40 mg(GAE)/g.

Total flavonoids

Determination of total flavonoids is related to the formation of complex between flavonoid and $AlCl_3$ that produces a yellow colored complex. The absorbance is measured spectrophotometrically at maximum wavelength of 415 nm.

The absorbance of series of solutions with different concentration of quercetine were plotted against the yield to give a linear calibration curve of quercetine with coefficient of correlation value of 0.9984. Flavonoid content (Table 1) was the highest in peel extract (9.17 \pm 0.19 mg(QE)/g), while the lowest was in gel extract isolated by Soxhlet extraction (0.29 \pm 0.03 mg(QE)/g).

In comparison with plant extract, commercial product of *Aloe vera* had significantly lower content of total phenolic and flavonoid compounds, 0.11 \pm 0.01, and 0.005 \pm 0.0003.

The results published by Hu, Y., Xu, Hu, Q., 2003, showed that three-year-old *Aloe vera* plant contained significantly higher levels of polysaccharides and flavonoids than two- and four-year-old *Aloe vera*, and no significant differences in flavonoid levels were found between three- and four-year-old *Aloe vera*.

Antioxidant activity

Antioxidant activity of isolated extract was determined by DPPH and ABTS methods.

The DPPH free radical scavenging activity of the *Aloe* extract was evaluated using an ethanol solution of the stable free radical, DPPH. A freshly prepared DPPH solution exhibits a deep purple color with a maximum absorption at 517 nm. This purple color disappears when

an antioxidant is present in the medium. Therefore, antioxidants molecules can quench DPPH free radicals and convert them to a yellow product, resulting in a decrease in absorbance at 517 nm. Free radical scavenging activity was proportional to the concentration of the extract. Concentration of sample at which the inhibition percentage reaches 50% is its IC₅₀ value. IC₅₀ value is negatively related to the antioxidant activity, as it expresses the amount of antioxidant needed to decrease its radical concentration by 50%. The lower IC₅₀ value, the higher is the antioxidant activity of the tested sample. Most methods used for evaluation of radical trapping properties often utilize stable model free radicals as indicators for radical scavenging abilities, among which DPPH is recommended as easy and accurate with regard to measuring the antioxidant activity of fruit and vegetable juices or extracts (Katalinić, Milos, Modun, et. al, 2004).

Reduction power of all extracts showed that sample prepared from peel had stronger antioxidant activity than extracts of gel. Gel extracts reduced the concentration of DPPH free radical, with efficiency significantly lower than quercetin and thymoquinone, well known as good antioxidants.

The 50% inhibition of DPPH radical obtained for ethanol extracts of peel yield 45.6±5.8mg/mL. Gel extract had significantly higher concentration for 50% inhibition, and their values were 80.2±4.2 mg/mL for ultrasound extract and 558.9±55.2 mg/mL for Soxhlet extract.

The reducing ability of *Aloe* extracts on DPPH radical was determined by López *et al.*, 2013, where extract of leaf skin was more active than the flower extract. There are some data which suggest that growth stage plays a very important role in the composition and antioxidant activity of *Aloe vera* (Hu, et. al, 2003). Etanolic and methanolic *Aloe vera* gel extracts possess maximum DPPH free radical scavenging activities (Khaing, 2011). Among three isolated samples, the best antioxidant activity for ABTS method had extract of peel (10.4±0.5 mg/mL), while Soxhlet extract of gel (55.4±3.3 mg/mL) had the lowest antioxidant activity.

Commercial product is used in a form which one can find at the market. The best result for %AA of this sample was 20% for ABTS method and less than 10% for DPPH method.

Aloe peel is a part from aloe plant which has the best ability as antioxidant agent.

Table 2: Antioxidant activity of isolated extracts

Compds	ABTS	DPPH
	IC ₅₀ (mg/mL)	IC ₅₀ (mg/mL)
U-g	24.1±0.7	80.2±4.2
S-g	55.4±3.3	558.9±55.2
S-p	10.4±0.5	45.6±5.8

U-g – Ultrasound extract of gel, S-g – Soxhlet extract of gel, S-p – Soxhlet extract of peel

These results are in agreement with literature data (Ozsoy, Candoken, Akev, 2009) for antioxidant activity of aqueous extract from *Aloe vera* were IC₅₀ values are significantly higher than those for ascorbic acid and α -tocopherol.

CONCLUSIONS

The antioxidant activity of isolated extracts is in correlation with the content of their phenolic compounds. The best antioxidative properties have Soxhlet extract of peel, and this sample have the highest content of phenolic and flavonoid compounds. This suggested that scavenging effect of *Aloe* extract may depend on hydrogen atom donation by the different phenolic and flavonoid compounds, and their hydrogen donor capacity, most probably accounts in large part for the antioxidant activity and may provide a basis for the pharmacological activity and therapeutic applications of this extract (Ozsoy, et. al, 2009). The activity of the extracts is not only dependent on the concentration of the phenolic compounds but also on the structure and nature of the compounds. Comparing results for antioxidant activity of samples and standards, it can be concluded that *Aloe* extracts obtained in this study could not be classified as good antioxidants.

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Summary/Sažetak

Etanolni ekstrakti kore lista i gela *Aloe* spp. su analizirani kako bi se odredio ukupan sadržaj fenola i flavonoida kao i procijenila njihova antioksidacijska aktivnost. Sadržaj ukupnih fenola u tri različita ekstrakta i jednom komercijalnom proizvodu *Aloe vera*, su određeni Folin-Ciocalteu metodom, dok je sadržaj flavonoida određen metodom sa $AlCl_3$. Ekstrakt kore je imao najveći sadržaj fenola (7.99 mg ekvivalenata galne kiseline (GAE)/g ekstrakta) kao i sadržaj flavonoida 9.17 mg ekvivalenata kvercetina (QE)/g ekstrakta. Najniži sadržaj fenola i flavonoida je određen u Soxhlet ekstraktu *Aloe* gela. Antioksidacijska aktivnost je određena *in vitro* 1,1-difenil-2-pikrilhidrazil (DPPH) metodom i 2,2'-azino-bis(3-etilbenzotiazolin-6-sulfonska kiselina (ABTS) metodom. Svi ekstrakti su pokazali nisku antioksidacijsku aktivnost u poređenju sa kvercetinom i timokinonom kao standardima. Najbolju antioksidacijsku aktivnost ima ekstrakt kore lista što je u korelaciji sa sadržajem fenola i flavonoida.