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MACERATION AND HEAT-ASSISTED EXTRACTION OF POLYPHENOLS FROM *ALOE VERA*

Natalija Čutović[1], Aleksandra A. Jovanović[2], Muna Rajab Elferjane[3], Violeta Milutinović[4], Predrag Petrović[5], Aleksandar Marinković[6], Branko Bugarski[6]

[1]*Institute for Medicinal Plants Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia*
[2]*Univeristy of Belgrade, Institute for the Application of the Nuclear Energy INEP, Banatska 31b, 11080 Zemun, Belgrade, Serbia*
[3]*University of Misurata, Faculty of Nursing and Health Sciences, Misurata, Libya*
[4]*University of Belgrade, Faculty of Pharmacy, Vojvode Stepe 450, 11000 Belgrade, Serbia*
[5]*University of Belgrade, Innovation centre of the Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia*
[6]*University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia*

Abstract

Aloe vera (L.) Burm. f. (Asphodelaceae) contains anthraquinones, their glycosides, flavonoids, tannins, terpenoids, saponins, resins, mono- and polysaccharides, polypeptides, lectins, enzymes, vitamins, and minerals. The plant is used in traditional medicine due to its antitumor, antioxidant, anti-inflammatory, antiulcerogenic, immunomodulatory, analgesic, and dermal protection properties. Maceration and heat-assisted extraction (HAE) are frequently used extraction procedures because of their simple operation and low costs. In the present study, A. vera dried leaves were extracted using ethanol (50%, V/V; drug solvent ratio 1:30, w/V). The influence of different extraction times (30-120 min for maceration and 15-60 min for HAE), and various extraction temperatures (40, 60, and 80ºC) in HAE on the total polyphenol content (TPC) and radical scavenging potential (ABTS and DPPH methods) was evaluated, while for the most prominent extracts (with the highest TPC) zeta potential and conductivity were additionally analyzed. Regarding maceration, the TPC was correlated with the rise in extraction time up to 45 min (from 7.8±0.72 to 9.66±0.51 mg gallic acid equivalents (GAE)/g of plant material) and after that reached the steady state (~9.1 mg GAE/g). In HAE, no statistically significant differences between the TPC of the extracts obtained after 15, 30, and 45 min (9.77±0.32, 9.76±0.18, and 9.95±0.50 mg GAE/g) were observed, while the extract prepared after 60 min showed significantly lower TPC (8.14±0.08 mg GAE/g). The TPC was in correlation with the increase of extraction temperatures (9.08±0.21 at 40ºC, 9.38±0.10 at 60ºC, and 9.86±0.24 mg GAE/g at 80ºC). As in the case of TPC results, anti-ABTS activity was significantly different between 30 and 45 min of maceration and reached the steady state after 45 min (~2.01 mmol Trolox/g of plant material). In HAE, the anti-ABTS potential of the extracts obtained at different extraction times was comparable (1.44-1.64 mmol Trolox/g), whereas the activity of the extract obtained at 40ºC was lower. DPPH radical scavenging activity rose after 45 min of maceration when also reached the steady state (~40.4 mg/mL), but there were no significant differences between the extracts prepared at different extraction times in HAE, and IC_{50} was lower for the extract obtained at 80ºC. Hence, the macerate prepared after 45 min and HAE extract obtained after 15 min at 80ºC exhibited very low zeta potential (0.14±0.06 and 0.50±0.01 mV), and conductivity (1.05±0.07 and 0.98±0.01 mS/cm). This study was an initial step in the production

of A. vera polyphenol extracts aimed to be used for the formulation of foodstuffs, medicines, and cosmetics.

Key words: *Aloe vera, antiradical activity, conductivity, polyphenols, zeta potential.*

Introduction

Aloe vera L. (Liliaceae) contains anthraglycosides, aloesin and its aglycone aloesone, free anthraquinones, resins, mono- and polysaccharides, polypeptides, flavonoids, terpenoids, tannins, sterols, organic acids, enzymes, saponins, vitamins, and minerals (Hęś et al., 2019; Khan and Abourashed, 2011). Aloe extracts have shown antitumor, antimicrobial, antiviral, anti-inflammatory, antioxidant, hypocholesterolemic, hypoglycemic, antiulcerogenic, immunomodulatory, analgesic, dermal protection, wound healing, burn healing, and frostbite healing activities (Hęś et al., 2019; Khan and Abourashed, 2011; Lee et al., 2000; Pugh et al., 2001). Carboxypeptidase, a serine carboxypeptidase enzyme, is a primary antithermic agent that showed analgesic activity and dermoprotectant activity against burns (Khan and Abourashed, 2011). Bioactive compounds can be effectively extracted from different plant materials using traditional extraction techniques, such as maceration and heat-assisted extraction. The mentioned extraction procedures are frequently used extraction procedures due to simple operation and low costs (Jovanović et al., 2017).

In the present study, *A. vera* extracts were prepared using dried leaves and two extraction procedures (maceration and heat-assisted extraction) *via* varying extraction times and temperatures. The prepared extracts were examined in terms of total polyphenol content (TPC) and antioxidant capacity (ABTS and DPPH methods), while for the most prominent extracts (with the highest TPC), zeta potential and conductivity were additionally analyzed.

Materials and Methods

Plant material and reagents

A. vera was purchased at ASC Garden d.o.o., Belgrade, Serbia. The following reagents were used: Folin-Ciocalteu reagent and gallic acid (Merck, Germany), ethanol (Fisher Scientific, UK), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) - ABTS, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid - Trolox, and 2,2-diphenyl-1-picrylhydrazyl - DPPH (Sigma-Aldrich, USA), and ultrapure water.

Extraction

The extracts were prepared using maceration (25ºC, 30-120 min) and heat-assisted extraction (40, 60, or 80ºC, 15-60 min for HAE) in the incubator shaker at 200 rpm, while the solid-to-solvent ratio was 1:30 g/mL and extraction medium was 50% ethanol. The flasks were covered with aluminium foil to avoid light exposure and ethanol evaporation.

Measurement of total polyphenol content

The TPC was evaluated spectrophotometrically using the modified Folin-Ciocalteu method (Galván d'Alessandro et al., 2012). The results are expressed as milligrams of gallic acid equivalents per gram of plant material (mg GAE/g).

Measurement of antioxidant activity (ABTS and DPPH assays)

The ABTS assay was based on the procedure described by Re et al. (1999) with a slight modification and the absorbance was measured at 734 nm. The antioxidant activity was expressed as mmol Trolox equivalent per g of plant material (mmol TE/g).

The DPPH assay was based on the procedure described by Horžić et al. (2009) with a slight modification and the absorbance was measured at 517 nm. The results were expressed as IC_{50} (mg/mL), defined as the concentration of the extract required to scavenge 50% of DPPH free radicals.

All spectrophotometric measurements were performed in a UV-1800 spectrophotometer (Shimadzu, Japan).

Measurement of zeta potential and conductivity

The measurements of zeta potential and conductivity of the selected extracts (samples with the highest TPC) were performed using photon correlation spectroscopy in Zetasizer Nano Series, Nano ZS (Malvern Instruments Ltd., UK). Each extract was measured three times at room temperature.

Statistical analysis

The statistical analysis was done by using analysis of variance (one-way ANOVA) and Duncan's *post hoc* test in STATISTICA 7.0. The differences were considered statistically significant at $p<0.05$.

Results and Discussion

The influence of different temperatures and times using two extraction procedures (maceration and HAE) on TPC and antioxidant capacity of ethanol *A. vera* extracts was examined; the results are shown in Figure 1 (for TPC) and Figures 2 and 3 (ABTS and DPPH antioxidant activity, respectively). Additionally, values of zeta potential and conductivity of the selected extracts (samples with the highest TPC) are presented in Table 1.

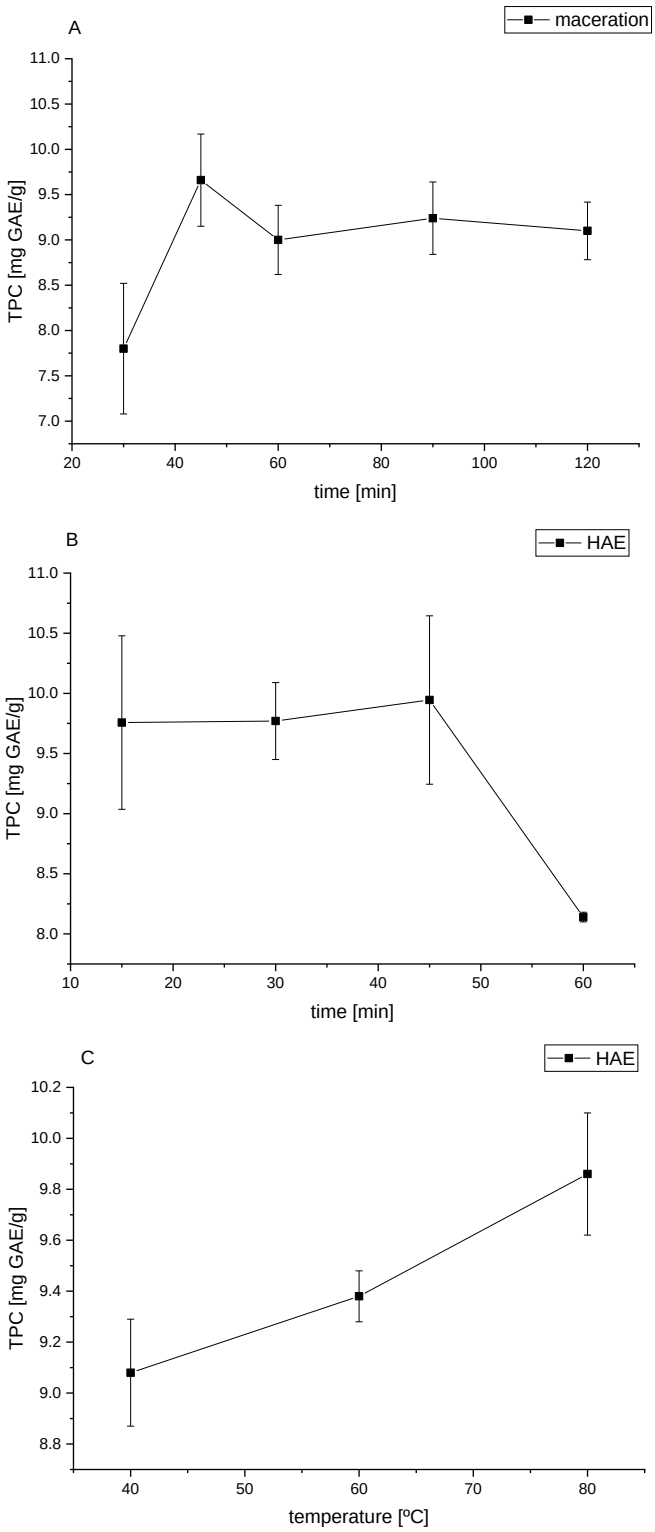

Figure 1. Total polyphenol content (TPC) of ethanol Aloe vera extracts prepared using maceration (A, influence of time) and heat-assisted extraction, HAE (B, influence of time and C, influence of temperature); gallic acid equivalent, GAE.

As can be seen in Figure 1A, there was a statistically significant difference between the TPC of the extracts prepared at different extraction times in maceration. Namely, the TPC rose for 45 min (from 7.8±0.72 to 9.66±0.51 mg GAE/g) and then reached a steady state (~9.1 mg GAE/g). According to Laib et al. (2019) study, maceration time had a significant effect on the TPC of *A. vera* extracts. In HAE, no statistically significant differences were observed in the TPC of the extracts obtained after 15, 30, and 45 min (9.77±0.32, 9.76±0.18, and 9.95±0.50 mg

GAE/g), while the extract prepared after 60 min showed significantly lower TPC (8.14±0.08 mg GAE/g, Figure 1B). The absence of a significant impact of extraction time on the polyphenol concentration of the extracts obtained in HAE can be explained by the occurrence of two stages in the recovery of polyphenols: an initial increase at the beginning (for 15 min) and slow extraction (60 min, Ćujić et al., 2016). Furthermore, according to the literature data, extraction time after 15 min at high temperatures did not have a significant and positive influence on polyphenol content (Fecka and Turek, 2008; Vergara-Salinas et al., 2012). Additionally, the TPC increased with the increase of extraction temperature (9.08±0.21 at 40ºC, 9.38±0.10 at 60ºC, and 9.86±0.24 mg GAE/g at 80ºC, Table 1C), which is in agreement with the literature data (Miron et al., 2011).

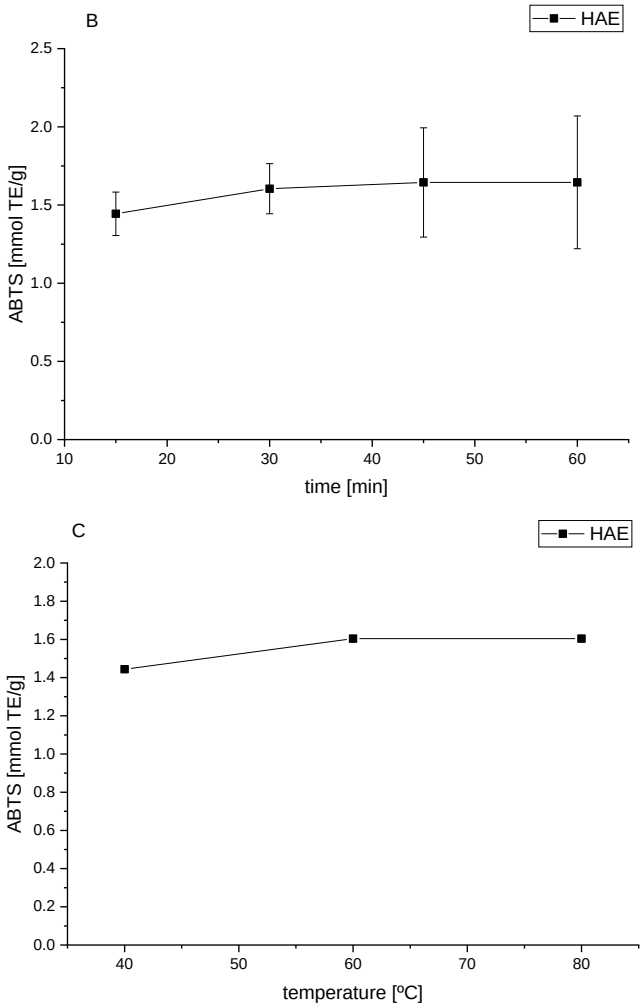

Figure 2. ABTS radical scavenging activity of ethanol Aloe vera extracts prepared using maceration (A, influence of time) and heat-assisted extraction, HAE (B, influence of time and C, influence of temperature); Trolox equivalent, TE.

As in the case of TPC results, ABTS radical scavenging activity was significantly different between 30 and 45 min of maceration and reached the steady state after 45 min (~2.01 mmol Trolox/g, Figure 2A). In HAE, the anti-ABTS potential of the extracts obtained at different extraction times was comparable (1.44-1.64 mmol Trolox/g, Figure 2B) and in correlation with TPC values. The ABTS radical scavenging potential of the extract obtained at 40ºC was significantly lower in comparison to other used temperatures (Figure 2C).

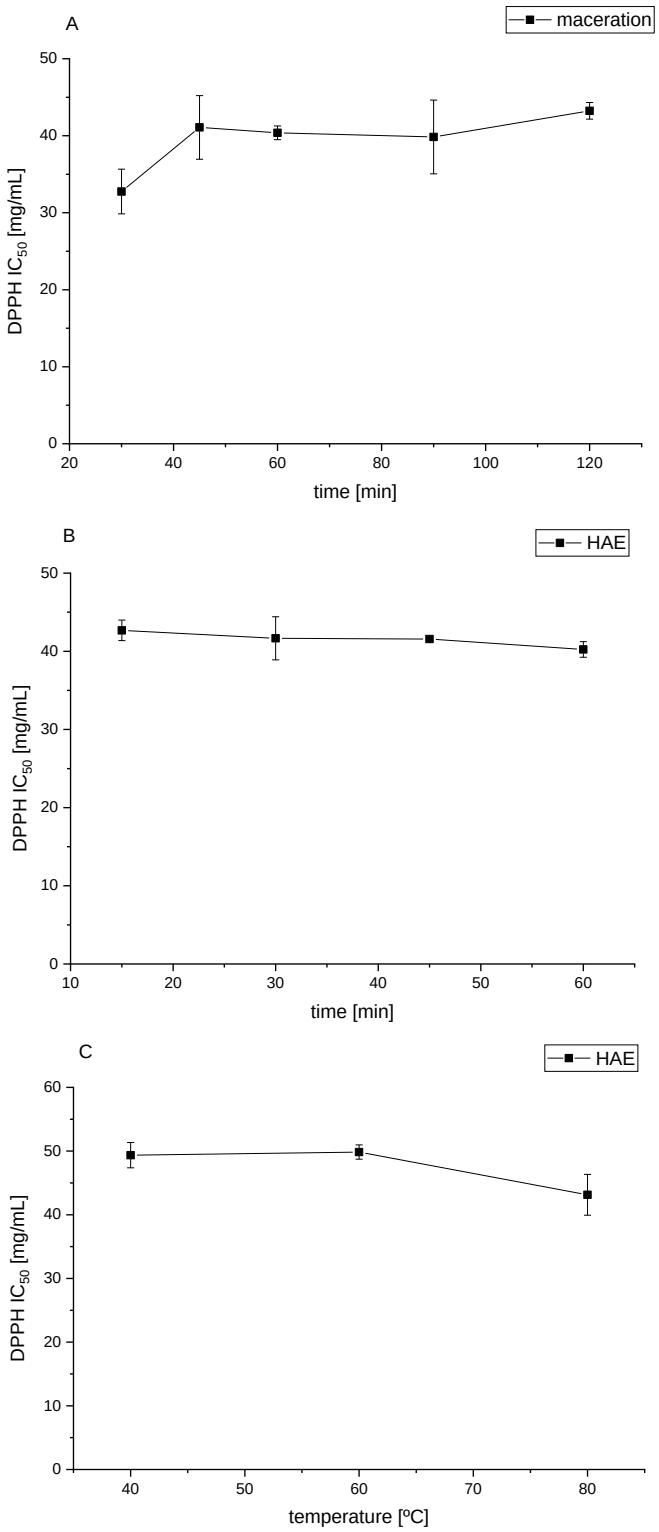

Figure 3. DPPH radical scavenging activity of ethanol Aloe vera extracts prepared using maceration (A, influence of time) and heat-assisted extraction, HAE (B, influence of time and C, influence of temperature; IC_{50}, the concentration of the sample requires to scavenge 50% of free DPPH radicals.

The DPPH radical scavenging capacity of the extract rose after 45 min of maceration when it also reached the steady state (~40.4 mg/mL, Figure 3A), as in the case of TPC values. On the other hand, no significant differences were observed between the extracts prepared at different extraction times in HAE (Figure 3B, the same trend as in the case of polyphenol concentration). The value of IC_{50} was significantly lower (higher antioxidant capacity) for the extract prepared at 80ºC (Figure 3C). Hirano et al. (2001) have reported that the neutralization of

DPPH radicals was correlated with the flavonoid content.

Table 1. Zeta potential (ζ) and conductivity (G) of ethanol Aloe vera extracts prepared using maceration and heat-assisted extraction (HAE).

variables	maceration, 45 min	HAE, 80ºC, 15 min
ζ [mV]	0.14±0.06[b]	0.50±0.01[a]
G [mS/cm]	1.05±0.07[a]	0.98±0.01[a]

**Values with different letters (a-b) in each row showed statistically significant differences ($p<0.05$; $n=3$; analysis of variance, Duncan's post-hoc test.*

As can be seen from Table 1, the extract prepared after 45 min of maceration and the HAE extract obtained after 15 min at 80ºC exhibited very low zeta potentials (0.14±0.06 and 0.50±0.01 mV, respectively). Measurement of the zeta potential of plant extracts is important from the aspect of their future implementation, such as encapsulation and application in coagulation and flocculation in the treatment of drinking water or wastewater. The zeta potential of plant extracts depends on extraction conditions and used solvents and varies between 2 mV and 15 mV (Skaf et al., 2021). However, the zeta potential of selected *A. vera* extracts was significantly lower, probably due to a lower amount of proteins in the mentioned extracts (data not shown).

The conductivity (as a predictor of the antioxidant potential of the herbal extract) of the selected *A. vera* extracts was also very low and amounted to 1.05±0.07 mS/cm for the macerate and 0.98±0.01 mS/cm for the extract obtained in HAE. Although the extracts with a higher conductivity have a higher antioxidant capacity (Jurinjak Tušek et al., 2018), the use of antioxidant tests is necessary in the case of *A. vera* extracts because ions from the samples can influence the conductivity but not change their antioxidant activity.

Conclusions

The aim of the study was the development, physicochemical characterization, and investigation of the antioxidant capacity of *A. vera* extracts. The highest TPC was achieved after 45 min of maceration, whereas in HAE, there was no statistically significant difference between various extraction times. On the other hand, the highest TPC was in the extract prepared at 80ºC. ABTS and DPPH radical scavenging activities followed the trend of TPC values. The selected *A. vera* extracts exhibited very low zeta potential and conductivity. This study was an initial step in the production of *A. vera* polyphenol extracts aimed to be used for the formulation of food, functional food, pharmaceutical, and cosmetic products.

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Conflict of interest

The authors declare that they have no financial and commercial conflicts of interest.

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