sciendo

ASN, Vol. 7, No 3, Pages 11–25, 2020

Acta Scientifica Naturalis

Former Annual of Konstantin Preslavsky University of Shumen: Chemistry, Physics, Biology, Geography

Journal homepage: asn.shu.bg

Effect of *in vitro* gastrointestinal digestion on phenolic compounds and the antioxidant activity of *Aloe vera*

Imen Laib [1,2]*, Farida Kehal [2], Nour Elyakine Haddad [2], Taous Boudjemia [2], Malika Barkat [2]

[1] *Department of Natural and Life Sciences, Faculty of Sciences, University August 20, 1955, SKIKDA, Algeria*

[2] *BIOQUAL Laboratory, Institute of Nutrition, Food and Agri-Food Technologies (I.N.A.T.A.A.), University of Frères Mentouri-Constantine 1, Route de Ain El-Bey, 25000 Constantine, Algeria*

Abstract: *The aim of this work is to study the effect of digestion on the total polyphenol content, flavonoids and the antioxidant activity of Aloe vera. Total polyphenol contents and flavonoid spectrophotometric methods: The evaluation of the antioxidant activity was carried out by three methods, DPPH, ABTS and CUPRAC. To confirm the results obtained we carried out an analysis by ATR-FTIR. The total phenol content found in the Aloe vera extract studied was 1.3638 mg EAG/100 g, while the content of flavonoids found in the Aloe vera extract studied was 0.690 mg EQ/100 g. The values of total polyphenols and flavonoids decreased under the effect of gastrointestinal digestion. The spectra obtained during the ATR-FTIR analysis show that Aloe vera is rich in phenolic compounds and flavonoids. Intense bands corresponding to O–H bonds, C=C bond, C–H, CO, CH_3 and CH_2 confirm the presence of these bioactive compounds. For both the DPPH and CUPRAC methods, Aloe vera extract reveals a strong antioxidant activity, which gradually decreases during the oral and gastric phase and then increases after the intestinal digestion. For the ABTS method, the antioxidant activity decreases during the oral phase, increases during the gastric phase and then decreases again during the intestinal phase.*

Keywords: polyphenols; flavonoids; *in vitro* digestion; *Aloe vera*; antioxidant activity

Corresponding author: mina.laib@gmail.com Full Paper

DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

Introduction

The importance of naturally occurring antioxidants has grown steadily in recent times. These antioxidants, as constituents of food, seem to contribute significantly to the prevention of certain diseases such as cancer, cardiovascular diseases and infectious diseases [1]. The mechanisms of action of antioxidant activity are diverse. They involve the neutralization of free radicals, the complexity of ions, transition metals and the reduction of oxidative phenomena and cellular aging [2]. The phenolic compounds of medicinal plants are counted among these natural active ingredients. Over the last ten years, they have been attracting increasing interest from nutritionists, agri-food manufacturers and consumers. One of the main reasons is the recognition of their antioxidant properties.

Aloe vera is one of the most used medicinal and food plants since ancient times for its therapeutic properties [3, 4]. In addition, *Aloe vera* extracts contain considerable amounts of polyphenols and flavonoids and have an antioxidant capacity comparable to that of the synthetic antioxidant butylated hydroxytoluene (BHT) and vitamin E [5, 6]. To obtain a beneficial effect of this plant on health, these polyphenols must be stable during digestion to reach the blood circulation through the intestinal wall [7]. Polyphenols are ingested as complex mixtures immersed in a food matrix. Therefore, it is important to determine how this digestion process affects polyphenols and their stability as this, in turn, affects their bioaccessibility to uptake as well as their possible beneficial effects on the cells lining the intestine [8].

The impact of gastrointestinal digestion *in vitro* on the stability of polyphenolic compounds, and on their antioxidant properties, has been one of the most widely discussed topics in the last decade. This is the case with a wide variety of fruits, including citrus fruits [9, 10], different types of berries [11, 12], tomato [13], grape berry [9], apple [14] and figs [15] as well as phenolic extracts from carob pulp [16].

However, few studies have addressed the effect of *in vitro* digestion on polyphenols in *Aloe vera*. In this context, the objective of this study is to evaluate the effect of gastrointestinal digestion *in vitro* on the phenolic content and antioxidant potential of *Aloe vera.*

Materials and methods

The plant used in the present study was *Aloe vera* is harvested in the garden of Institute of Nutrition, Food and Agri-Food Technologies (INATAA), Constantine University, in February 2019. The leaves of the plant were washed, cut and lyophilized.

Polyphenol extraction

In this work, a composite central experiment design (CCD) was used to determine the effect of solvent concentration ($X1$) and maceration time ($X2$) on total polyphenol content (Y) of *Aloe vera*. CCD requires three

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

types of trials, i.e. four factorial trials, four axial trials and five center point trials, giving a total of 13 trials. A second order polynomial equation reflects the dependence of the studied answer *Y* according to the two variables coded (*X*1 and *X*2). This model takes into account linear effects, quadratic effects and interactions between the studied factors, and is written:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1X_1 + b_{22}X_2X_2 + b_{21}X_1X_2,$$

Where:

Y is response studied;

X_1, X_2 are coded forms of the explanatory variables;

b_0 is the constant that expresses the general average effect;

b_1, b_2 are linear coefficients (the main effect of each variable);

b_{11}, b_{22} are quadratic coefficients and b_{12} is the interaction coefficient (the interaction between the two variables).

For each coded variable (Xi) five levels, −α, −1, 0 and +1, + α were assigned. −α and +α represent the extreme values (minimum, maximum). For the plane to be rotatable, the coded value of α was set at 1.414 [17, 18, 19]. The choice of actual levels of maceration time and solvent concentration is based on preliminary tests. The polyphenols were extracted by the method described by Elfalleh et al. [20] with some modifications. The plant was previously ground; a 100 g sample was macerated with 500 ml of methanol. Then, the samples were centrifuged at 4500 rpm for 15 min. The extracted supernatants obtained were filtered, the *Aloe vera* plant was extracted three times and the combined extracts were used for the experiments. Then, the solvent was evaporated by a rotary evaporator. Finally, the extracts were lyophilized.

***In vitro* gastrointestinal digestion**

In vitro gastrointestinal digestion of *Aloe vera* was performed according to the method described by Ydjedd et al. [16] with some modifications. It consists of a three-step procedure that simulates digestion in the mouth (oral phase), stomach (gastric phase) and small intestine (intestinal phase).Digestion was performed on the powder of the *Aloe vera* plant. The liquids that make up the different phases of digestion used in this study are presented in Table 1.

Table 1. Composition of three digestive liquids (oral, gastric and intestinal) [16]

Solution	Salivary fluid(ml)	gastric fluid (ml)	Intestinal fluid (ml)
KCL (0.5 M)	7.55	3.45	3.4
KH_2PO_4 (0.5 M)	1.85	0.45	0.4
$NaHCO_3$ (1M)	3.4	6.25	21.25
NaCl (2M)	-	5.9	4.8

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

$MgCl_2(H_2O)_6$ (0.15M)	0.25	0.2	0.55
$NH_4(CO_3)_2$	0.03	0.25	-
HCl (6M)	0.045	0.65	0.7

Digestion was simulated by the introduction of 20 ml of salivary fluid (LS) containing 15 mg of α-amylase and 75 µl of $CaCl_2(H_2O)_6$ (0.3 M) to 1 g of sample (powder of *Aloe vera*. The mixture (oral phase) at pH 7 was incubated with stirring for 2 min at 37°C and then centrifuged with a Sigma centrifuge at 4500 rpm for 30 minutes. Next, 10 ml of the oral phase (PO) was taken, 10 ml of gastric fluid (LG) composed of 40 mg of pepsin and 5 µl of $CaCl_2(H_2O)_6$ (0.3 M) was added to the oral phase, the pH was adjusted to 3 with HCl (6 M) and the mixture (gastric phase) was incubated at 37°C in a shaking water bath for 2h. Then, the PG was centrifuged for 30 minutes at 4500 rpm. Ten ml of the PG gastric phase was taken for further analysis. To simulate intestinal digestion, 10 ml of intestinal fluid (LI) with 37.5 mg of pancreatin and 40 mg of bile salts and 20 ml $CaCl_2(H_2O)_6$ (0.3 M) were added to the gastric phase, the pH was adjusted to 7 with NaOH (1 M) and the mixture (intestinal phase) was incubated with stirring for 2 h. The intestinal phase (PI) was centrifuged for 30 minutes at 4500 rpm. Supernatants obtained after centrifugation were collected, lyophilized and stored until further use.

Determination of phenolic compounds

The determination of total polyphenols was carried out according to the method of Folin–Ciocalteu (FC) described by Waterhouse [21] with some modifications. Two ml of *Aloe vera* extract was mixed with 0.4 ml of FC reagent and 1.2 ml of 2% (w/v) Na2CO3. The mixture was stirred and incubated in the dark and at room temperature for two hours, and the absorbance reading was measured at 760 nm. The results are expressed in mg of gallic acid per 100 grams of dry matter by reference to the calibration curve of gallic acid carried out with 6 concentration values (ranging from 0 to 1 mg/ml).

Determination of total flavonoids

The determination of total flavonoids was carried out according to the method described by Dehpeur et al. [22]. A total of 0.5 ml of the *Aloe vera* extract was added to 1.5 ml of 80% ethanol and 0.1 ml of 10% $AlCl_3$ with 0.1 ml of 1 M sodium acetate. The mixture was stirred and then incubated in the dark and at room temperature for 30 minutes. The absorbance is measured at 415 nm. The results are expressed in mg equivalent quercetin/100 g of dry matter by reference to the standard curve of quercetin (0–1 mg/ml).

Attenuated Total Reflectance-Fourier transform infrared spectroscopic analysis (ATR-FTIR) of *Aloe vera* extracts before and after digestion

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

The phenolic extracts of digested and undigested digested *Aloe vera* were analyzed by Attenuated Total Reflectance-Fourier transform infrared spectrometry (ATR-FTIR) using an Agilent Cary 600 series FTIR spectrometer. Two milligrams of sample were analyzed, and the spectra were recorded at wave numbers ranging from 400–4000 cm^{-1} and at a resolution of 4 cm^{-1}.

Determination of antioxidant activity

The antioxidant activity of *Aloe vera* extracts was evaluated by three methods, DPPH, ABTS and CUPRAC, and compared with antioxidants of BHA and BHT references.

Determination of antioxidant activity by the DPPH method

DPPH method: The evaluation of the antioxidant activity was done by the method of BLOIS [23].The inhibition power is expressed in% and determined by applying the following formula:

$$I\% = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \text{x}100$$

where A blank isabsorbance of white (DPPH in ethanol)and A sample isabsorbance of the test compound.

Reference extract and antioxidant concentrations (BHA and BHT), based on the percentages of inhibited DPPH, were plotted at the end of the reaction to obtain the IC50 index [24].

Determination of antioxidant activity by the ABTS method

The following method of trapping the free radical ABTS ° is that described by Re et al. [25]. The inhibition percentages of the ABTS radical were plotted against extract concentrations to determine the IC50 index.

Determination of cupric reductive antioxidant capacity (CUPRAC)

We realized the evaluation of the antioxidant activity by the method of Özyürek et al.[26]. The results were calculated as A0.5 (µg/ml) corresponding to the concentration indicating 0.50 absorbance.

Statistical analysis

The means plus or minus the standard deviation of the three replicates of determination of total polyphenol content, evaluation of antioxidant activity, as well as graphic representations, were performed with Excel 2013 (Microsoft Excel Version 3. 2013, Microsoft Corp., Redmond, WA, USA). Means were compared by single factor analysis of variance (ANOVA) analysis followed by a post-hoc Tukey test using STATISTICA 7.0 software (StatSoft, Inc., Tulsa, OK, USA). The superscript letters a, b, c, d, and e indicate a significant difference at the 0.05 significance level. The generation of the test matrice, the analysis of the results of the experimental design (the central composite design) is generated with Minitab 17 software (Minitab Inc., State College, PA, USA). The generation of surface plots and the optimization of the factors

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

were made by STATISTICA 7.0 software (StatSoft, Inc., Tulsa, OK, USA). Ftir spectra were plotted using Origin Pro 9.1 software (Origine Lab Corporation, Northampton, MA, USA).

Results and Discussion

The analysis of variance partitioned the variability of total polyphenol levels for each effect. The model is significant ($P<0.05$) and has a high R2 (85.45%). The response surface is shown in Figure 1. The optimal values of the total polyphenol content are in the red zone bounded by the point's solvent concentration (98%–100%) and maceration time (1189–1320 min).

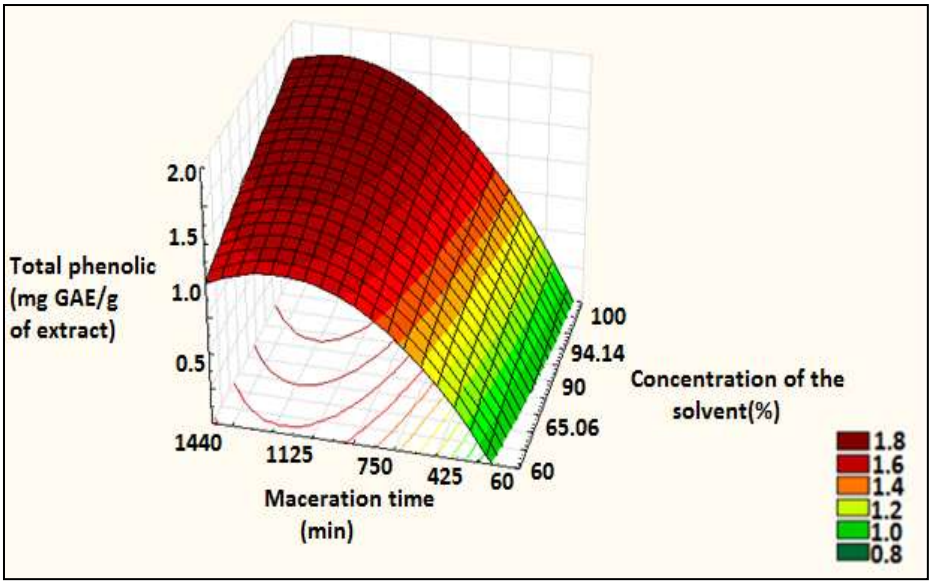

Figure 1. *Aloe vera* polyphenol rate response surface as a function of solvent concentration and maceration time

The prediction of optimal conditions is solvent concentration 100% and maceration time 1226 min. The results of the optimization are confirmed by the determination of the total polyphenols experimentally with the factors selected at the optimum point. The measured value (1.36 mg EAG/g dry extract) is close to that predicted by the mathematical model (1.98 mg EAG/g dry extract), which confirms the validation of the model.

Effect of gastrointestinal digestion on levels of total polyphenols and flavonoids

The determination of the total polyphenols gives us an overall estimate of the content in different classes of phenolic compounds contained in the extract. Colorimetric methods were used to evaluate the

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

number of phenolic compounds in the plant material. Maceration and choice of the solvent used are the main criteria to consider for profitable extraction [27].

Before proceeding to the determination of the content of phenolic compounds, we established a calibration curve using gallic acid. The results are expressed in milligram (mg) gallic acid equivalent per 100 gram of dry matter (mg EAG/100 g MS) and are represented in Figure. 2. The FT assay was performed according to the aluminum trichloride method ($AlCl_3$) usingthe quercetin calibration curve. The absorbance was read in a wavelength of 415 nm. The results are expressed in milligram (mg) equivalent of quercetin per 100 grams of the dry matter (mg EQ/100 g MS) and are represented in Figure 2.

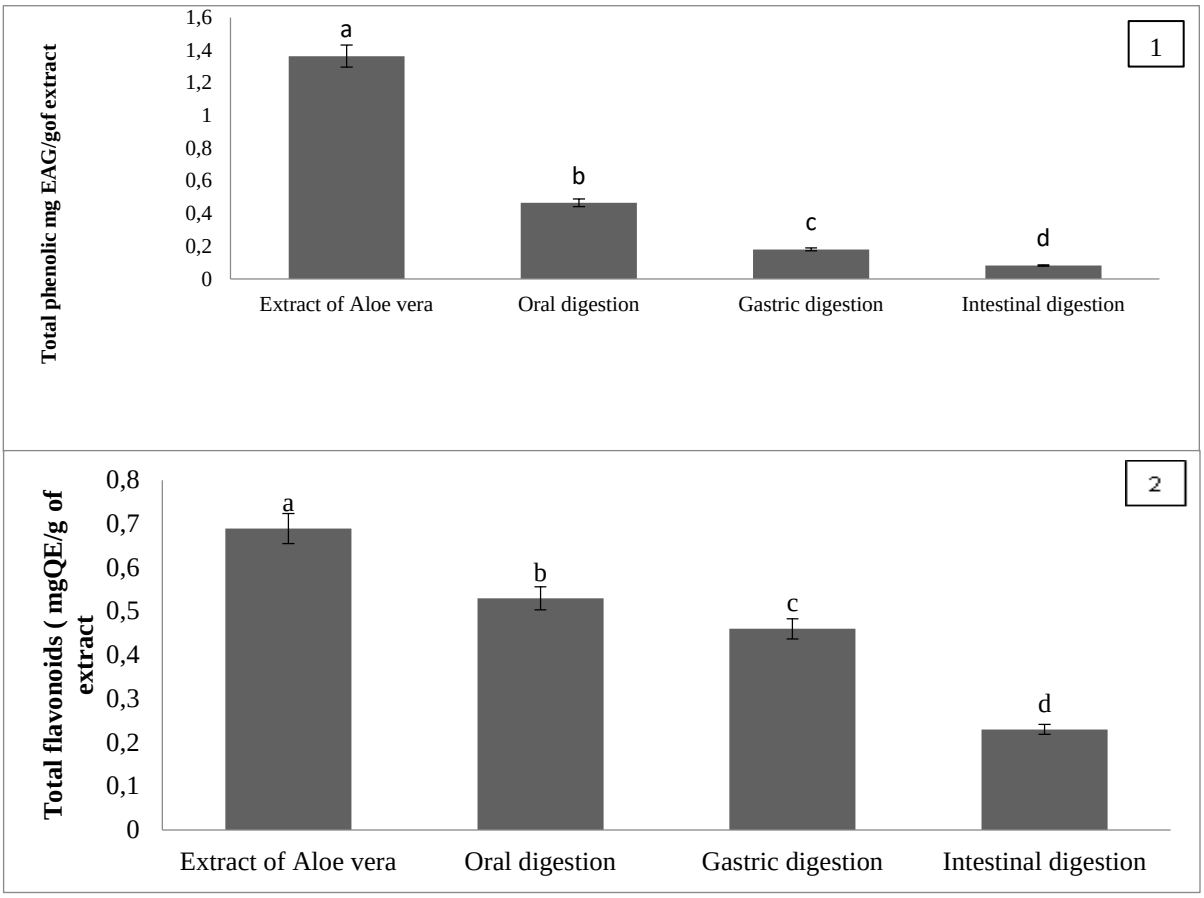

Figure 2. Effect of invitro digestion on the total polyphenol and flavonoid contents of *Aloe vera*: (1) total polyphenols, (2) flavonoids. The letters a, b, c and d indicate a significant difference between the antioxidant activities of undigested and digested *Aloe vera* at the 0.05 level of significance

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

According to the results obtained the content of total polyphenols and flavonoids of the extract before gastrointestinal digestion invitro was 1.36 ± 0.25 mg EAG/g and 0.690 ± 0.5 mg EQ/100 g. On reading the results obtained (Figure 3), we note that the three phases of digestion (salivary, gastric and intestinal) seem to cause a decrease in the levels of total polyphenols and flavonoids. This behavior may be attributed to the interaction of polyphenols with digestion enzymes: α-amylase in the oral phase (salivary), pepsin in the gastric phase and pancreatin in the intestinal phase. In addition, digestion conditions (especially pH) play an important role in the increase or decrease of phenolic compounds [28]. It can therefore be assumed that the decrease in the total phenolic concentration of extracts observed during the gastric digestion phase could be attributed to the acid hydrolysis of phenolic glycosides in their aglycons during gastric digestion [29].

During the intestinal phase, polyphenols are degraded because they are very sensitive to alkaline conditions, which result in the loss of these compounds during this digestion stage [30, 31].

Interactions between antioxidant compounds and food constituents, such as minerals and proteins, can also lead to complex formations and consequently cause changes in the chemical structure of polyphenols, molecular weight and solubility during digestion when simulated, leading to a decrease in their levels [29].

Attenuated Total Reflectance-Fourier transform infrared spectroscopic analysis (ATR-FTIR) of *Aloe vera* extracts before and after *in vitro* digestion

Attenuated Total Reflectance-Infrared spectrophotometric analysis was performed in order to monitor the change in the overall chemical composition of the sample (*Aloe vera*) after digestion (Figure 3). We found the following absorption bands: A band at 3324.641 cm^{-1} signifying an absorption band corresponding to the OH bond vibrations of the phenolic compounds [32]; a band at 2923.810 cm^{-1} was detected, indicating the presence of a CH bond [33]; a band was found at 1638.850 cm^{-1}, which corresponds to the vibration of the group C=C [34]; the band at 1415.770 cm^{-1} could be linked to CH_3, CH_2, flavonoids and aromatic rings [35]; the band at 1252 cm^{-1} is attributed to the vibration of the CO group of polyols, such as hydroxy flavonoids, and the band at 920.181 cm^{-1} could be due to a vibration of the aromatic rings [32]. The digestion has a negative effect on the detected links, and a progressive decrease of the intensity of the bands of absorption was observed during the three phases of digestion (oral, gastric and intestinal) until their disappearance after the intestinal phase.

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

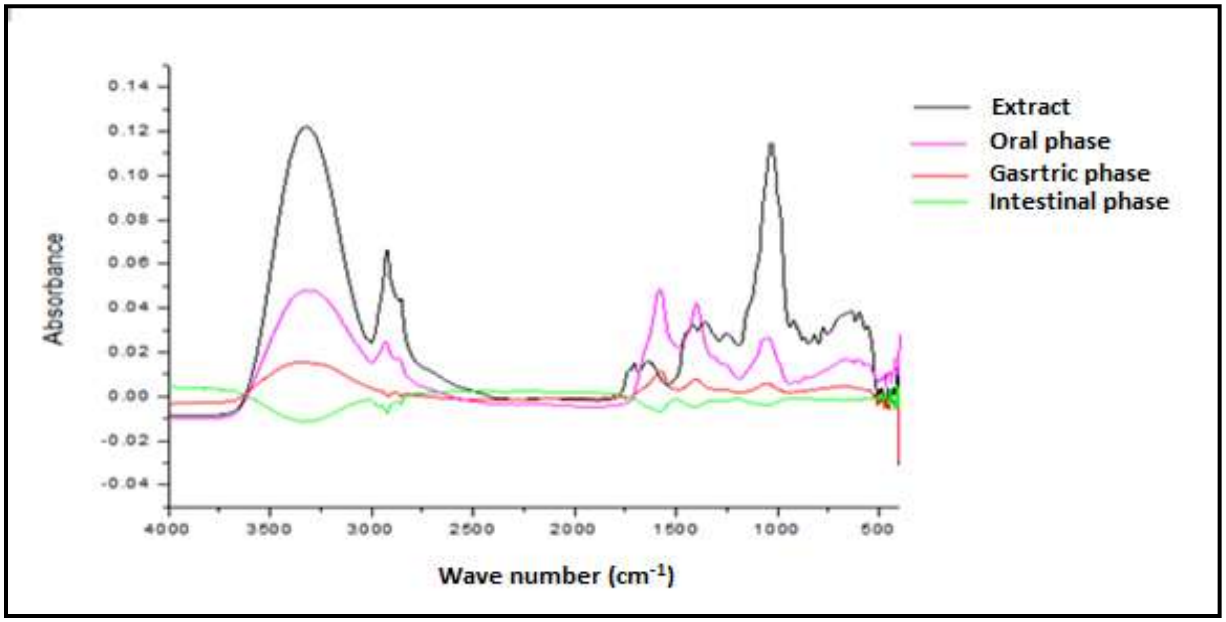

Figure 3. FTIR spectra of undigested and digested *Aloe vera* extracts (oral, gastric and intestinal phase)

Effect of gastrointestinal digestion on antioxidant activity

The values obtained from IC50 for (DPPH and ABTS) and A0.5 for the CUPRAC method are shown in Table 2.

Table 2. Values of IC50 and A0.5 (µg/ml)

	DPPH (IC50 µg/ml)	ABTS (IC50 µg/ml)	CUPRAC (IC50 µg/ml)
Extract	24.85 ± 0.95^{d}	22.92 ± 0.08^{d}	25.35 ± 0.71^{d}
Oral phase	645.45 ± 0.42^{c}	279.57 ± 0.26^{b}	5555.46 ± 0.72^{c}
Gastric phase	3370.59 ± 0.90^{a}	92.48 ± 0.17^{c}	16666.59 ± 0.33^{d}
Intestinal phase	962.79 ± 0.28^{b}	1002.41 ± 0.76^{a}	12499.92 ± 1.90^{b}
BHA	6.14 ± 0.41^{f}	1.81 ± 0.10^{e}	5.35 ± 0.71^{f}
BHT	12.99 ± 0.41^{e}	1.81 ± 0.10^{e}	8.97 ± 3.94^{e}

The letters a, b, c, d, e, and f indicate a significant difference between IC50 (µg/ml) undigested and digested *Aloe vera* at the 0.05 level of significance.

The three methods of evaluating antioxidant activity, DPPH, ABTS and CPRAC, resulted in the same outcome. *Aloe vera* showed a strong antioxidant activity. This same finding has been reported by several authors [19, 36-37]. This activity may be related to the wealth of *Aloe vera* in phenolic compounds. This activity was reduced to different degrees during digestion. The results obtained for the two methods DPPH

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

and CUPRAC are identical; we notice the antioxidant activity decreased gradually during the oral and gastric phase followed by a slight increase after intestinal digestion. The gastric phase has the highest value of IC50 and A0.5, indicating the lowest antioxidant activity.

For the ABTS method, the antioxidant activity decreased during the oral phase, increased during the gastric phase and then decreased again during the intestinal phase. For this method the highest IC50 value was found after intestinal digestion.

The difference obtained between the results obtained for the two methods DPPH and ABTS can be attributed to the reaction media. The DPPH test is reactive in a medium consisting of organic solvents, while the ABTS test is carried out under aqueous conditions. In addition, the solubility of flavonoids in both media should be taken into account. Some bioactive compounds that may not be soluble in the reaction medium cannot perform radical removal activities [38].

The observed decrease in antioxidant activity after digestion can be attributed to the instability of polyphenols in digestion conditions. This result is confirmed by FTIR spectroscopic analysis.

According to the literature, several authors report that there is a relationship between the content of phenolic compounds and the antioxidant properties [39-41]. According to HAYES et al. [42], the antioxidant activity generally depends on the number and position of the hydroxyl groups relative to the functional carboxyl groups. Of the phenolic compounds, flavonoids are compounds with high antioxidant activity [43]. Destruction of the latter decreases the antioxidant activity.

The antiradical activity of phenolic compounds is strongly dependent on the pH of the reaction medium, where an increase in its capacity can occur with an increase in pH values [44]. This may be related to the deprotonation of the hydroxyl groups present on the aromatic rings of the phenolic compounds by decreasing the energy of O and dissociations of H bonds, which facilitates the hydrogen donation reactions as well as the decrease of the ionization potential and the increase in the ability to donate electrons. However, the duodenal medium can also induce structural modifications such as the ionization of phenolic compounds, which allows the increase of the antioxidant activity during this phase compared to the gastric phase and this can explain the increase of the observed antioxidant potential for both methods DPPH and CUPRAC [45].

On the other hand, the values found by the ABTS show that the antioxidant activity of *Aloe vera* decreases in the oral phase, then increases in the gastric phase and decreases again in the intestinal phase. Previous studies have discovered that a number of polyphenols increased after the gastric phase *in vitro* digestion process because polyphenols are very sensitive to pH changes.

The increase in antioxidant activity during the gastric phase can be attributed to the decrease in pH that allows the release of insoluble phenolic compounds. Previous studies have shown that a number of polyphenols increased after the gastric phase of the *in vitro* digestion process [30].

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

After the intestinal digestion phase, the antioxidants are degraded by the alkaline pH, which leads to an overall loss of antioxidant capacity after *in vitro* digestion [12]. It is possible that when these compounds are exposed to such conditions, they are transformed into different structural forms with different chemical properties and different degrees of bioaccessibility and biological activities.

In the study by Henning et al. [46], in some samples the antioxidant activity after *in vitro* digestion remained unchanged, but in other samples it was increased by 50%, compared to undigested controls. These modifications were attributed to the hydrolysis of some of these compounds during digestion and the formation of other metabolites with higher or lower antioxidant activity. In a study to evaluate polyphenolic stability and the change in antioxidant potential of berries during *in vitro* digestion, Lucas-González et al. [12] demonstrated that polyphenolic compounds are released, especially in the early stages of gastrointestinal digestion, where they can exert biological activity as antioxidant compounds after absorption in gastric digestion.

Conclusion

Aloe vera has several therapeutic and nutritional virtues, some of which are little known. This plant is rich in bioactive molecules such as phenolic compounds, the content of which are influenced by several intrinsic parameters (effect of digestion). This work aims to evaluate the effect of gastrointestinal digestion *in vitro* on the content of phenolic compounds and the antioxidant activity of *Aloe vera*. The values of total polyphenols and flavonoids decrease under the effect of gastrointestinal digestion. This can be attributed to the interaction of phenolic compounds with digestion enzymes, the variation of chemical structures of *Aloe vera* polyphenols and the pH of the reaction medium. The antioxidant activity is decreased after digestion in the three phases comparing with the results obtained in *Aloe vera* extract. This simulation study of gastrointestinal digestion *in vitro* predict human conditions *in vivo*, but it would be interesting to carry out studies and feeding trials *in vivo* to confirm the results of the present studyas well as the concentration on digestion of *Aloe vera* polyphenols using a model including a microbial digestion of the colon in order to obtain qualitatively well correlated results with human studies. In addition, encapsulation technology can be used to improve the bioavailability of polyphenols or to achieve controlled release of these digestion compounds.

Conflicts of interest

The authors declare no conflict of interest.

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

Acknowledgements

The authors are thankful to CRBT (Research center of biotechnology, Constantine) for its support in this study.

References

[1]. Berger, M.M., Antioxidant micronutrients in major trauma and burns: evidence and practice, *Nutr Clin Pract*, **2006**, *21*, 438-49.

[2]. Djeridane, A.; Yous, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P.; Vidal, N., Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds, *Food Chem*, **2006**, *97*, 654-660.

[3]. Guo, X.; Mei, N., *Aloe vera*: a review of toxicity and adverse clinical effects, *J Environ Sci Health*, **2016**, *34*, 77-96.

[4]. Minjares- Feuentes, R.; Femenia, A.; Comas-Serra, F.; Rosséllo, C.; Rodrigue Gonzalez, V.M.; González Laredo, R.F.; Gallegos-Infante, J.A.; Medina-Torres, L., Effect of different drying procedures on physicochemical properties and flow behavior of *Aloe vera* (*Aloe barbadensis* Miller) gel., *LWT−Food Science and Technology*, **2016**, *74*, 378-386.

[5]. Choi, S.; Chung, M.H., A review on the relationship between *Aloe vera* components and their biologic effects, *Semin Integr Med*, **2003**, *1*, 53-62.

[6]. Kaithwas, G.; Singh, P.; Bhatia, D., Evaluation of *in vitro* and *in vivo* antioxidant potential of polysaccharides from *Aloe vera* (*Aloe barbadensis* Miller) gel, Drug *Chem Toxicol*, **2014**, *37*, 135-143.

[7]. Soriano Sancho, R.A.; Pavan, V.; Pastore, G.M., Effect of *in vitro* digestion on bioactive compounds and antioxidant activity of common bean seed coats, *Food Res Int*, **2015**, *76*, 74-78.

[8]. Kamiloglu, S., Bioavailability and bioactivity of black carrot polyphenols using *in vitro* digestion models combined with a co-culture model of intestinal and endothelial cell lines. Ph.D. Dissertation, Faculty of Bioscience Engineering, Ghent University, Belgium, **2016.**

[9]. Chen, G.L.; Chen, S.G.; Zhao, Y.Y.; Luo, C.X.; Li, J.; Gao, Y.Q., Total phenolic contents of 33 fruits and their antioxidant capacities before and after *in vitro* digestion, *Industrial Crops and Products***, 2014**, *57*, 150-157.

[10]. De Ancos, B.; Cilla,A.; Barberá, R.; Sánchez-Moreno, C.; Cano, M. P., Influence of orange cultivar and mandarin postharvest storage on polyphenols, ascorbic acid and antioxidant activity during gastrointestinal digestion, *Food Chemistry*, **2017**, *225*, 114-124.

[11]. Liang, L.; Wu, X.; Zhao, T.; Zhao, J.; Li, F.; Zou,Y.; Mao, G.; Yang, L., *In vitro* bioaccessibility and antioxidant activity of anthocyanins from mulberry (*Morus atropurpurea* Roxb.) following simulated gastro-intestinal digestion, *Food Research International*, **2012,** *46*, 76-82.

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

[12]. Lucas-González, R.; Navarro-Coves, S.; Pérez-Álvarez, J.A.; Fernández-López, J.; Muñoz, L.A.; Viuda-Martos, M., Assessment of polyphenolic profile stability and changes in the antioxidant potential of maqui berry (*Aristotelia chilensis* (Molina) Stuntz) during *in vitro* gastrointestinal digestion, *Industrial Crops and Products*, **2016**, *94*, 774-782.

[13]. Talens, P.; Mora, L.; Bramley, P.M.; Fraser, P. D., Antioxidant compounds and their bioaccessibility in tomato fruit and puree obtained from a DETIOLATED-1 (DET-1) down-regulated genetically modified genotype, *Food Chemistry*, **2016**, *213*, 735-741.

[14]. Bouayed, J.; Deußer, R.; Hoffmann, L.; Bohn, T., Bioaccessible and dialyzable polyphenols in selected apple varieties following *in vitro* digestion vs. their native patterns, *Food Chemistry*, **2012**, *131*, 1466-1472.

[15]. Kamiloglu, S.; Capanoglu, E., Investigating the *in vitro* bioaccessibility of in fresh and sun-dried figs (*Ficus carica* L.), *Journal of Food Science and Technology*, **2013**, *48*, 2621-2629.

[16]. Ydjedd, S.; Bouriche, S.; López-Nicolás, R.; Sánchez-Moya, T.; Frontela-Saseta, C.; RosBerruezo, G.; Rezgui, F.; Louaileche, H.; Kati, D.E., Effect of *in vitro* gastrointestinal digestion on encapsulated and nonencapsulated phenolic compounds of carob (*Ceratonia siliqua* L.) pulp extracts and their antioxidant capacity, *J Agric Food Chem*, **2017**, *65*, 827-835.

[17]. Goupy, J.; Creignton, L., Introduction aux plans d'expériences, **2006**, Donud, France.

[18]. Laib, I.; Barkat, M., Optimization of Conditions for Extraction of Polyphenols and the Determination of the Impact of Cooking on Total Polyphenolic, Antioxidant, and Anticholinesterase Activities of Potato, Foods, **2018**, *7*, 36.

[19]. Laib, I.; Boubrik, F.; Barkat, M., Optimization of the extraction parameters of *Aloe vera* polyphenols and study of antioxidant and antifungal activities: application to molds isolated from durum wheat, *Acta Scientifica Naturalis*, **2019**, *6*, 79-90.

[20]. Elfalleh, W.; Hannachi, H.; Tlili, N.; Yahia, Y.; Nasri, N.; Ferchichi, A., Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower, *J Med Plants Res*, **2012**, *6*, 4724-4730.

[21]. Waterhouse, A., Folin-Ciocalteu Micro Method for Total Phenol in Wine, *Food Anal Chem*, **1999**, *299*, 152-178.

[22]. Dehpour, A.A.; Ibrahimzadeh, M.A.; Fazel, S.N.; Seyed, M.N.,Antioxydant activity of the methanol extract of Ferula assafoetida and its essential oil composition, *Grasas Y Aceites*, **2009**, *60*, 405-412.

[23]. Blois, M.S., Antioxidant determinations by the use of a stable free radical, *Nature*, **1958**, *181*, 1199-1200.

[24]. Sharififar, F.;Mosh afi, M.H.; Mansouri, S.H.; Khodashenas, M.; Khoshnoodi, M., *In vitro* evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic *Zataria multiflora* Boiss, *Food Cont*, **2007**, *18(7)*, 800-805.

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

[25]. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang M.; Rice-Evans, C., Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic Biol Med*, **1999**, *26*, 1231.

[26]. Özyürek, M.; Güçlü, K.; Apak, R., The main and modified CUPRAC methods of antioxidant measurement, *TrAC-Trends Anal Chem*, **2011**, *30*, 652-664.

[27]. Turkmen, N.; Velioglu, Y.S.; Sari, F.; Polat, G., Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea, *Molecules*, **2007**, *12*, 484-496.

[28]. Velderrain-Rodríguez, G.; Quirós-Sauceda, A.; Mercado-Mercado, G.; Ayala-Zavala, J.F.; Astiazarán-García, H.; Robles-Sánchez, R.M.; Wall-Medrano, A.; Sayago-Ayerdi, S.; González-Aguilar, G.A., Effect of dietary fiber on the bioaccessibility of phenolic compounds of mango, papaya and pine apple fruits by an *in vitro* digestion model, *Food Science and Technology (Campinas)*, **2016**, *36*, 188-194.

[29]. Seraglio, S.K.T.; Valese, A.C.; Daguer, H.; Bergamo, G.; Azevedo, M.S.; Nehring, P.; Gonzaga, L.V.; Fett,R.; Costa, A.C.O., Effect of *in vitro* gastrointestinal digestion on the bioaccessibility of phenolic compounds, minerals, and antioxidant capacity of *Mimosa scabrella* Bentham honeydew honeys, *Food Research International*, **2017**, *99*, 670-678.

[30]. Lucas-González, R.; Viuda-Martos, M.; Pérez-Alvarez, J.A.; Fernández-López, J., *In vitro* digestion models suitable for foods: Opportunities for new fields of application and challenges, *Food Research International*, **2018**, *107*, 423-436.

[31]. Bermúdez-Soto, M.J.;Tomás-Barberán, F.A.; García-Conesa, M.T., Stability of polyphenols in chokeberry (*Aronia melanocarpa*) subjected to *in vitro* gastric and pancreatic digestion, *Food Chemistry*, **2007**, 102, 865-874.

[32]. Bouayed, J.; Hoffmann, L.; Bohn, T., Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake, *Food Chemistry*, **2011**, *128*, 14-21.

[33]. Sharaf, S.; Higazy, A; Hebeish, A., Propolis induced antibacterial activity and other technical properties of cotton textiles, *International Journal of Biological Macromolecules*, **2013**, *59*, 408-416.

[34]. Gutierrez-Gonçalves, M.E.J., Marcucci, M.C., Atividades Antimicrobiana e Antioxidante da Própolis do Estado do Ceará, Revista Fitos, **2009**, *4(1)*, 81-86.

[35]. Barud, H.; Júnior, A.; Saska, S.; Mestieri, L.; Campos, J.A.D.B.; de Freitas, R.M.;Ferreira, N.U.; Nascimento, A.P.; Miguel, F.G.; de Oliveira Lima Leite Vaz, M.M.; Barizon, E.A.; Marquele-Oliveira, F.; Gaspar, A.M.M.; Ribeiro, S.J.L.; Berretta, A.A. Antimicrobial Brazilian propolis (EPPAF) containing biocellulose membranes as promising biomaterial for skin wound healing. *Evidence Based Complementary and Alternative Medicine*, **2013**, 1-10.

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved

[36]. Silva, A.J.; Silva, J.R.; de Souza, N.C.; Souto, P.C.S., Membranes from latex with propolis for biomedical applications, *Mater Lett*, **2014**, *116*, 235.

[37]. Saritha, M., Efficacy of topical *Aloe vera* in patients with oral lichen planus: à randomized double-blind study, *J Oral Pathology and Medicine*, **2010**, *39(10)*, 735-740.

[38]. Salawu, K.M.; Ajaiyeoba, E.O.; Ogbole, O.O.; Adeniji, J.A.; Faleye, T.C.; Agunu, A., Antioxidant, brine shrimp lethality and antiproliferative properties of gel and leaf extracts of *Aloe schweinfurthii* and *Aloe vera*, *J Herbs Spices Med Plants*, **2017**, *23(4)*, 263-270.

[39]. Floegel, A.; Kim, D.O.; Chung, S.J.; Koo, S.I., Chun, O.K., Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US, foods, *J Food Compos Anal*, **2011**, *24*, 1043-1048.

[40]. Zhao, H.; Dong, J., Lu, J.; Chen, J.; Li, Y.; Shan, L.; Lin, Y.; Fan, W.; Gu, G., Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (*Hordeum vulgare* L.), *J Agric Food Chem*, **2006**, *54(19)*, 7277-7286.

[41]. Lachman, J.; Hamouz, K.; Sulc, M; Pivec, V.; Hejmankova, A.; Dvorak, P.; Cepl, J. Cultivar differences of total anthocyanins and anthocyanidins in red and purple coloured potatoes and their relation to antioxidant activity, *Food Chem*, **2009**, *114*, 836-843.

[42]. Hamouz, K.; Lachman, J.; Pazderu, K.; Tomášek, J.; Hejtmánková, K.; Pivec, V., Differences in anthocyanin content and antioxidant activity of potato tubers with different flesh color**,** *Plant Soil Environ*, **2011**, *57*, 478-485.

[43]. Hayes, J.E.; Stepanyan, V.; Allen, P.; O'Grady, M.N.; Kerry, J.P., Evaluation of the effects of selected plant-derived nutraceuticals on the quality and shelf-life stability of raw and cooked pork sausages, *LWT Food Sci Technol*, **2011**, *44*, 164-172.

[44]. Montoro, P.; Tuberoso, C.I.G.; Piacente, S.; Perrone, A.; De Feo, V.; Cabras, P.C., Stability and antioxidant activity of polyphenols in extracts of *Myrtus communis* L. berries used for the preparation of myrtle liqueur, *J Pharm Biomed*, **2006**, *41(5)*, 1614-1619.

[45]. Tagliazucchi, D.; Verzelloni, E.; Bertolini, D.; Conte, A., *In vitro* bio-accessibility and antioxidant activity of grape polyphenols, *Food Chemistry*, **2010**, *120*, 599-606.

[46]. Henning, S.M.; Zhang, Y.; Rontoyanni, V.G.; Huang, J.; Lee, R.P.; Trang, A.; Nuernberger, G.; Heber, D.J., Variability in the antioxidant activity of dietary supplements from pomegranate, milk thistle, green tea, grape seed, goji, and acali: Effects of *in vitro* digestion, *Journal of Agricultural and Food Chemistry*, **2014**, *62*, 4313-4321.

Corresponding author: mina.laib@gmail.com Full Paper
DOI: 10.2478/asn-2020-0030 ©2020 Konstantin Preslavsky University of Shumen. All rights reserved