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COMPARATIVE PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT PROPERTIES OF EXTRACTS FROM *ALOE VERA* LEAF PEEL AND GEL

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Introduction. For the industrial production of extracts, *Aloe vera* gel is usually used as raw material, and the outer shell, which makes up more than 30% of the total leaf mass, is mostly burned or composted. However, recent studies indicate that *Aloe vera* skin also contains valuable biologically active substances, which makes it a promising material for further research and use in medical and cosmetic products.

Objective. To evaluate the phytochemical properties and compare the content of polyphenols, flavonoids, antioxidant compounds, and antioxidant activity of extracts from the peel and gel of *Aloe vera* leaves.

Materials and methods. To obtain the extracts, dry raw materials of *Aloe vera* were used. 2 g of homogenized dried raw materials were weighed and poured with a water-ethanol mixture with an ethanol content of 30%, 50%, 70% and 90%; 2% dimethyl sulfoxide (DMSO); distilled water in a ratio of raw materials: extractant 1:10. Phytochemical screening, determination of the content of extractive substances, polyphenols, flavonoids, and antioxidants were carried out using appropriate methods.

Results. Comparative analysis of *Aloe vera* peel and gel extracts showed a higher content of bioactive compounds in the peel. Phytochemical screening confirmed the presence of tannins, flavonoids, anthraquinones, mucilage, inulin, and reducing sugars.

Among the investigated solvents, the 70% ethanol extract of *Aloe vera* peel demonstrated the most promising results, with the highest levels of bioactive substances and the strongest antioxidant activity.

Conclusions. The study established that *Aloe vera* peel extracts contain a significantly higher concentration of bioactive compounds compared to gel extracts, particularly in terms of polyphenols, flavonoids, and antioxidant components. These findings highlight the potential of *Aloe vera* peel as a valuable source of biologically active compounds, supporting its sustainable, zero-waste utilization in pharmaceutical and cosmetic applications.

Keywords: *Aloe vera*, polyphenols, flavonoids, antioxidant activity, spectroscopy.

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ПОРІВНЯЛЬНИЙ ФІТОХІМІЧНИЙ АНАЛІЗ ТА АНТИОКСИДАНТНІ ВЛАСТИВОСТІ ЕКСТРАКТІВ ЗІ ШКІРКИ ТА ГЕЛЮ ЛИСТЯ *ALOE VERA*

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Метою досліджень був фітохімічний скринінг і порівняння вмісту поліфенолів, флавоноїдів, антиоксидантної активності екстрактів зі шкірки та гелю листя *Aloe vera.* Об'єктом для досліджень були шкірка та гель листя *Aloe vera*; екстрагент – вода, диметилсульфоксид, 30%, 50%, 70%, 90% етанол; співвідношення сировина : екстрагент – 1:10. Екстракти одержували методом мацерації.

Порівняльне дослідження екстрактів шкірки та гелю листя *Aloe vera* показало, що екстракти саме зі шкірки містять більшу кількість екстрактивних речовин, поліфенольних сполук, флавоноїдів, антиоксидантів. Серед спиртових екстрактів за всіма досліджуваними показниками лідером виявився 70%-й етанольний екстракт зі шкірки, у якому вміст екстрактивних речовин 0,017 мг/мл, поліфенолів – 0,091 мгГК/мл, флавоноїдів – 0,0725 мгК/мл, антиоксидантних речовин – 0,085 мгАК/мл, відсоток інгібування цього екстракту становив 90,6 %.

Ключові слова: *Aloe vera*, поліфеноли, флавоноїди, антиоксидантна активність, спектроскопія.

Introduction

Many products derived from *Aloe vera* raw materials have long been used in healthy nutrition, as well as for medical and cosmetic purposes. It is also known that *Aloe vera* has been used topically to heal wounds and various skin conditions, as well as orally as a laxative. The extract of whole *Aloe vera* leaves, including the gel, contains more than 200 chemicals [1]. The chemical composition of the plant depends on many factors: type and conditions of cultivation, time of harvest, climate, position of the leaves on the stem, type of aloe and method of harvesting the leaves [2]. *Aloe vera* contains pharmacologically active ingredients associated with various biological effects, including

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fungicidal, antiviral, antibacterial, anti-inflammatory, laxative, immunomodulatory and anticancer, wound healing, hepatoprotective, anti-diabetic, anti-ulcer, and anti-hyperlipidemic [3; 4]. The inner transparent gel of the leaves is believed to be responsible for most of the plant's therapeutic properties. In dry form, the gel contains sugars, minerals, proteins, lipids and phenolic compounds. The middle layer consists of a bitter, yellow latex that contains anthraquinones and glycosides. The outer thick layer (peel) performs a protective function and synthesises a number of valuable biologically active compounds in addition to carbohydrates and proteins [5].

Cosmetic products with *Aloe vera* are very popular due to their moisturising, anti-inflammatory, soothing and regenerating properties. *Aloe vera* is included in face masks, moisturising creams and lotions, face and body gels, sunscreens, and hair care products [6].

The combination of research and practical applications points to the significant potential of *Aloe vera* in pharmaceuticals and cosmetics, emphasising the importance of both a medical and cosmetic resource. Further scientific research could expand the use of *Aloe vera* and confirm new therapeutic possibilities, opening up broader prospects for the health and beauty industries.

Traditionally, the gel of the plant's leaves is used to make medicines and cosmetics, while the peel ends up as production waste.

The aim of the study. Evaluation of phytochemical properties and comparison of the content of polyphenols, flavonoids, antioxidant compounds and antioxidant activity of extracts from the peel and gel of *Aloe vera* leaves.

Materials and methods of research

Preparation of plant material. The raw material for the study was fresh *Aloe vera* leaves, which were washed with running water and left in it for 15 minutes to remove alloyin. The narrow ends of the leaves, which contain a small amount of gel, were cut off. The leaves were filleted, separating the skin and gel. For drying, the gel was cut into small cubes with a face length of 2 x 2 mm, and the peel was cut into 5 x 5 mm squares. The raw materials were dried for 48 hours at 50°C [7].

Preparation of extracts. To obtain the extracts, dry *Aloe vera* raw materials were used. 2 g of homogenised dried raw materials were weighed and poured into an ethanol-water mixture containing 30%, 50%, 70% and 90% ethanol; 2% dimethyl sulfoxide (DMSO); distilled water in the ratio of raw material: extractant 1:10. The extraction was carried out for 12 days at 8 °C. For further studies, the resulting extracts were filtered and centrifuged at 5,000 rpm for 12 min and stored at 4 °C [8].

Determination of the content of extractive substances. 1 ml of the respective extracts were placed in a pre-weighed and constant weight buret. The solvent was evaporated in a water bath, then dried at 105 °C to constant weight, cooled in a desiccator and weighed [9].

Study of the qualitative composition of the extracts. Free anthraquinones: 250 µl of the extract was put into a test tube and 250 µl of dilute ammonia solution was added and the contents were mixed. The appearance of a red colour was observed [10].

Combined anthraquinones: 3 drops of 10% iron (III) chloride solution were added to 250 µl of the extract, heated for 5 min in a water bath, then cooled and mixed with 5 ml of chloroform. Next, 100 µl of dilute ammonia solution was added and stirred. The intensification of the red colour of the solution was observed [10].

Sterols and triterpenes: 250 µl of the alcohol extract was evaporated to dryness in a test tube, the residue was dissolved in 20 µl of acetic acid and 20 µl of chloroform was added. The resulting mixture was carefully layered on 100 µl of concentrated sulfuric acid. A brown or violet-red ring formed in the contact zone of the two liquids [10].

Saponins: 10 ml of aqueous extract was poured into a test tube and stirred for 15 s, then settled for 15 min. The presence of saponins is indicated by the appearance of a stable foam above 1 cm in height [10].

Coumarins: 250 µl of the alcoholic extract was evaporated, and 200 µl of hot water was added to the residue. After that, 0.5 ml of 25% ammonia solution was added to the resulting solution. The appearance of fluorescence under UV light at a wavelength of 366 nm was observed [10].

Mucus: 5 ml of 96% ethanol was added to 1 ml of aqueous extract. After 10 min, the formation of a flaky precipitate was observed [13].

Reducing sugars: 5 ml of the aqueous extract was evaporated to dryness. To the residue was added 1 ml of Fehling's reagent. The appearance of an orange precipitate was observed [10].

Tannins: 250 µl of extracts were saturated with sodium acetate, and then 3 drops of $FeCl_3$ were added. The appearance of a blue-black colour was observed [10].

Flavonoids: Method 1: A few drops of a 20% alcohol-water NaOH solution were added to 250 µl of extracts. For comparison, a similar reaction was performed with a 0.1% rutin solution. The appearance of a yellow colour was observed [11].

Method 2: 3 drops of 10% $FeCl_3$ solution were added to 250 µl of the studied extracts and a similar reaction with 0.1% rutin solution was performed for comparison. The appearance of a brown or dark green colour was observed at a high concentration of flavonoids [12].

Inulin: 3 drops of α-naphthol were added to 250 µl of the studied extracts, mixed, and slowly poured into a test tube with concentrated sulfuric acid. The appearance of a purple ring was observed [12].

Quantitative determination of polyphenolic compounds. To 20 µl of extracts were added 1580 µl of water and 100 µl of Folin-Ciocalteu reagent. Incubated for 5 min, after which 300 µl of sodium carbonate solution was added. The reaction was incubated for another 1.5 hours in a dark place to complete the reaction. Measurements of the optical density of the solutions with a layer thickness of 10 mm were carried out at a wavelength of 765 nm using an Ulab 108 spectrophotometer. All measurements were performed in triplicate [13].

Quantitative determination of flavonoids. The total flavonoid content was determined by the spectrophotometric method [14]. For the study, 0.8 ml of the extract was mixed with 8.4 ml of an alcohol-water mixture with the corresponding ethanol content and 0.8 ml of a 2% aluminium chloride solution. It was kept in the dark at room

temperature for 40 min. A mixture of 0.8 ml of the extract and 9.2 ml of the alcohol-water mixture was used as a compensation solution. The total flavonoid content, expressed in milligrams of quercetin equivalents (QE) per 1 ml of extract (mgQE/ml), was calculated according to the calibration graph of solutions of the standard compound quercetin. All measurements were performed in triplicate.

Determination of the antioxidant effect by the DPPH method. 1800 µL of ethanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) with a concentration of 0.04 mg/ml was mixed with 200 µL of the extract under study [15; 16]. After mixing, the samples were kept in a dark place for 30 minutes. For the control, 200 µl of 70% ethanol and 1800 µl of DPPH solution were mixed. The optical density of the studied solutions was measured using an Ulab 108 spectrophotometer at a wavelength of 517 nm. Measurements were performed in triplicate.

The antioxidant activity was calculated by the formula:

$$AOA(\%) = 100 \cdot (A_0 - A) / A_0,$$

where A_0 – is the optical density of a solution of DPPH in ethanol with a concentration of 2 mg/50 ml; A – is the optical density of the solution of the studied extract.

The total antioxidant content, expressed in milligrams of ascorbic acid (AA) equivalents per 1 ml of extract (mgAA/ml), was calculated according to the calibration graph of solutions of the standard compound, ascorbic acid. Measurements were performed in triplicate.

Research results and their discussion

As a result of maceration, the following extracts were obtained: on the basis of distilled water, 2% DMSO, 30%, 50%, 70% and 90% water-ethanol mixture, using the following raw materials – dried gel and dried peel.

The determination of extractive substances was carried out by the gravimetric method at a temperature of 105 °C. The results are shown in Fig. 1.

The highest content of extractives in peel extracts was found in the aqueous extract, followed by 2% DMSO, and the leader among alcohol extracts was 70% ethanol. In all extracts from the gel, the content of extractive substances is lower than in similar extracts from the peel.

Phytochemical screening was carried out for aqueous and 70% water-ethanol extracts from *Aloe vera* peel and gel. The results of the phytochemical screening of the studied extracts are given in Table 1.

Tannins, flavonoids, mucilage and inulin were found in all the extracts studied. However, coumarins and starch were absent. Reducing sugars were found in aqueous and alcoholic extracts. It was found that saponins were absent

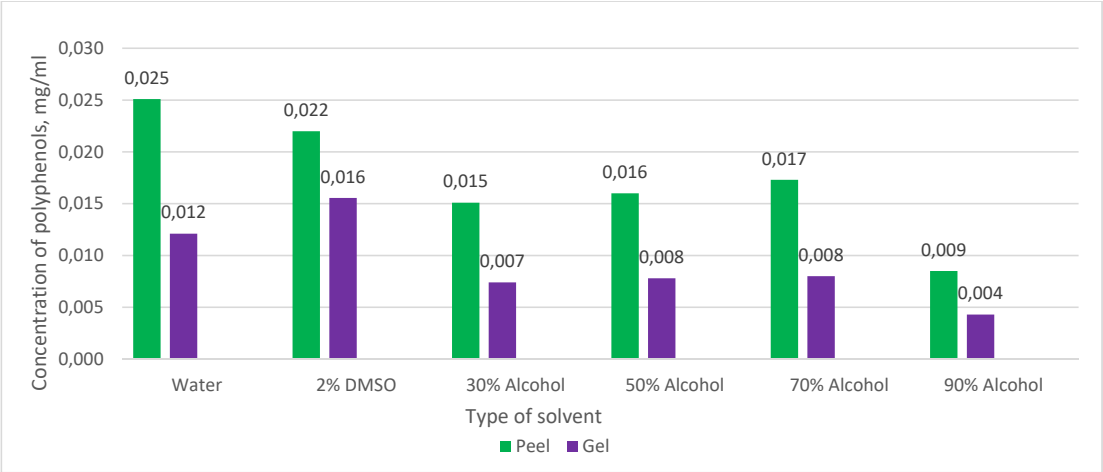

Fig. 1. Quantitative content of extractive substances

Table 1

Phytochemical screening of the studied extracts

Biologically active substances	Type of extract			
	Water from gel	Alcohol from gel	Water from peel	Alcohol from peel
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Anthraquinones	+	+	+	+
Sterols and triterpenes	–	+	–	+
Saponins	+	–	+	–
Coumarins	–	–	–	–
Mucus	+	+	+	+
Starch	–	–	–	–
Inulin	+	+	+	+
Reducing sugars	–	–	+	+

in the alcohol extracts, but sterols and triterpenes were present.

The infrared spectra of *Aloe vera* peel and gel were studied to identify the functional active groups of substances [17; 18]. These studies help to better understand the similarities and differences in the chemical composition of *Aloe vera* peel and gel (Fig. 2).

In both spectra, there is a broad absorption band (3200-3500 cm^{-1}), indicating the valence vibrations of O-H groups. In the IR spectrum of the peel, the broad band is shifted towards short wavelengths, indicating the absence of free water.

Sharp absorption bands (pprox.. 2800–3000 cm^{-1}) – valence vibrations of the C-H bond in alkanes – are present in the IR spectra of the peel and gel.

Similarly, the following absorption bands are present in both IR spectra obtained: at 1720 cm^{-1}, which corresponds to the valence vibrations of C = O, indicating the presence of carbonyl compounds such as ketones, aldehydes or carboxylic acids; Absorption bands in the range of 1500–1600 cm^{-1} are associated with C=C valence vibrations in aromatic rings and N-H strain vibrations, indicating the presence of aromatic compounds and proteins; Absorption bands in the range of 1300–1400 cm^{-1} are associated with C-H strain vibrations, indicating the presence of methyl or methylene groups.

The absorption band in the infrared spectrum of the gel at 1240.7 cm^{-1} is indicative of valence vibrations of C-O in alcohols, simple or esters.

Additional absorption bands in the range of 1000–1100 cm^{-1} of the peel IR spectrum may indicate C-O valence vibrations in alcohols, simple or esters.

In general, the spectra for both the gel and the peel are quite similar, indicating the presence of key functional groups such as hydroxyl, carbonyl and aliphatic hydrocarbon groups.

The total polyphenol content of Aloe vera extracts was determined using the Folin-Cocalteu spectrophotometric method. Gallic acid (GA) was used as a standard, and the total amount of polyphenols was expressed as mgGA/ml of gallic acid equivalents. The calculation was carried out according to the calibration graph equation $y = 1.339x + 0.0315$ with $R^2 = 0.9832$, where x is the concentration of gallic acid solution (mg/ml) and y is the optical density. The results of the calculations are shown in Fig. 3.

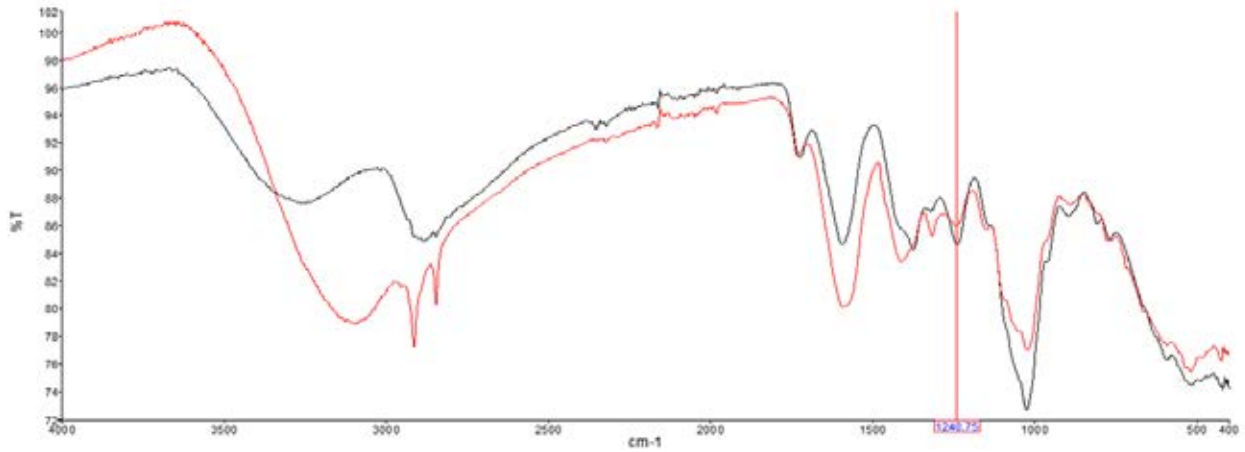

Fig. 2. Infrared spectra of *Aloe vera* peel (red) and gel (black)

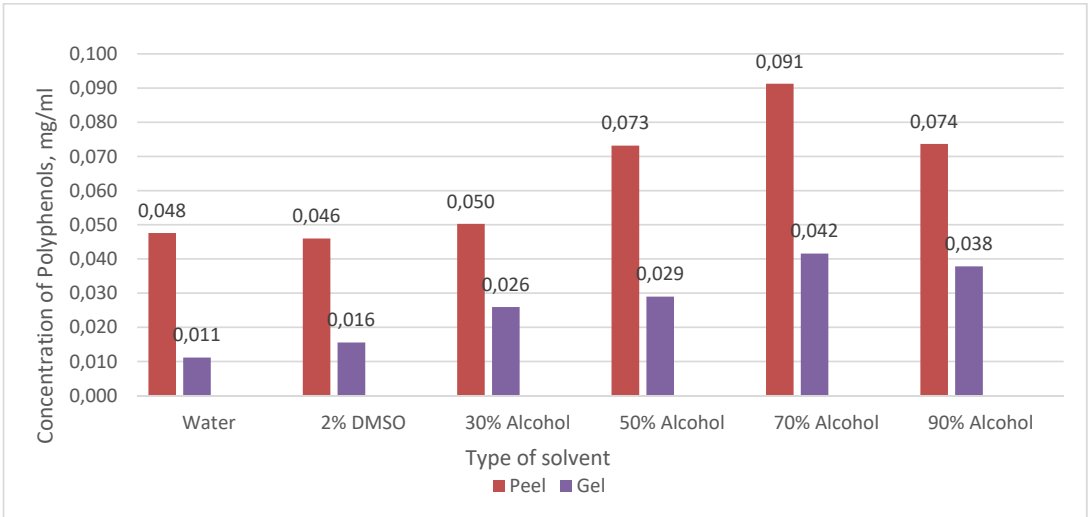

Fig. 3. Quantification of polyphenols

70% Ethanolic extract from the peel showed the highest polyphenol content (0.091 mg/ml). This indicates that this extract may be particularly useful for studies of antioxidant properties. Lower polyphenol content was found in the gel extracts, which indicates an uneven distribution of polyphenolic compounds in the plant leaves.

One of the widely used methods for the determination of flavonoids in plant extracts is spectrophotometric analysis using aluminium chloride, where Al^{3+} is used as a complexing agent. When $AlCl_3$ is added, yellow-coloured Al^{3+}-flavonoid complexes are formed, which are recorded at a wavelength of 420 nm.

The content of flavonoids in the samples was calculated according to the equation of the calibration graph of quercetin solutions $y = 5.1012x + 0.0346$ with $R^2 = 0.9823$, the X-axis is the concentration of quercetin expressed in milligrams per millilitre (mg/ml), the Y-axis is the optical density measured by a spectrophotometer. Fig. 4 shows the results of the study on the determination of flavonoids in *Aloe vera* extracts.

The study showed that the peel of *Aloe vera* has a higher flavonoid content than the gel, regardless of the type of solvent used. The highest concentration of flavonoids was found in the 70% ethanol extract from the peel (0.0725 mg/ml) and in the 90% ethanol extract from the gel (0.036 mg/ml).

These results indicate the feasibility of using *Aloe vera* peel to obtain extracts with a high content of flavonoids, which are interesting for further research and practical application in medicine or cosmetology.

The antioxidant activity of *Aloe vera* extracts was assessed by the DPPH method and expressed as a percentage of free radical inhibition by such biologically active substances as polyphenols, including flavonoids. The percentage of free radical inhibition is calculated by the formula:

$$\%DPPH^{+} = \left(\frac{A_0 - A_1}{A_0}\right) \cdot 100$$

where, A_0 – is the absorbance of the control solution, $A_{1_}$ is the absorbance of the test extract.

The measurement results are shown in Fig. 5.

The highest percentage of inhibition was observed in the 70% ethanol extract from both the peel and the gel of the plant leaves. 2% DMSO extracts have the lowest antioxidant activity.

The total content of antioxidant substances in terms of ascorbic acid (mgAA/ml) was calculated according to the regression equation $y = 1089.6 \times -2.1341$ with $R^2 = 0.9995$ of the calibration curve of optical density versus ascorbic acid concentration (AA concentrations: 0.08, 0.06, 0.04, 0.02, and 0.01 mg/ml).

The measurement results are shown in Fig. 6.

Ethanolic peel extracts contain the most antioxidant substances, including polyphenolic compounds, including flavonoids. In particular, the 70% ethanolic peel extract has the highest antioxidant content in terms of ascorbic acid (0.08512 mgAA/ml), making it the best choice for further research.

Since the preliminary results of quantitative studies showed that the content of polyphenols, flavonoids, and antioxidants was higher in peel extracts, these extracts were chosen for the study of UV spectra in the visible region.

According to Table 2, all UV spectra of the studied extracts have peaks at a wavelength in the range of 370–385 nm. According to the literature [19; 20], the following substances give absorption peaks in this range:

1) flavonoids: quercetin – can have a maximum absorption in the range of 370–380 nm; camphor – can have a maximum absorption of about 375–380 nm;

2) anthraquinones: alloin – may have an absorption in the ultraviolet region of the spectrum close to 375–380 nm; emodin – may have a maximum absorption of about 380 nm;

3) polyphenols: a broad group of compounds that includes flavonoids, phenolic acids and other polyphenolic structures that can have absorption maxima in the range of 350–400 nm. For example, caffeic acid and its derivatives can have an absorption maximum in the range of 370–380 nm.

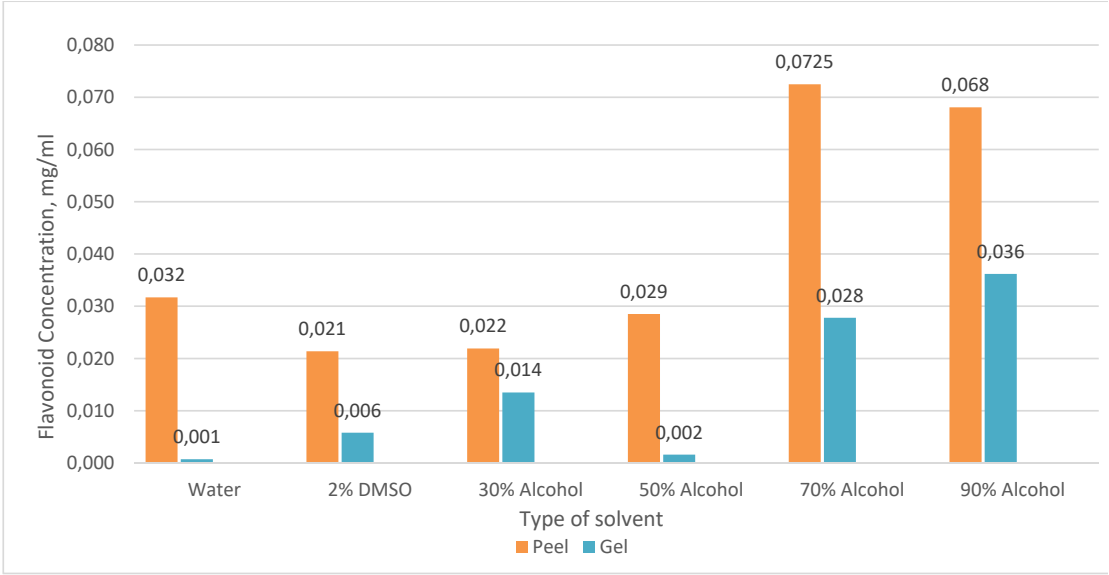

Fig. 4. Quantitative content of flavonoids in the studied extracts

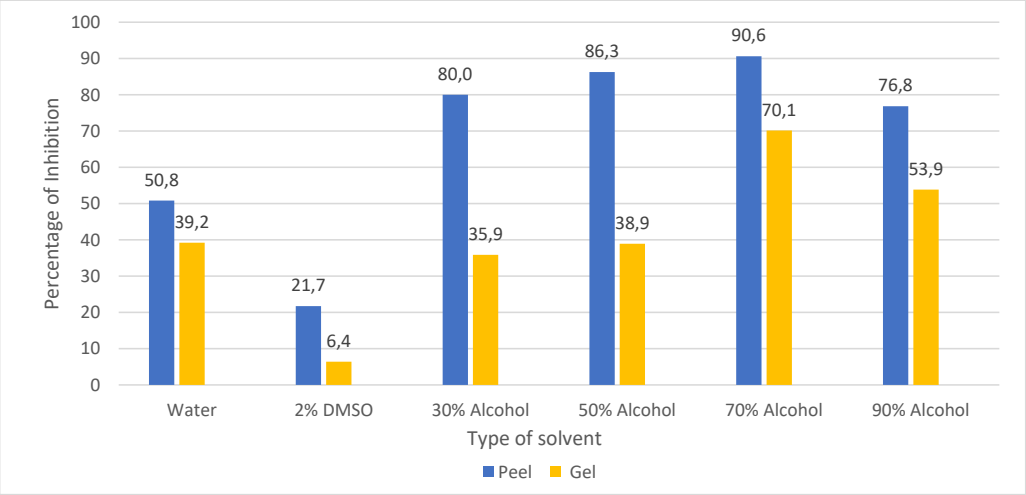

Fig. 5. Antioxidant activity of *Aloe vera* extracts

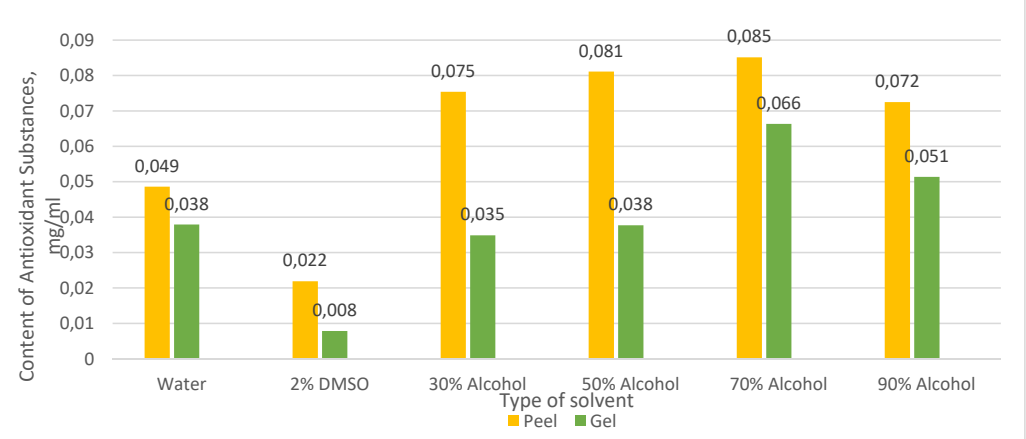

Fig. 6. Concentration of antioxidant substances

Table 2

Results of spectrophotometric analysis of peel extracts

Solvents	Wavelengths, nm	Absorption
Aqueous	370.1	1,3892
2% DMSO	371.5	1,139
30% ethyl alcohol	375	1,435
50% ethyl alcohol	375	2,0785
70% ethyl alcohol	385.5; 665	2.2702; 0.2199
90% ethyl alcohol	372.5; 665	1.8837; 0.2907

The appearance of a peak at 665 nm may indicate the presence of certain compounds that absorb light in the red part of the visible spectrum. The most likely compound to produce an absorption peak in this range is chlorophyll.

Conclusions

As a result of a comparative study of *Aloe vera* leaf peel and gel extracts, it was found that aqueous, 2% DMSO, 30%, 50%, 70%, 90% ethanol extracts from the peel contain a higher amount of extractive substances, antioxidants, including polyphenolic compounds, including flavonoids. According to phytochemical tests, it was found that peel extracts are rich in tannins, flavonoids, anthraquinones, mucilage, inulin and reducing sugars. Among the alcoholic extracts, the leader in all studied parameters was a 70% ethanol extract from the peel, which contained 0.017 mg/ml of extractives, 0.091 mgGC/ml of polyphenols, 0.0725 mgC/ml of flavonoids, 0.085 mgAC/ml of antioxidants, and a percentage of inhibition of this extract of 90.6%. All these results indicate the feasibility of further studies of *Aloe vera* peel extracts as a valuable source of biologically active substances, which will ensure the comprehensive waste-free use of *Aloe vera* leaves.

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