

## EXPERIMENTAL PAPER

# Effect of flavonoids content on antioxidant activity of commercial cosmetic plant extracts

PAULINA MALINOWSKA

Department of Technology and Instrumental Analysis  
Poznan University of Economics  
Al. Niepodległości 10  
61-875 Poznań, Poland

e-mail: paulina.malinowska@ue.poznan.pl, phone: +4861 8569054

### Summary

The aim of this work was to evaluate the antioxidant capacity and flavonoids content in 10 commercial cosmetic plant extracts used in cosmetics industry. Antioxidant activity of plant extracts were measured using two methods: FRAP (Ferric Reducing Antioxidant Power) and TEAC (Trolox Equivalent Antioxidant Capacity). The relationship between flavonoids content and antioxidant capacity of plant extracts were checked. As a result of this research it was found that FRAP and TEAC values of plant extracts significantly depend on the flavonoids content. The highest antioxidant activity, both in FRAP and TEAC assays, was observed for arnica flowers, hawthorn flowers and lungwort herb extracts. These extracts can be used as source of natural antioxidants for the prolongation of the oxidative stability of cosmetic products. Additionally, they can replace synthetic antioxidants.

*Key words:* plant extracts, antioxidant activity, FRAP, TEAC, cosmetics

## INTRODUCTION

Flavonoids are a group of polyphenolic compounds broadly distributed as a secondary metabolites in plant kingdom. Due to their reported biological activity, flavonoids are interesting for pharmaceutical, cosmetic and food industry [1].

Botanical extracts are often rich in flavonoids which applications in cosmetology, pharmacology and food supplements have been well developed recently. In

most publications first priority is given to the antioxidant properties of these compounds, although, it is more and more evident that flavonoids have also significant role in topically applied products. Many flavonoids can act as cofactors of all kinds of enzymes. They are also used in anti-aging, anti-cellulite, anti-couperose and skin-lightening products. Other important forms of activity of flavonoids are: strengthening of capillaries, anti-inflammatory effect, protective against radiation, moistening, softening, soothing, antiseptic and other [2-6]. Scientifically proven skin benefits of flavonoids and other polyphenols as cosmetic ingredients refer, for example, to prevention of the lipid oxidation [3], stimulation of fibroblast proliferation [7], reduction of collagen breakdown [8] or 5 $\alpha$ -reductase [9].

Antioxidant activity is one of the most important properties of plant extracts, because scientists have looked for the sources of natural antioxidants which can replace synthetic ones in many cosmetic, pharmaceutical and food formulations. In search of novel sources of antioxidants in the past, medicinal plants have been extensively studied.

This work is aimed at a preliminary screening of antioxidant activity of extracts isolated from plants used in cosmetics industry. It was decided to conduct the analysis of extracts that have healing properties, and simultaneously they are applied in many known cosmetics, such as: *Arnica montana*, *Crataegus monogyna*, *Pulmonaria officinalis*, *Helichrysum italicum*, *Sophora japonica*, *Arctium lappa*, *Polygonum aviculare*, *Centella asiatica*, *Melilotus officinalis* and *Hippophae rhamnoides*. Moreover, listed plants exhibit stronger or weaker antioxidant activity. There are some data in the literature concerning the antioxidant properties of these medicinal plants. There is a lot of information on antioxidant properties of *Arnica montana*, *Sophora japonica*, *Crataegus monogyna*, *Hippophae rhamnoides*, and less data on antioxidant properties of *Pulmonaria officinalis*, *Helichrysum italicum*, *Arctium lappa*, *Polygonum aviculare*, *Centella asiatica* and *Melilotus officinalis*.

Independent studies have shown that *Arnica montana* showed a good free radical scavenging activity and cytoprotective effect against oxidative damage in fibroblast-like cells [10-12]. Extracts of *Crataegus monogyna* have been found to be cytoprotective by scavenging superoxide anion, hydroxyl radical, hydrogen peroxides and reducing lipid peroxidation as compared to standards such as BHA and  $\alpha$ -tocopherol [13-16]. Moreover, literature provides data indicating that *Pulmonaria officinalis* may have antioxidant effects on bacteria simultaneously through several different pathways, including direct inhibition of reactive oxygen species, iron chelation and antioxidant genes induction [17, 18]. In previous studies, it turned out that *Helichrysum italicum* inhibited enzymatic and non-enzymatic lipid peroxidation and has free-radical scavenging properties [19, 20]. In turn *Sophora japonica*, known as anti-tyrosinase extract, was especially potent in HEMn cells in terms of free radical scavenging effects [21, 22]. There are also scientific reports indicating that *Arctium lappa* extracts are the strong free radical

scavengers and exhibit selective antiproliferative activity [23-25]. Medicinal uses of burdock in treating chronic diseases such as cancers and diabetes have been reported [26]. Scientific literature provides also data indicating that *Polygonum aviculare* showed a good superoxide and hydroxyl radical scavenging activities [27]. The extract of *Centella asiatica* possesses confirmed antioxidant, cognitive-enhancing, antiepileptic, antinociceptive and anti-inflammatory properties [28, 29]. Free radical scavenging activity of *Melilotus officinalis* was confirmed in ORAC (Oxygen radical absorbance capacity) [30]. In turn *Hippophae rhamnoides* has the ability to scavenge peroxy radicals and to protect against UV radiation. Studies indicated that it is a safe and effective antioxidant nutraceutical product [31].

The aim of this work was to determine and compare the content of flavonoids and the antioxidant capacity of 10 abovementioned plant extracts which are used in commercially available cosmetic products. In order to evaluate total antioxidant activity of these extracts two tests: Ferric Reducing Antioxidant Power (FRAP) assay and Trolox Equivalent Antioxidant Capacity (TEAC) method were chosen. Moreover the relationship between flavonoids content and the antioxidant capacity of the plant extracts were determined.

## MATERIAL AND METHODS

### Chemicals

The following chemicals: Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma Chemicals), TPTZ (2,4,6-tripyridyl-s-triazine, Fluka), ABTS (2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulphonic acid, Roche Diagnostics), potassium persulphate, chloride aluminium hexahydrate, sodium nitrate(V), sodium hydroxide (Merck), ferric(III) chloride hexahydrate and ferric(II) sulphate heptahydrate (POCH S.A.), ethanol, methanol, acetic acid, sodium acetate trihydrate and hydrochloric acid (Chempur) were used as supplied without any purification before use. All chemicals used in this work were of analytical grade.

### Plant material

Ten glycolic, hydroglycolic and/or hydroethanolic commercial cosmetic plants extracts (table 1) were obtained from two cosmetics companies: L'Angelica (Italy) and Ennagram (France). They were stored at room temperature in the dark. For the determination of flavonoids, extracts were diluted 5, 10 and 20 times; for the determination of total antioxidant capacity – 5, 10, 20, 50, 100, 200, 500 times. All dilutions of plant extracts were prepared in methanol.

Table 1.

The characteristics of plant extracts used in the study

Common name of plant	Latin name of plant	Part of plant Solvent	Flavonoids naturally occurring in the plant
Arnica	<i>Arnica montana</i> L.	flowers G	astragalinal, luteolin, kaempferol, quercetin, isoquercetrin, hispidulin, apigenin, isorhamnetin, patuletin, spinacetin [32,33]
Hawthorn	<i>Crataegus monogyna</i> L.	flowers WG	kaempferol, quercetin, rutin, vitexin, hyperoside, orientin, vicenin [32, 34]
Lungwort	<i>Pulmonaria officinalis</i> L.	herb G	quercetin, kaempferol [35]
Catkin	<i>Helichrysum italicum</i> L.	flowers WG	kaempferol, apigenin, naringenin, luteolin [36]
Japanese Pagoda Tree	<i>Sophora japonica</i> L.	buds WG	rutin, quercetin, kaempferol [37]
Burdock	<i>Arctium lappa</i> L.	root G	isoquercetrin, rutin, kaempferol, quercetin [37]
Knot-grass	<i>Polygonum aviculare</i> L.	herb WE	quercetin, hyperoside, avicularin, vitexin, kaempferol, miricetin [32, 34, 37]
Gotu-kola	<i>Centella asiatica</i> L.	herb G	kaempferol, quercetin, rutin, apigenin, naringin, castilliferol, castillicetin [38, 39]
Sweet clover	<i>Melilotus officinalis</i> L.	herb WE	kaempferol, quercetin [34, 40]
Buckthorn	<i>Hippophae rhamnoides</i> L.	fruit WE	quercetin, isorhamnetin, kaempferol, miricetin [41, 42]

Symbols used for solvents: W – water, E – ethanol, G – propylene glycol

## Flavonoids content

The amount of flavonoids in plant extracts was determined according to Karadeniz procedure [43] using (-)epicatechin as a standard. One milliliter of extract was placed in a 10 ml volumetric flask, and 5 ml of distilled water and 0.3 ml of 5% NaNO<sub>2</sub> were added and mixed. After 5 min, 0.6 ml of 10% AlCl<sub>3</sub>·6H<sub>2</sub>O was added. Two milliliters of 1 M NaOH were added 5 min later and then the volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured immediately at 510 nm. Flavonoid contents were calculated using a standard calibration curve, prepared using (-)epicatechin (fig. 1). The resulting values were expressed in mg epicatechin per gram of extract [mg ECE/g of extract]. Data are presented as mean ± SD of three replicates.

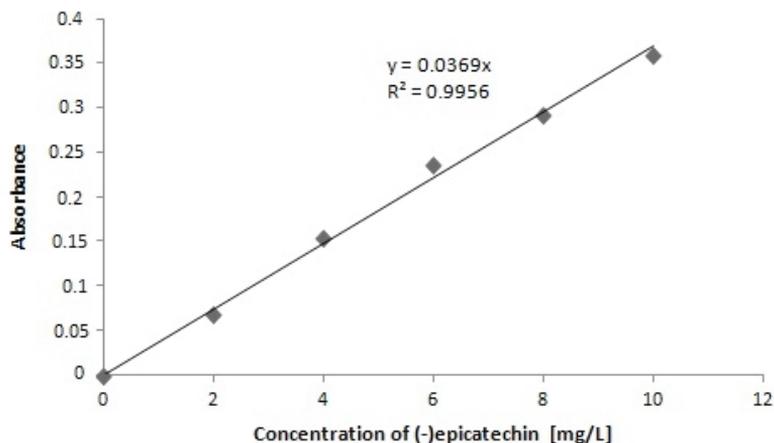


Figure 1.

Calibration curve for determination of flavonoids content in plant extracts

### FRAP (Ferric Reducing Antioxidant Power) assay

The reduction power of plant extracts was measured according to the procedure described by Benzie and Strain [44]. The method is based on the reduction of  $\text{Fe}^{3+}$ - TPTZ (2,4,6-tripyridyl-s-triazine) complex (FRAP reagent) to the ferrous ( $\text{Fe}^{2+}$ ) form. The appearance of a blue colour of the solution with an absorption maximum located at 593 nm is observed. Briefly, the FRAP reagent was prepared by mixing 25 ml of 300 mM acetate buffer (pH 3.6), 2.5 ml of 10 mM TPTZ solution in 40 mM HCl and 2.5 ml of 20 mM  $\text{FeCl}_3$ . For determination 30  $\mu\text{l}$  of various dilutions of plant extract or standard were added to 2970  $\mu\text{l}$  of FRAP reagent and mixed. After keeping at a room temperature for 4 min, the increase in absorbance was measured at 593 nm using UV-VIS spectrophotometer Genesis 2 (Milton-Roy, USA).  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  was used as a standard. The measurements of antioxidant capacity by means of FRAP assay were repeated three times for each concentration of the plant extract. FRAP value was expressed in  $\mu\text{mol}$  ferrous ( $\text{Fe}^{2+}$ ) form per gram of extract [ $\mu\text{mol Fe}^{2+}/\text{g}$  extract]. Data are presented as mean  $\pm$  SD of three replicates.

### TEAC (Trolox Equivalent Antioxidant Capacity) assay

Antioxidant capacity of plant extracts was measured according to the procedure described by Re et al. [45]. The method is based on the ability of antioxidant molecules to quench the long-lived  $\text{ABTS}^{\cdot+}$ , a blue-green chromophore

having a characteristic absorption band at 734 nm. Before proper determination, ABTS radical cation ( $\text{ABTS}^{\cdot+}$ ) was produced by mixing 0.2 ml 24.5 mM potassium persulphate with 7.7 mg ABTS and 1.8 ml redistilled water. Final concentrations were: for ABTS – 7 mM, for potassium persulphate – 2.45 mM. This solution was kept in the dark at a room temperature for 14–16 h before use. Prior to determination, this solution was diluted with methanol to obtain the solution with the absorbance of 0.7 – 0.8 measured at 734 nm. Briefly, 792  $\mu\text{l}$  of  $\text{ABTS}^{\cdot+}$  solution was placed in a 1cm glass cuvette and the absorbance was measured at 734 nm, using UV-VIS spectrophotometer Genesis 2 (Milton-Roy, USA). Next 8  $\mu\text{l}$  of different dilutions of plant extract or standard were added to  $\text{ABTS}^{\cdot+}$  solution and mixed. After keeping the reaction mixture at room temperature for 6 min the absorbance was measured again at 734 nm. The measurements of antioxidant capacity by means of TEAC assay were repeated three times for each concentration of the plant extract. In all measurements Trolox was used as a standard. The resulting TEAC values were expressed in  $\mu\text{mol}$  Trolox per gram of extract [ $\mu\text{mol}$  Trolox/g of extract]. Data are presented as mean  $\pm$  SD of three replicates.

## Statistical analysis

All analyses were performed in triplicate. The recorded results were subjected to statistical analysis using SPSS Statistics 14.0 software. The results were interpreted at the significance level  $p=0.05$ .

## RESULTS AND DISCUSSION

The content of flavonoids and the antioxidant activity of 10 plant extracts measured using FRAP and TEAC methods are presented in table 2. The amount of flavonoids varied widely in plant extracts tested and ranged from 0.16 mg ECE/g in buckthorn fruit extract to 2.15 mg ECE/g in arnica flowers extract. The plant extracts with the highest level of flavonoids were arnica flowers, hawthorn flowers, lungwort herb and catkin flowers extracts. Flavonoids content in these extracts were in several cases higher than in gotu-kola herb, sweet clover herb and buckthorn fruits extracts.

Similarly as observed for flavonoids content, FRAP values varied widely for plant extracts tested – they ranged from 3.68 [ $\mu\text{mol}$   $\text{Fe}^{2+}$ /g of extract] in gotu-kola herb extract to 32.22 [ $\mu\text{mol}$   $\text{Fe}^{2+}$ /g of extract] in arnica flowers extract, respectively (tab. 2). The glycolic extract of arnica flowers contained the highest flavonoids amount. It also exhibited the highest reducing potential among all extracts. It was observed that the most effective reducing extracts such as arnica flowers,

hawthorn flowers and lungwort herb extracts had several times higher reducing potential than gotu-kola herb, sweet clover herb and buckthorn fruits extracts. The order of reducing potential of plant extracts was as follows: arnica flower > lungwort herb > hawthorn flower > catkin flower > knot-grass herb > Japanese pagoda tree buds > burdock root > buckthorn fruit > sweet clover herb > gotu-kola herb.

Table 2.

Flavonoids content and antioxidant capacity of 10 commercial cosmetic plant extracts

Common name of plant	Flavonoids [mg ECE <sup>1</sup> /g of extract]	FRAP [ $\mu\text{mol Fe}^{2+}$ /g of extract]	TEAC [ $\mu\text{mol Trolox}$ /g of extract]
Arnica	2.15±0.09 <sup>a</sup>	32.22±2.10 <sup>a</sup>	20.53±1.48 <sup>a</sup>
Hawthorn	1.70±0.05 <sup>b</sup>	16.36±0.56 <sup>b</sup>	18.23±0.34 <sup>a</sup>
Lungwort	1.34±0.03 <sup>c</sup>	16.61±0.19 <sup>b</sup>	10.41±0.34 <sup>b</sup>
Catkin	1.02±0.05 <sup>d</sup>	11.78±0.09 <sup>c</sup>	10.51±0.36 <sup>b</sup>
Japanese Pagoda Tree	0.65±0.02 <sup>e</sup>	10.42±0.11 <sup>d</sup>	10.76±1.22 <sup>b</sup>
Burdock	0.62±0.01 <sup>e</sup>	6.95±0.17 <sup>f</sup>	4.91±0.44 <sup>d</sup>
Knot-grass	0.54±0.01 <sup>f</sup>	10.51±0.32 <sup>d</sup>	11.69±0.42 <sup>b</sup>
Gotu-kola	0.33±0.03 <sup>g</sup>	3.68±0.33 <sup>h</sup>	2.28±0.24 <sup>f</sup>
Sweet clover	0.27±0.02 <sup>g</sup>	5.88±0.23 <sup>g</sup>	6.82±0.25 <sup>c</sup>
Buckthorn	0.16±0.01 <sup>h</sup>	6.20±0.11 <sup>e</sup>	3.02±0.15 <sup>e</sup>

<sup>1</sup>ECE – (-)epicatechin

Values (mean ±SD) are average of three independent experiments.

a, b, c... – mean with different letters in columns are statistically different at  $p \leq 0.05$ .

A positive correlation between FRAP values and the flavonoids content in 10 plant extracts was found ( $r=0.931$ ;  $p \leq 0.05$ , fig. 2). The reliable correlation obtained indicates that the reducing power (FRAP) of plant extracts depends on their flavonoids content significantly.

Similarly as observed for FRAP, TEAC values obtained in the TEAC method, respectively (tab. 2) varied widely for plant extracts tested – they ranged from 2.28 [ $\mu\text{mol Trolox}$ /g of extract] in gotu-kola herb extract to 20.53 [ $\mu\text{mol Trolox}$ /g of extract] in arnica flowers extract. The highest TEAC values were observed for arnica flowers, hawthorn flowers and knot-grass herb extracts, which also contained the highest amount of flavonoids. The lowest TEAC values were obtained for gotu-kola herb, buckthorn fruits and burdock root extracts. The order of antioxidant capacity of plant extracts was as follows: arnica flower > hawthorn flower > knot-grass herb > Japanese pagoda tree buds > catkin flower > lungwort herb > sweet clover herb > burdock root > buckthorn fruit > gotu-kola herb.

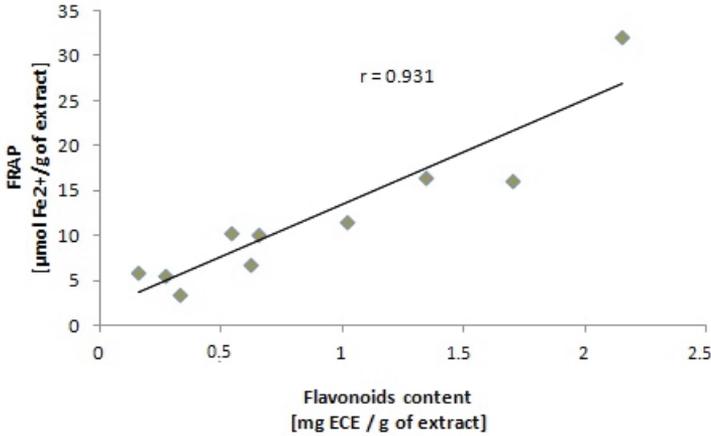


Figure 2.

The relationship between flavonoids content and FRAP values found for plant extracts ( $r=0.931$ ;  $p \leq 0.05$ )

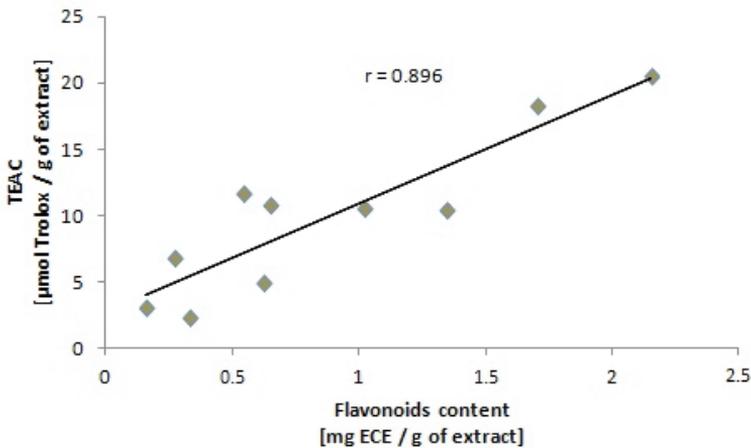


Figure 3.

The relationship between flavonoids content and TEAC values found for plant extracts ( $r=0.896$ ;  $p \leq 0.05$ )

Obtained high correlation between TEAC values and flavonoids content in plant extracts ( $r=0.896$ ;  $p \leq 0.05$ , fig. 3) again indicates that flavonoids are the main antioxidants in researched plant extracts.

On the basis of these results it can be concluded that the higher value of antioxidant activity of plant extract is related to their higher flavonoids content. From the obtained high correlations between flavonoids content and the antioxidant

capacity of plant extracts it may be deduced that we may approximately estimate flavonoids content in researched plant extracts using FRAP and TEAC assays.

Moreover, on the basis of this research commercial plant extracts with the highest antioxidant activity: arnica flowers, hawthorn flowers and lungwort herb extracts can be proposed as a good source of natural antioxidants for cosmetic and pharmaceutical topical formulations.

Regardless from the antioxidant activity, these plant extracts have many other properties, so they can be applied in many kinds of cosmetics (tab. 3). Recently, arnica has become popular as topical treatment in gel or cream form to reduce inflammatory skin conditions, bruising and heal chronic wounds [46]. Literature provides data indicating that it is more popular cosmetic ingredient than hawthorn and lungwort [47], but due to the growth of “natural trend” in cosmetics, the interest in natural resources is growing as well. Therefore, cosmetologists have become interested in plant extracts which were not applied before.

**Table 3.**

Commercial plant extracts with the highest antioxidant activity – cosmetic properties and the application in cosmetic formulations

Plant extract	Properties	Application of plant extracts by cosmetic producers [48]
Arnica	<ul style="list-style-type: none"> <li>- regenerating - reducing flaking and restoring suppleness [49, 50]</li> <li>- sealing capillary vessels [50, 51]</li> <li>- anti-reddening [51]</li> <li>- anti-swelling [50, 52, 53, 54]</li> <li>- anti-inflammatory [50, 52, 55]</li> <li>- antimicrobial [56]</li> <li>- astringent [57]</li> <li>- soothing [57]</li> <li>- antiseptic [51]</li> <li>- UV protective [58]</li> <li>- stimulate the hair follicles [51]</li> </ul>	<ul style="list-style-type: none"> <li>- anti-couperose products</li> <li>- anti-inflammatory and regenerating cosmetics</li> <li>- stimulating shampoos</li> <li>- shower and bath gels</li> <li>- foot care cosmetics</li> <li>- deodorants</li> </ul>
Hawthorn	<ul style="list-style-type: none"> <li>- anti-irritant [59]</li> <li>- anti-inflammatory [60]</li> <li>- anti-couperose [59]</li> <li>- anti-itching [51]</li> <li>- UV protective [60]</li> <li>- skin and hair regenerating and stimulating [61, 62]</li> </ul>	<ul style="list-style-type: none"> <li>- sensitive skin care products</li> <li>- shampoos and conditioners for dry, damaged hair</li> <li>- anti - dandruff shampoos and conditioners</li> <li>- relaxing shower and bath gels</li> </ul>
Lungwort	<ul style="list-style-type: none"> <li>- regenerating [62, 63]</li> <li>- anti-irritant [50]</li> <li>- anti-inflammatory [62, 63]</li> <li>- anti-bacterial [62, 63]</li> </ul>	<ul style="list-style-type: none"> <li>- anti-acne products</li> <li>- oily and sensitive skin care cosmetics</li> <li>- regenerating products</li> </ul>

These plant extracts can be a good solution for anti-allergic cosmetics, which are made of only a few compounds in order to avoid allergy. In these cosmetics

only one plant extract can be used instead of few synthetic compounds, but care effect will be the same.

Nowadays, a few hundreds of plant extracts with various flavonoids content, different antioxidant activity and simultaneously various quality are available on Polish cosmetics market. In the research conducted it was found that some plant extracts known generally for high flavonoids or ascorbic acid content, such as buckthorn fruits extract, in this work showed very low antioxidant activity. Similarly extracts from exotic plants, such as Japanese Pagoda Tree buds or gotu-kola herb, also revealed relatively low antioxidant activity. The reason may be the quality of the available commercial cosmetic plant extracts. The problem can be bound with low quality of some of them. It is supposed that it can be caused by extraction method of active ingredients from the plant raw material or by excessive dilution of the extracts by their producers.

## CONCLUSION

Commercial cosmetic plant extracts are available and used in cosmetic industry, however, their flavonoids content and the antioxidant capacity vary among different extracts significantly.

As a result of this research it was found that antioxidant capacity of plant extracts is significantly affected by their flavonoids content. The highest antioxidant activity, both in FRAP and TEAC assays, was observed for: arnica flowers, hawthorn flowers and spotty bugloss herb extracts. They can be used as natural antioxidants for prolonging the stability of cosmetic products and replace synthetic antioxidants such as BHT or BHA, which can cause skin allergy.

In this work, it was turned out that FRAP and TEAC methods can be effective and credible tests for evaluation the antioxidant activity of commercial plant extracts used in cosmetics industry. FRAP and TEAC values are sufficiently good parameters to assess the quality of plant extracts used in cosmetics industry, in relation to their flavonoids content. These methods could be successfully applied for quick, preliminary quality evaluation of plant extracts. It is very important, as the quality of cosmetics depends on the quality of their active ingredients.

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## WPLYW ZAWARTOŚCI FLAWONOIDÓW NA AKTYWNOŚĆ PRZECIWUTLENIAJĄCĄ HANDLOWYCH KOSMETYCZNYCH EKSTRAKTÓW ROŚLINNYCH

PAULINA MALINOWSKA

Katedra Technologii i Analizy Instrumentalnej  
Wydział Towaroznawstwa  
Uniwersytet Ekonomiczny w Poznaniu  
Aleja Niepodległości 10  
61-875 Poznań

e-mail: paulina.malinowska@ue.poznan.pl, tel.: +4861 8569054

### Streszczenie

Celem badań była ocena aktywności przeciwutleniającej i zawartości flawonoidów w 10 handlowych kosmetycznych ekstraktach roślinnych stosowanych w przemyśle kosmetycznym. Ich aktywność przeciwutleniającą zmierzono przy użyciu dwóch metod: Ferric Reducing Antioxidant Power (FRAP) i Trolox Equivalent Antioxidant Capacity (TEAC). Ponadto sprawdzono zależność między zawartością flawonoidów w ekstraktach roślinnych a ich aktywnością przeciwutleniającą. Stwierdzono, iż wartości wskaźników FRAP i TEAC wyznaczone dla ekstraktów roślinnych w sposób istotny zależą od zawartych w nich flawonoidów. Najwyższą aktywność przeciwutleniającą, zarówno w teście FRAP, jak i TEAC, zaobserwowano dla ekstraktu z arniki górskiej, głogu jednoszyjkowego i miodunki plamistej. Ekstrakty te mogą być użyte jako źródła naturalnych przeciwutleniaczy dla przedłużenia stabilności oksydacyjnej produktów kosmetycznych i ponadto mogą zastępować syntetyczne przeciwutleniacze.

**Słowa kluczowe:** ekstrakty roślinne, aktywność przeciwutleniająca, FRAP, TEAC, kosmetyki