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ANTIRADICAL ACTIVITIES OF THE EXTRACT OF *PASSIFLORA INCARNATA*

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Abstract: The objective of this work was to investigate the aqueous and ethanolic extracts of passionflower and the influence of the flavonoids they contain on the antiradical activity by DPPH· and ABTS·+ methods. The data show that the *Passiflora* extract has not only sedative but also antiradical activity. The ethanol extract catches free radicals more effectively than the water extract. The strongest antiradical effect among the investigated flavonoids (chlorogenic acid, hyperosid, isovitexin, caffeic acid, quercetin, luteolin, orentin, rutin, scutelarein, vicenin and vitexin) was predetermined by vicenin, isovitexin and orentin. The antiradical activity increases with the increase of the concentration of the mentioned materials.

Keywords: *Passiflora incarnata,* flavonoids, free radical scavenging activities

Multiple studies show that herbal preparations of the passionflower are widely used in medicine as sedatives and tranquilizers (1). It has been determined that *Passiflora incarnata* lowers the level of HDL and can be used as a prophylactic means against atherosclerosis. It has an anti-atherogenic and cardioprotective effect (2, 3). The wide range of pharmacological action of passionflower is determined by its bioactive ingredients: alkaloids and flavonoids (4). Flavonoids have been shown to act as scavengers of various oxidizing species i.e. superoxide anion ($O_2^{\cdot-}$), hydroxyl or peroxy radicals (5). They may also act as quenchers of singlet oxygen (6). Another possible contributory mechanism to the antioxidant activity of flavonoids is their ability to stabilize membranes by decreasing membraine fluidity (5). It is important to neutralize the free radicals, which are the products of the metabolic processes, since they may play a role in cardiac insufficiency. The neutralization can be performed not only by the antioxidant protective enzymes but also by different bioactive materials found in herbal preparations. There are multiple data in literature, which show that bioactive compounds neutralize free radicals in different ways. The data how free radicals interact with *Passiflora incarnata,* and the data how its antiradical activity is predetermined by flavonoid ingredients, were not found. Therefore, it is an important field of investigation, since *Passiflora incarnata* is a popular ingredient in numerous phytopharmaceutical preparations.

The objective of this work was to investigate the aqueous and ethanolic extracts of passionflower and the influence of the flavonoids they contain on the antiradical activity by DPPH· and ABTS·+ methods.

EXPERIMENTAL

Plant material

The herb of *Passiflora incarnata* was harvested from the collection of the medicinal plants in Kaunas Botanical Garden (Vytautas Magnus University, Lithuania). The raw material was sorted out and dried at ambient temperature in a dry room with active ventilation.

Flavonoids

These flavonoid standarts (chlorogenic acid, hyperosid, isovetixin, caffeic acid, quercitin, luteolin, orentin, rutin, scutelarein, vitexin) were used for this experiment (producer Carl Roth GmbH, Karlsruhe, Germany).

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Extraction

Aqueous extraction: dried and crushed passionflower herb (500 g) was extracted by boiling water (5.0 L) for 20 min in a water bath shaker (Shaking Bath 5B-16) (Techne Ltd., UK). After cooling the extract was centrifuged at 5000 rpm for 10 min and filtered by a Millipore filter with a nylon membrane under vacuum at 25°C. The filtrate was stored at 4°C until use within 24 h (7).

Ethanol extraction: 50.0 g of raw material was extracted with 100 mL of 70% ethanol by the method of repercolation.

Evaluation of antioxidant activity

2.2-Diphenyl-1-picrylhydrazide (DPPH·) radical scavenging assay

Free radical scavenging capacity was measured using the radical chromogen 2.2-diphenyl-picrylhydrazide photometric assay (8). The antioxidant activity of herbal tinctures and flavonoids was determined by measuring what percentage of DPPH· radical is needed to neutralize the investigated specimens of compounds with antioxidant activity. 1 mL of 0.1 mM methanol DPPH· solution is mixed with the investigated preparations. In 5 min after mixing the diminution of absorption at 517 nm was measured. The antiradical activity is measured by the percentage of inactive DPPH:

inactive percentage = ((Ab-Aa)/Ab)×100

where : Ab – the absorption of a blank specimen (t = 0 min), Aa – the absorption of the specimen of the investigated material.

2.2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS·+) radical cation decolorization assay

ABTS·+ radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature (23°C) in dark for 16 h. The ABTS+ solution was diluted with 80% ethanol to an absorbance of 0.700 ± 0.050 at 734 nm. The filtered sample was diluted with ethanol to give 20-80% inhibition of the blank absorbance with 0.1 mL of sample. ABTS·+ solution (3.9 mL; absorbance of 0.700 ± 0.050) was added to 0.1 mL of the tested samples and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 10 min and the absorbance was recorded at 734 nm. The antioxidant activity was measured by ABTS·+ inactivity percentage according to the same formula as when applying DPPH· radical connection method (8).

The quantitative measurement of phenolic compounds

This was estimated using the Folin-Ciocalteau colorimetric method described previously with a little modification (9). Briefly, the appropriate dilutions of the filtered extracts were oxidized with 0.2 M Folin-Ciocalteu reagent and then the reaction was neutralized with saturated sodium carbonate (75 g/L solution). The absorbance of the resulting blue color was measured at 760 nm after incubation for 2 h at 23°C. Quantification was done on the basis of the standard curve of gallic acid. The results were expressed as gram of gallic acid equivalent (GAE) per 100 mL weight of herbal tincture.

Determination of the amount of flavonoids using high-performance liquid chromatography (HPLC)

For the study, we used the chromatographic system Waters 2690 with UV/Vis detector Waters 2487 (Waters, Milford, USA), column XTerra RP18 150 × 3.9 mm, 3.5 µm. The mobile phase A was 0.1% aqueous trifluoroacetic acid (TFA) solution, and the mobile phase B – 0.1% TFA solution in acetonitrile. The change in the concentration of the solvents of the phase was a direct gradient from A +5% B to 45% B per 45 min, and the flow rate was 0.4 mL/min. Chromatograms were recorded at 360 and 275 nm wavelength. The amount of flavonoids was calculated according to the peak areas, using flavonoid standard calibration charts.

The preparation of the flavonoid solutions

Identified by HPLC method, flavonoids in ethanol and water extracts of passionflower (Table 1) were dissolved in 1 mL of methanol and used for the investigation of antiradical activity by DPPH· and ABTS·+ methods.

The preparation of flavonoid mixture

The preparation of the mixture of flavonoids found in ethanol extract of the passionflower: 284 µg of chlorogenic acid, 178 of µg hyperosid, 4963 µg of isovitexin, 175 µg of caffeic acid, 7 µg of quercetin, 21 µg of luteolin, 3299 µg of orentin, 164 µg of rutin, 42 µg of scutelarein, 6102 µg of vicenin and 1436 µg of vitexin were dissolved in 1 mL of methanol and used for the investigation of the antiradical activity.

The preparation of the mixture of flavonoids found in aqueous extract of the passionflower: 26 µg of chlorogenic acid, 14 µg of hyperosid, 410 µg of isovetixin, 11 µg of caffeic acid, 0.2 µg of quercetin, 0.75 µg of luteolin, 12 µg of rutin, 465 µg of vicenin and 96 µg of vitexic were dissolved in 1 mL of ethanol for the investigation of the antiradical activity.

Data analysis

Statistical analysis was performed using statistical software package Statistica 5.5. The data were

presented as the means ± S.E.M. Statistical analysis was performed using Student's *t* test, and $p < 0.05$ was used as the level of significance.

RESULTS AND DISCUSSION

The flavonoids from *Passiflora incarnata* were extracted with water and 70% ethanol, because they are usually used for making *Passiflora* preparations – teas, tinctures and extracts. Y. Cai et al. investigated 112 Chinese medicinal plants using water and methanol as extracting materials (10). G. Miliauskas et al. used for extraction three solvents: acetone, ethyl acetate and methanol (11).

Antiradical activity of the preparations was investigated by DPPH. and ABTS.+ methods. So the most common and reliable method involves the spectrophotometric determination of the disappearace of free radicals (12). The scientists from Italy investigated the methanol passiflora extract, which was made from five species of *passiflora* obtained by zygotic embryo culture and evaluated for their capacity to quench DPPH· and ABTS· radicals (13). They determined that only *P. nitida and P. palmeri* also showed high antioxidant activity (14).

The results obtained by us show that the aqueous extract of passionflower (APE) and ethanol passionflower extract (EPE) reliably neutralized the free radicals, what was statistically significant if compared with the control group. EPE conjugated the free radicals by DPPH· method 2.5 times, while ABTS·+ – 2.3 times stronger than APE (Figure 1).

Zheng and Wang determined that phenolic compounds had a major contribution to antioxidant activity (15). There are scientific papers which show that the quantity of phenolic compounds is directly proportional to antioxidant activity (16). Other literature sources do not show the direct correlation between the quantity of phenolic compounds and the antioxidant activity., e.g. Masteikova et al. determined that *Ginkgo biloba* tincture contained phenolic compounds at higher concentration than in *G. biloba tinctura*, while its antioxidant activity was much smaller than in Ginseng and Echinacea (17). It was found that in EPE phenolic compounds were higher (1476 ± 33 mg/100 mL) in comparison with

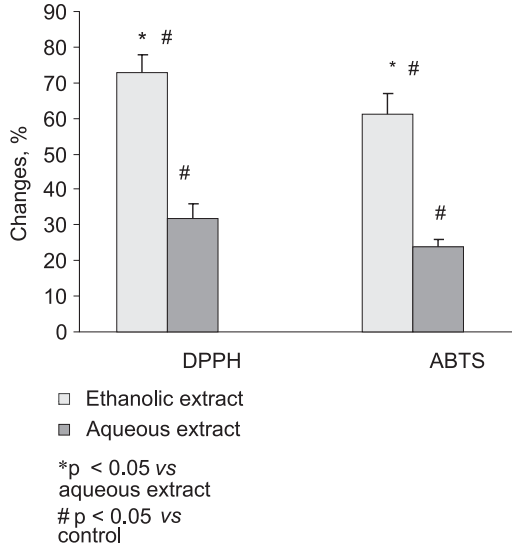

Figure 1. The antiradical activity of ethanolic and aqueous extracts as investigated by DPPH· and ABTS·+ methods, n = 4-5.
*p < 0.05 *vs.* aqueous extract

Table 1. Main components of ethanolic and aqueous *Passiflora* extracts, n = 4

Component	Ethanolic passiflora extract µg/mL	Aqueous passiflora extract µg /mL
Chlorogenic acid	284 ± 7.0	26 ± 3.0
Hyperoside	178 ± 8.0	14 ± 0.6
Isovitexin	4963 ± 142.0	410 ± 0.7
Caffeic acid	175 ± 11.0	11 ± 2.0
Quercetin	7 ± 0.5	0.2 ± 0.0
Luteolin	21 ± 4.0	0.75 ± 0.0
Orentin	3299 ± 89.0	287 ± 12.0
Rutin	164 ± 9.0	12 ± 2.0
Scutelarein	42 ± 3.0	-
Vicenin	6102 ± 45.0	465 ± 8.0
Vitexin	1436 ± 21.0	96 ± 5.0

APE (138 ± 11 mg/100 mL) and bigger antiradical activity ($p < 0.05$) (Figure 1). Literary sources confirm that the antioxidant activity of phenolic compounds depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic molecules and the activity of the phenolic compounds depends on their chemical structure (18), therefore the flavonoids in the passionflower extracts were identified (Figure 2, Table 1). HPLC results show that the identified chemical compounds – flavonoids – and their quantities are similar to the chemical structure of the passionflower extract mentioned in other scientific papers (3). Mostly vicenin, vitexin and isovitexin were found in EPE and APE and least of all quercetin.

In further investigations the attempt was made to determine whether free radicals are destroyed by flavonoids found in the tinctures (Figure 3). The concentration of flavonoids used in this experiment was identical to the quantity of active ingredients in the investigated preparations. The concentration of vicenin (6102 µg/mL), isovitexin (4963 µg/mL) and orentin (3299 µg/mL) bind the free radicals statistically more significantly than the concentration of

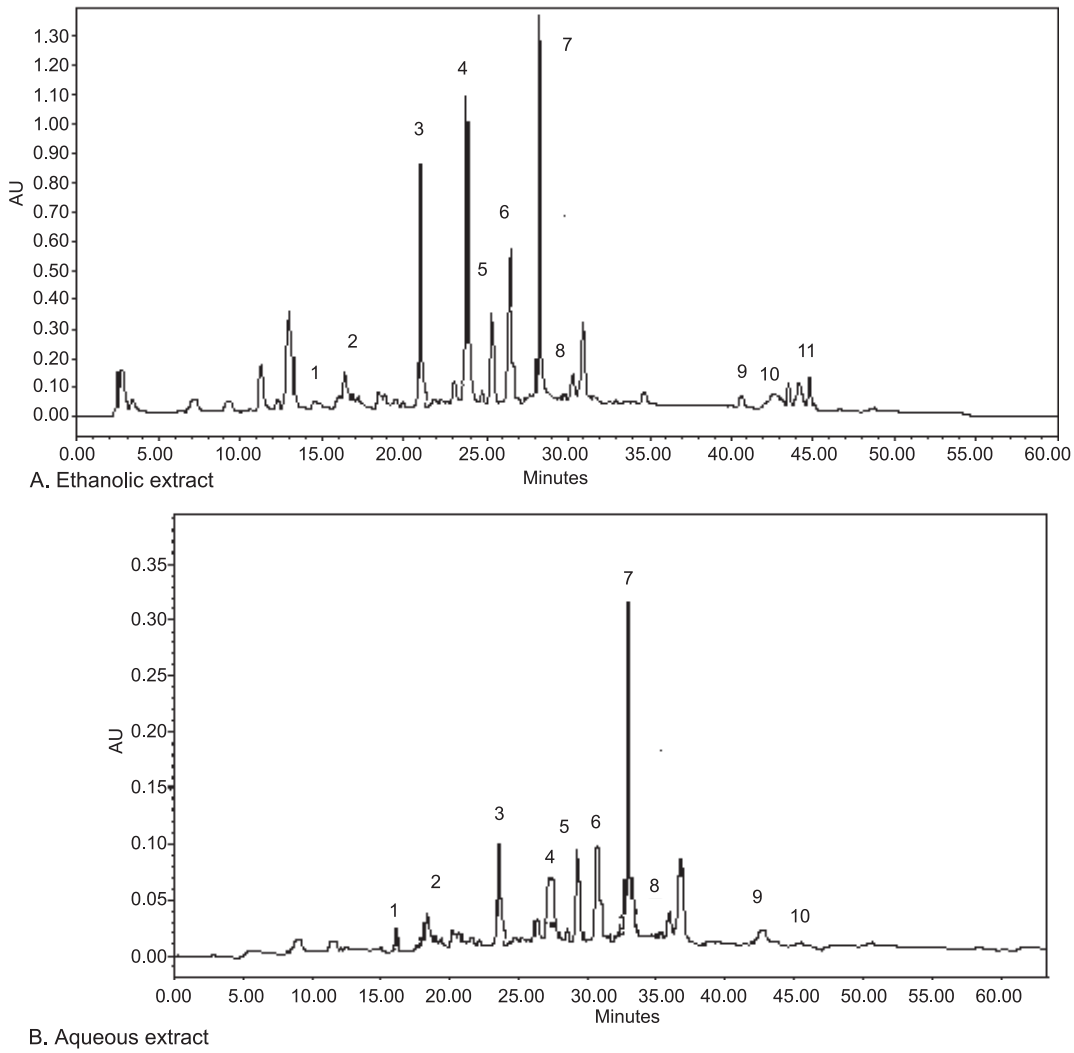

Figure 2. Chromatogram of flavonoids in ethanolic (A) and aqueous (B) *Passiflora* extracts under the optimal conditions. Column XTerra RP18; mobile phase, 0.1% aqueous trifluoracetic acid solution and 0.1% trifluoracetic acid solution in acetonitrile; flow rate 0.4 mL/min; injection volume, 20 µl; detection, 360 nm.
Peak 1, chlorogenic acid; peak 2, caffeic acid; peak 3, vicenin; peak 4, orentin; peak 5, vitexin; peak 6, rutin; peak 7, isovitexin; peak 8, hyperosid; peak 9, luteolin; peak 10, quercetin; peak 11, scutelarein.

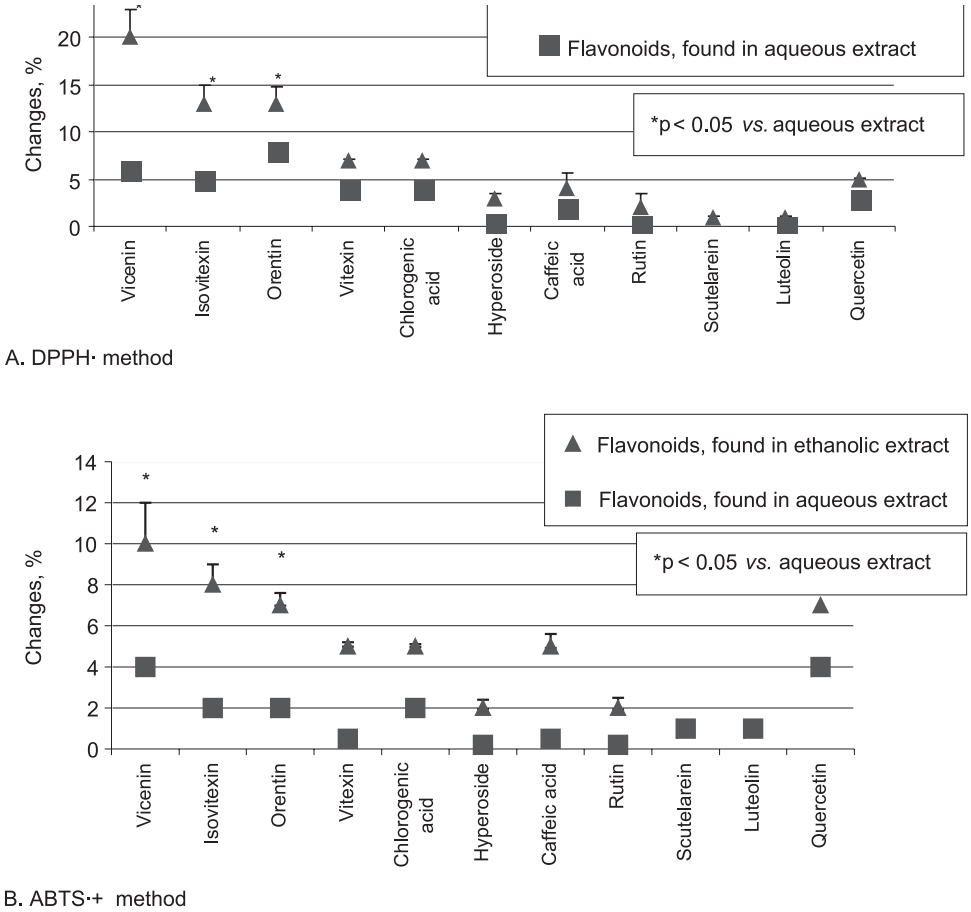

Figure 3. The antiradical activity of flavonoids, as investigated by DPPH· (A) and ABTS·+ (B) methods. *p < 0.05 *vs*. aeqeuos extract, n = 4.
The flavonoid concentrations, identified in ethanolic and aqueous extract are shown in Table 1.

flavonoids found in the aqueous extractof the passionflower, i.e. vicenin 465 µg/mL, isovitexin 410 µg/mL and orentin 287 µg/mL.

There are data which show that flavonol aglycone, quercetin, has a lot of hydroxyl groups and its antioxidant activity is higher than of its glycosides such as rutin (19). Our results confirm this fact, since we found 23 times smaller quantity of quercetin in EPE than that of rutin ($p < 0.05$), but the antiradical activity of quercetin was the same as that of rutin (Figure 3). The scientists determined that quercetin is found to be the most active of the flavonoids and many medicinal plants owe much of their activity to their high quercetin content (19). In addition, it exerts potent antioxidant activity and vitamin C-sparing action. The results of the investigation show that quercetin has very high antiradical activity, because 7 µg/mL and 0.2 µg/mL of quercetin had more antiradical activity than 42 µg/mL of scutalerein and 21 µg/mL of luteolin.

Many papers prove the antioxidant activity of hyperoside (20). It was found that hyperoside most effectively protects the low density lipoprotein oxidation in humans (20). It was also determined that this flavonoid used in concentrations from 100 µg/mL to 160 µg/mL, effectively reduces the concentration of hydrogen peroxide in cancerous cells (18). In EPE and APE, 178 µg/mL and 14 µg/mL of hyperoside was found, respectively, and its antiradical activity, as determined by two investigations, was not statistically significantly different from chlorogenic acid (284 µg/mL (in EPE) and 26 (in APE) µg/mL and caffeic acid (175 µg/mL (in EPE) and 11µg/mL (in APE) antiradical activity. So the

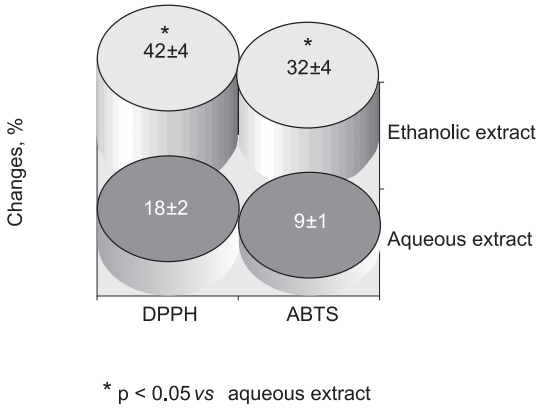

Figure 4. The antiradical activity of flavonoid mixture found in ethanolic and water passionflower extracts, n = 4.
The flavonoid ingredients found in 1 mL aqueous *Passiflora* extract: 26 μg chlorogenic acid, 14 μg hyperosid, 410 μg isovitexin, 11 μg caffeic acid, 0.2 μg quercetin, 0.75 μg luteolin, 287 μg orentin, 12 μg rutin, 465 μg vicenin and 96 μg vitexin
The flavonoid ingredients found in 1 mL ethanolic *Passiflora* extract: 284 μg chlorogenis acid, 178 μg hyperosid, 4963 ěg isovitexin, 175 μg caffeic acid, 7 μg quercetin, 21 μg luteolin, 3299 μg orentin, 164 μg rutin, 42 μg scutelarein, 6102 μg vicenin and 1436 μg vitexin.

data are identical with the data of other investigators. They show the antiradical activity of hyperoside.

In further investigation the antiradical activity of separate flavonoids in mixtures, found in EPE and APE, was investigated (Figure 3). APE and EPE by DPPH· method were catching the free radicals 1.33 times and 3.5 times more actively ($p < 0.05$) and by ABTS·+ method 1.45 and 2.28 times more actively ($p < 0.05$) in comparison with flavonoids identified in APE and EPE mixtures (Figures 1 and 4). This allows an assumption that the antiradical activity is also predetermined by other biologically active substances, which can be found in raw medicinal material. The aforementioned scientists determined that the antioxidant activity of *Passiflora* herb correlated with high amounts of *o*-diphenol and catechin (20). Since we were unable to find the data about the influence of different flavonoid concentrations on the antiradical activity, we cannot compare the obtained results with the results obtained by other investigators.

CONCLUSIONS

1. The data of the investigation show that the *Passiflora* extract has not only sedative but also antiradical activity. The ethanol extract binds free radicals more effectively than the water extract.

2. The strongest antiradical effect among the investigated flavonoids (chlorogenic acid, hyperosid, isovitexin, caffeic acid, quercetin, luteolin, orentin, rutin, scutelarein, vicenin and vitexin) was predetermined by vicenin, isovitexin and orentin. The antiradical activity increases with the increase of the concentration of the mentioned materials.

3. Quercetin catches the free radicals more effectively than rutin, scutelarein and luteolin.

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