ORIGINAL ARTICLE

CONTRIBUTIONS TO THE PHARMACOGNOSTICAL AND PHYTOBIOLOGICAL STUDY OF *PRUNUS PERSICA* (L.) BATSCH FLOWERS

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Abstract

The flowers of *Prunus persica* (L.) Batsch, a species with nutritive and therapeutical importance, were studied. The specific morpho-anatomical characteristics were established: pointy multicellular trichomes (sepal), epidermis with striated cuticle and papillae with rounded tips (petal). The following active substances were identified: reducing compounds, mucilage, polyphenolcarboxylic acids, flavones, condensed tannins, triterpenes. Flavones and polyphenolcarboxylic acids were assayed (105.033 ± 4.228 mg *per* 100 g herbal drug expressed as rutin; 1.605 ± 0.181 g *per* 100 g herbal drug expressed as caffeic acid, respectively). A significant antioxidant activity was established (0.130 ± 0.01 mmols Trolox/g herbal product). The aqueous extract of flowers significantly inhibited the growth of embryonic *Triticum* radicles at 5.00%, 3.33% and 1.67% concentrations and showed genotoxicity on root tip cells.

Rezumat

S-au luat în studiu florile de *Prunus persica* (L.) Batsch, specie cu importanţă alimentară, dar şi terapeutică. S-au stabilit caractere morfo-anatomice specifice: peri tectori unicelulari, cu vârf ascuţit (sepală), epidermă cu cuticulă striată, papile cu vârf rotunjit (petală). S-au decelat: compuşi reducători, mucilagii, acizi polifenolcarboxilici, flavone, taninuri catehice, triterpene. S-au analizat cantitativ flavonele (105,033 ± 4,228 mg la 100 g produs vegetal, exprimate în rutozidă), acizii polifenolcarboxilici (1,605 ± 0,181 g la 100 g produs vegetal, exprimaţi în acid cafeic) şi s-a determinat acţiunea antioxidantă (0,130 ± 0,01 mmoli Trolox/g produs vegetal). Extractul apos a inhibat semnificativ creşterea radiculară la concentraţiile 5,00%, 3,33% şi 1,67%, prezentând şi genotoxicitate asupra celulelor din vârful radicular.

Keywords: *Prunus persica* (L.) Batsch, flavones, polyphenolcarboxylic acids, genotoxicity

Introduction

Prunus persica (L.) Batsch is a tree of the *Rosaceae* Family, native of China and grown in temperate zones for its fruits with nutritional and therapeutic value. Fruits are considered functional food due to their low caloric content and high levels of anti-oxidants, vitamins, minerals and fibres with importance in preventing the onset of oxidative stress and degenerative diseases, including cardiovascular diseases and cancer [2, 15]. For fruit polyphenols, the ability to reduce the viability of tumour cells and inhibit their proliferation without affecting normal cells was reported [28]. *In vivo* preclinical studies showed anti-allergic and anti-inflammatory effects [23]. The polysaccharides from fruits have shown an immuno-stimulatory effect due to the activation of macrophages [25]. The aqueous extract obtained from the seeds has inhibited the cholinesterase, with potential application in the treatment of the Alzheimer disease [26]. Seed glycosides (amygdalin, prunasin) showed a comparable antitumour effect with epigalocatehin from green tea [6]. The seeds are traditionally used in Korea, Japan, China and other Asian countries as anti-asthma, antitussive, emollient, laxative, analgesic and sedative [15]. The methanolic extract from the bark is anti-bacterial and antioxidant [21]. The bark is traditionally used as a demulcent, diuretic, expectorant and sedative [15]. An antioxidant action has been demonstrated for leaves conferred by prunasin, quercetin and kaempferol

[10]. A preclinical experiment also showed a hypoglycaemic effect by inhibiting glucose absorption in the small intestine in mice [24]. Leaves are traditionally used as astringent, demulcent, diuretic, expectorant, febrifuge, laxative, vermicide, sedative and healing [15].

Peach flowers, which have been less studied, were shown to provide photoprotection against UV radiation when applied topically, the compounds responsible for this action being kaempferol glycosides, including multiflorin B [8, 11, 12]. Flowers are used in the traditional medicine as a diuretic, sedative, vermifuge and laxative [15].

The objectives of this study have been to establish the morphological and anatomical characters of flowers, identify the main classes of active compounds, quantitatively determine flavones and polyphenolcarboxylic acids. The study has also aimed to determine the antioxidant action of a methanolic flower extract and establish the genotoxicity of an aqueous extract by performing the *Triticum* bioassay.

Materials and Methods

Plant material and extract preparation

The peach flowers were collected from Bucharest in May 2014. To establish the morpho-anatomical flower characteristics, a botanical macroscopic and a microscopic examination on surface preparations (partially clarified with sodium hydroxide 80 g/L) were performed. The main active compounds were identified by specific chemical reactions in solutions obtained with solvents with different polarities: ethyl ether, alcohol and purified water.

Considering that in the literature several polyphenols (flavones, caffeic acid derivatives) are cited for their antioxidant potential [2], we carried out a phytochemical characterization of the raw material by assaying flavones and polyphenolcarboxylic acids using appropriate spectrophotometric methods.

The powdered raw material was extracted two times sequentially with 70% methanol (v/v) by refluxing for 20 minutes. The extractive solutions were separated by filtering and then combined, followed by filling up to the mark, the volumetric flask, with 70% methanol in order to obtain the assay solution (a final raw material: solvent ratio of 5:100). Five such replicates were carried out.

Methods

Flavone assay was carried out by a spectrophotometric method based on the capability of the flavonoid derivatives to form chelates with aluminium chloride in a medium of sodium acetate [7]. The intensity of the yellow colour of the complex was measured at 427 nm, after 40 minutes, using a UV-VIS Cecil 2000 spectrophotometer. The extinctions were interpolated on a calibration curve constructed with known amounts of rutin (linearity range: 1.0 - 4.0 μg/mL, $R^2 = 0.998$, n = 5).

The assay of polyphenolcarboxylic acids was performed by a spectrophotometric method based on the ability of caffeic acid derivatives to form nitroso-derivatives (nitrous acid being formed *in situ* by the reaction of sodium nitrite with hydrochloric acid) and their conversion to oximes (in an alkaline medium of sodium hydroxide) [7]. The intensity of oxime red colour was read with a spectrophotometer Cecil 2000 at 510 nm, within 5 minutes. The extinctions were interpolated on a standard curve constructed with known amounts of caffeic acid (range 13.36 to 66.80 μg/mL, $R^2 = 0.999$, n = 5). The final results of spectrophotometric measurements were expressed as mean values ± 95% confidence intervals, computed on 10 independent results (two replicates from each extractive solution).

The TEAC (Trolox equivalent antioxidant capacity) assay is based on the ability of antioxidant molecules to quench the long-lived ABTS, a blue-green chromophore with maximum absorption at 734 nm, compared with that of Trolox (a water-soluble vitamin E analogue). The addition of antioxidants to the preformed radical cation reduces it to ABTS determining a decolourization. A stable stock solution of ABTS was produced by reacting a 7 mmol/L aqueous solution of ABTS with 2.45 mmol/L potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12 - 16 h before use. The ABTS working solution was obtained by the dilution in ethanol of the stock solution to an absorbance of 0.70 ± 0.02 AU at 734 nm, verified by an UV-VIS spectrophotometer (UV mini 1240, Shimadzu, Kyoto, Japan). Results were expressed as TEAC in mmol of Trolox *per* g of sample [18, 19].

The effects of an aqueous extract obtained from flowers by refluxing with water for 30 minutes (100ºC) on plant cell division were evaluated using a *Triticum* bioassay (Constantinescu method). The method has been described in detail elsewhere [4]. Five test concentrations (5.00, 3.33, 1.67, 0.33 and 0.03%) and a negative control (distilled water) were used. The inhibition index was calculated according to R. Ancuceanu *et al*., on median values [1].

Statistical analyses

Statistical analysis was carried out on values measured in the third day, with the open source R software package, v. 3.1.3 and several R packages ("car", "fBasics", "simpleboot", "WRS2") [5, 17, 20, 22], graphs were generated with the ggplot2 and wq packages [9, 29]. Normality distribution of residuals was assessed visually by q-q plots, histograms and boxplots and objectively with the d'Agostino-Pearson, Jarque-Bera and Shapiro-Wilk tests (in all three, $p > 0.22$). Since the assumption of homoscedasticity (as assessed by Levene test) was not confirmed,

multiple group comparisons were carried out by one-way ANOVA with the White correction. Since all values were identical in the first group (5% concentration), the Welch correction could not be used, but for sensitivity analyses we applied the Welch correction on the other four groups and the results were very similar; various modifications of the White correction [3, 16] did not have any discernible impact on the results as compared with the classical White correction. Multiple relative contrasts were assessed with a Tukye-type non-parametric method based on pseudo-ranks and the logit asymptotic approximation (R package "nparcomp") [13]. Effect size for heteroscedastic data was computed using a robust method based on the 20% trimmed mean, according to Wilcox R. and Tian T.S. [30], using the R package WRS2 [17].

Results and Discussion

The macroscopic examination confirms the identity of the raw material based on the correspondence with the characters described in the scientific literature [14].

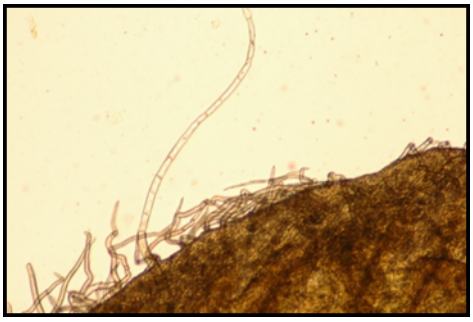

Figure 1.
Pointy multicellular tector hairs (sepal) (ob.4x)

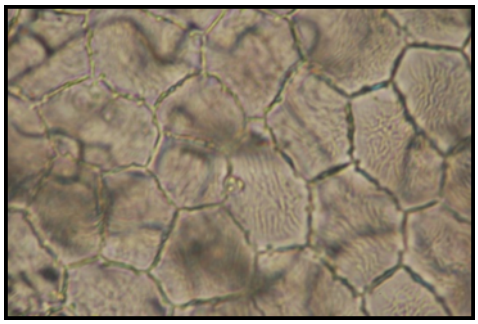

Figure 2.
Epidermis with striated cuticle (petal) (ob.40x)

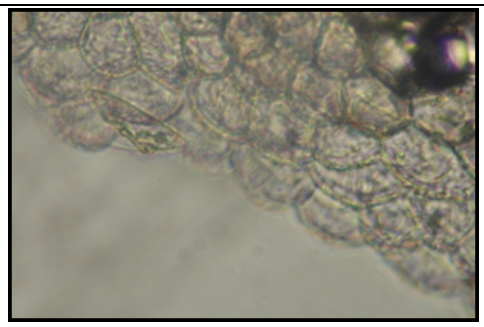

Figure 3.
Papillae (petal) (ob. 40x)

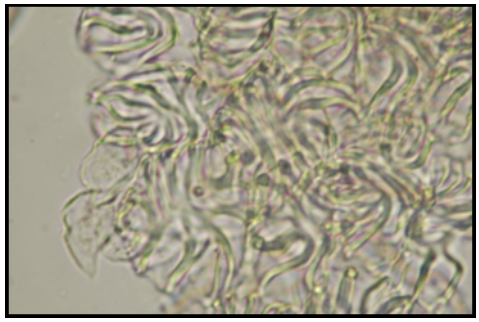

Figure 4.
Endothecium (stamen) (ob. 40x)

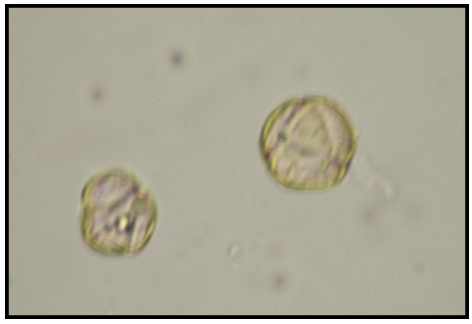

Figure 5.
Pollen grains (stamen) (ob. 40x)

Pointy multicellular trichomes (sepal), epidermis with striated cuticle, papillae with rounded tip (petal), endothecium and pollen grains with three germination pores (stamen) were observed.

Reducing compounds, mucilage, polyphenolcarboxylic acids, flavones, condensed tannins and triterpenes were identified. These compounds are cited by the scientific literature [15].

The results of the assays and antioxidant capacity determination are included in Table I.

Table I

The contents of flavones and polyphenolcarboxylic and antioxidant capacity

No.	Assay	Results (mean ± 95% CI)
1.	Flavones (mg rutin *per* 100 g herbal drug)	105.033 ± 1.512 (n = 10)
2.	Polyphenolcarboxylic acids (g caffeic acid *per* 100 g herbal drug)	1.605 ± 0.063 (n = 10)
3.	Antioxidant capacity (mmoli Trolox/g herbal product)	0.130 ± 0.010 (n = 10)

The flowers of *Prunus persica* analysed have an appreciable amount of flavones and especially polyphenolcarboxylic acids, mainly rutin and caffeic acid derivates. These contents of active principles lead to a significant antioxidant activity demonstrated by ABTS method.

The aqueous extract from flowers significantly inhibited the growth of embryonic *Triticum* radicles at 1.67% and higher concentrations, as compared with the negative control group ($p < 0.022$) (the inhibition index was 58%, 96% and 100% for the 1.67%, 3.33% and 5.00% concentrations, respectively). At lower concentrations (0.03, 0.33) no significant difference was seen among the test concentrations or against the negative control ($p > 0.22$) (Figure 6). The effect size was 0.724, which may be interpreted as a large one (according to the suggestion of Wilcox R. and Tian T.S. [17], based on a proposed equivalence to the Cohen's criteria applied under normality and homoscedasticity). The inhibitory effect is relatively modest, similar to those seen for many other plant extracts. The microscopical examination indicated cytotoxic effects at higher concentrations (5.00%, 3.33% and 1.67%) related probably to the tannin contents, known to be widely spread in *Rosaceae* species [27].

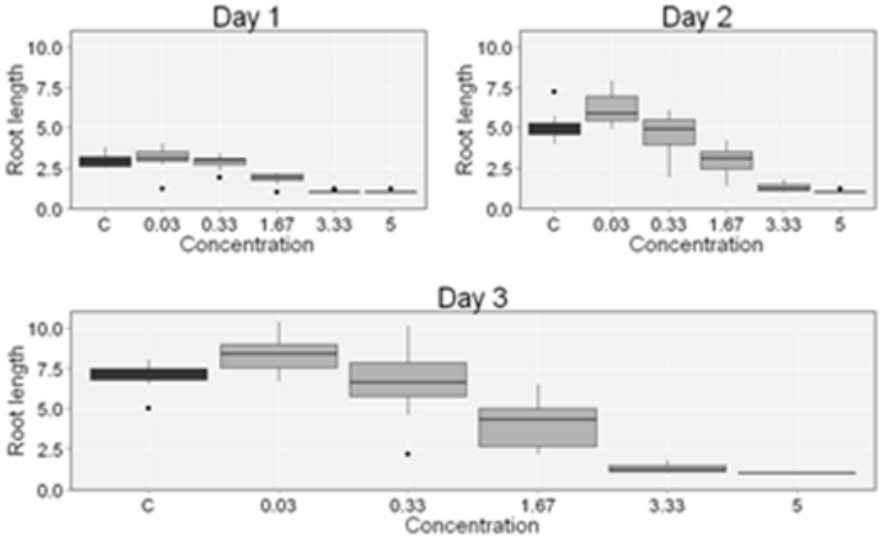

Figure 6.
Boxplots showing the variation of *Triticum* embryonic root length under the influence of an aqueous extract of *Prunus persica* flowers (C = control)

Conclusions

The flowers of *Prunus persica* (L.) Batsch have a moderate content of flavones and a high content of polyphenolcarboxylic acids, with appreciable antioxidant effects, as evaluated by the TEAC method. An aqueous extract has shown moderate genotoxic effects on plant cells, but these were only manifested at high levels of concentration, which suggests that this is an unlikely cause of concern for the potential use in therapeutics.

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