TITLE: Effects of bearberry, parsley and corn silk extracts on diuresis, electrolytes composition, antioxidant capacity and histopathological features in mice kidneys

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A B S T R A C T

The effects of corn silk (*Maydis stigma*), parsley (*Petroselinum crispum*) and bearberry leaves (*Uvae ursi* folium) extracts on diuresis, electrolytes composition , antioxidant capacity and histopathological features of pretreated mice kidneys were determined. The first group of ten animals drank corn silk, second parsley and third bearberry leaf extract. Fourth group was the control when animals drank water. Extracts and water were administrated *ad libitum*. On 0, 1, 7, 14 and 28th day of the experiment urine volume and electrolyte content were measured. Antioxidant status of kidneys was investigated by determining antioxidant enzymes and reduced glutathione quantity. Free radical scavenging capacity, lipid peroxidation and total antioxidant power were determined. Hystopahological examination of kidneys was performed at the end of the experiment. On the basis of the overall presented results parsley and corn silk extracts could be proposed for further investigations as new functional food ingredients with antioxidant and diuretic properties.

Keywords: Maydis stigma, Petroselinum crispum, *Uvae ursi,* pharmacodynamic-estimation, antioxidant-status, histopathological- features

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1. Introduction

Spices and herbs have been used since antiquity as food supplements to improve flavoru of food as well as in traditional medicine in many countries. Nowadays, they have also been the subject of study, particularly by the pharmaceutical and food industries as functional food ingredients because of their potential use for improving health.

Antioxidant properties are among the first links between chemical reactions and biological activity and have been extensively studied during the past years. It is generally accepted that free radicals play an important role in the development of oxidative stress and tissue damage and pathological events (Feher et al., 1987; Auroma 1988; Halliwel & Guteridge, 1989 ; Ahmad & Beg, 2013). In recent years, antioxidants from natural food, spices and herbs have attracted increasing interest compared with chemical antioxidants (Zhang et al.,2014; Luthria et al., 2015). Oxidative stress is frequently associated with, and is partly involved in, the pathogenesis of chronic renal failure, hypertension and their complications. Furthermore chronic kidney disease is a significant risk factor for premature cardiovascular disease and death. Increased oxidative stress in people with chronic kidney disease is a significant risk factor for diabetic nephropathy, premature cardiovascular disease and death (Rojas-Rivera et al., 2012).

There is an increasing interest in the natural antioxidants in different herbs and their role in health and disease (Shahidi, 1997; Štajner et al., 2006). In addition to the protective effects of the endogenous antioxidant defense system, natural products with antioxidant activity could retard the oxidative damage of a tissue by increasing those defenses (Keli et al., 1996; Schinella et al., 2000 ; Štajner et al., 2014-a; Štajner et al., 2014-b). A diet rich in fruits and vegetables has a positive impact on several chronic conditions, such as obesity, diabetes, cancer, cardiovascular, kidney and neurodegenerative diseases. In some cases fruits as functional foods, are consumed due to their antioxidant properties (Gustavo et al., 2013). Herbal medicines, one of the nature's gifts to

50 mankind, are used for many therapeutic and prophylactic effects, among others, as diuretics. Also,
51 medicinal plants are famous for their antioxidant properties. Herbal diuretics with the longest
52 tradition in folk medicine and modern phytotherapy in Europe, Asia and America include corn silk,
53 parsley and bearberry leaves. Investigation of corn silk, parsley and bearberry leaf extracts effects
54 on diuresis and antioxidant status may point toward new therapeutic approach of different chronic
55 conditions. Spices and other food ingredients used in ethnomedicine may be beneficial to the
56 patients with the kidney disease.

57 Kidney is one of vital organs in the body which removes metabolic waste products, such as
58 urea, creatinine and ammonia. Also kidney keeps a balance of extracellular fluid volume,
59 concentration of inorganic electrolytes in the extracellular fluid, extracellular fluid osmolarity, acid-
60 base balance and blood pressure. In addition, kidney plays a role in producing vitamin D and
61 hormones (National Kidney Federation, 2003). If kidney is damaged, it can no longer perform the
62 function of excretion properly, which leads to accumulation of metabolic waste products. In
63 addition, kidney failure is often followed by a variety of other disorders, such as cardiovascular
64 disease, anemia, osteodystrophy, acidosis, etc. The incident of kidney failure increases in the
65 diabetic and hypertension population (CDC, 2010). Treatments in already advanced kidney failure
66 are mainly symptomatic, and only kidney replacement therapy, such as dialysis or kidney
67 transplantation are treatment of choice in terminal stages. Therefore, safe and non-expensive
68 alternative therapies could be use to prevent the progression of kidney disease (Sukandar et al.,
69 2013).

70 Bearberry, parsley and corn silk are selected as plants which extracts are traditionally used as
71 diuretics. Also, recent works pointed the use of these plants as functional food ingredients
72 (Amarawicz & Pegg, 2013; Jia et al., 2014; Sarepoura et al., 2015). However, their biological
73 properties are actually not completely elucidated (Aw et al., 2014).

74 It is well known that bearberry (*Arctostaphylos uva-ursi* L. Sprengel), parsley (*Petroselinum*
75 *crispum*) and corn silk (*Zea mais* L.hair) are used in treatment and prevention of renal diseases
76 (Amarowicz et al., 2004; Markel, 2005). Folk medicine around the world has recommended *Uva*
77 *ursi* for nephritis, kidney stones, and chronic cystitis. Bearberry extract also known as *Uva ursi*, is
78 considered a potent diuretic so it can enhance the excretion of fluids from the body. Bearberry
79 decreases the accumulation of uric acid, a natural component of urine. Uric acid build up may
80 crystallize and get deposited into the kidneys, joints and blood stream. Bearberry supports the
81 membranes of the urinary system and express antioxidant and antimicrobial activities. Due the high
82 tannin content bearberry-leaf extract can decrease lipid oxidation (Carpenter et al., 2007). *Uva ursi*
83 juice is used to prevent some stomach and kidney problems by suppression of lipid peroxidation and
84 mobilizing of antioxidant activity (Shikov et. al., 2014). Although the use of *Uva-ursi* folium has a
85 historic tradition, controlled clinical studies are needed to verify if indeed *Uva ursi* is effective in
86 humans. It is also important to note that *Uva ursi* can be toxic since hydroquinone can cause serious
87 liver damage. Amarawicz and Pegg (2013) indicated that phenolic compounds extracted from
88 bearberry leaves are potential functional food ingredient with antiproliferative activities, especially
89 against colon carcinoma cell line.
90 *Petroselinum crispum* (parsley) aqueous seed extract was reported to produce a diuretic
91 effect in rats. The mechanism of action of parsley seems to be mediated through an inhibition
92 of the Na^+/K^+ pump that would lead to a reduction in Na^+ and K^+ reabsorption leading to an osmotic
93 water flow into the lumen, and diuresis (Kreydiyyeh & Usta, 2002). Also*, Petroselinum crispum*
94 prevented formation of calcium oxalate stones in rats with nephrolithiasis and reduced the number
95 of calcium oxalate deposits (Saeidi et al., 2012). Jia et al. (2014) proposed parsley as a nutraceutical
96 and functional food intervention in inflammatory bowel disease by multi-omics evaluation.

97 Health benefits of corn silk (*Zea mais* L., hair) have been reported in many investigations.
98 Corn silk extract could promote insulin production in animals, support the recovery of the injured
99 cells of the kidney, pancreas and control blood sugar level in rats (Sarepoua et al., 2013.) Corn silk,
100 a diuretic, can increase the flow of urine from the body and may reduce the risk of new stones
101 developing. It was indicated that corn silk might reduce or even prevent renal damage by defending
102 kidney against oxidative stress (Sukandar et al., 2013). Silk of corn, rich in polyphenol compounds
103 can be used as dietary fiber and as a food additive for the prevention of several diseases (Sarepoua et
104 al., 2015).

105 The aim of this study was to investigate corn silk (*Maydis stigma)*, parsley (*Petroselinum*
106 *crispum*) and bearberry leaf (*Uvae ursi* folium) extracts as potential functional food ingredients
107 with the effects on diuresis and electrolyte composition of urine and to evaluate the effects of these
108 extracts on antioxidant capacity of kidneys. Furthermore the effect of *Maydis stigma, Petroselinum*
109 *crispum* and *Uvae ursi* folium extracts on histopathological features of the kidneys of pretreated
110 experimental animals was also examined.

111

112 **2. Materials and methods**

113 *2.1. Chemicals and reagents*

114 Chemicals used in the experiments: 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-
115 *S*-triazine (TPTZ), nitro blue tetrazolium (NBT)**,** quaiacol (2-methoxyphenol), Folin & Ciocalteu-
116 phenol reagent, 2-thiobarbituric acid (TBA), ferric chloride and monobasic potassium phosphate
117 were obtained from Sigma-Aldrich (Beograd, Serbia). Methanol (HPLC, gradient grade), quercetin
118 and formic acid were supplied by Merck KGaA (Darmstadt, Germany). Standard substances
119 including gallic acid, protocatechuic acid, catechin, caffeic acid, vanillic acid, chlorogenic acid,
120 ferulic acid, rutin, quercetin, myricetin, luteolin, kaempferol, apigenin and orientin were purchased

from Sigma-Aldrich GmbH (Sternheim, Germany). Water used throughout the experiments was purified using a Millipore, Elix UV and Simplicity Water Purification System (Milford, MA, USA).

2.2. Experimental animals

The study was conducted on experimental animals body weight of 31-46 g and ages up to 3 months (*Mus musculus* variety albino NMRI), which were selected randomly from the litter of Pasteur Institute in Novi Sad, Serbia. Research was conducted in accordance to the principles established for research in animal models (European Council Directive of November 24, 1986, 86/609/EEC, III-2014-04). Animal care and all experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animal Resources, edited by Commission of Life Sciences, National Research Council, Male and Female Hanover National Medical Institute (Hann NMRI). Mice were bred in the vivarium at the Department of Pharmacology, Toxicology and Clinical Pharmacology, Medical Faculty, University of Novi Sad, Serbia. Animals were kept in the metabolic plexiglas cages at constant room temperature 21 ± 1ºC and humidity 55% ± 1.5%, with circadian rhythm (day/night). They were fed with the standard laboratory mice feed, produced by the Veterinary Institute in Zemun, Serbia. Animals were given free access to food and fluid (water or plants extracts). Experimental animals were divided into four groups of ten animals. During the experiment the first group of ten animals drank corn silk extract, the second group parsley extract, the third group bearberry leaf extract *ad libitum*. The forth group was control group in which animals drank water *ad libitum*. Urine of all groups was collected on 0, 1, 7, 14 and 28h day of experiment, and on the same date urine volume was measured as well as electrolyte contents. All animals were sacrificed under urethane anesthesia. Kidneys were removed and homogenized and after extraction, antioxidant status was determined using different contemporary methods.

2.3. Herbal material extraction

145 Herbal material which was used in this study was of controlled origin (mountain Rtanj,
146 2014) available in retail. The plant leaves were dried in air and ground in a mixer. An amount of 200
147 g of the finely powdered material (0.2-0.3 mm) was extracted with 4 L of 96% ethanol (EtOH),
148 during 72h at room temperature (25ºC). After that, extracts were vacuum evaporated at 40˚C. Dry
149 residues were used to prepare 5% aqueous extracts used in the experiment for polyphenol
150 characterization and total phenol determination.
151
152 *2.4. Biochemical Assays*
153 *2.4.1. Serum urine analyses*
154 Concentrations of blood urea nitrogen and creatinine were estimated as described by
155 Fossati et al. (1980), respectively using specific diagnostic kits (Sigma Aldrich, St. Louis, MO,
156 USA). Serum and urine levels of sodium and potassium were determined as described by Ali (2010).
157
158 *2.4.2. Preparation of kidney homogenate*
159 One gram of the right kidney tissue was collected, washed in ice-cooled 0.9% NaCl and
160 homogenized in ice-cooled 1.15% potassium chloride solution and 50 mM potassium phosphate
161 buffer solution (pH 7.4) to yield 10% homogenate (w/v). Homogenization was performed using
162 ultrasonic homogenizer. The homogenate was then centrifuged at 4000 x g for 5 min at 4°C. The
163 supernatant was collected and kept for further use.
164
165 *2. 5. Assessment of prooxidant / antioxidant activity*
166 Prepared kidney homogenate was used for antioxidant determinations (DPPH RSC, FRAP
167 and ·OH quantity) determination as well as enzymatic assays, glutathione and lipid peroxidation.

168 The superoxide dismutase, SOD activity was determined in aliquots by the method of Misra
169 and Fridovics (1972), based on the inhibition of transformation of adrenaline to adrenochrome at
170 pH 10.2 (Matkovics et al., 1977) ; guaiacol peroxidase GPX activity, using guiacol as substrate
171 (Matkovics et al., 1977).; glutathione peroxidase GSH-Px activity using cumene hydroperoxide and
172 reduced glutathione (GSH) as substrates (Chiu et al., 1976); catalase CAT activity
173 spectrophotometrically at 240 nm (Beers et al., 1952: Iwase et al., 2013); Lipid peroxidation was
174 measured by TBARS (quantities of thiobarbituric acid reactive substances) assay and values were
175 reported as equivalents of malonyldialdehyde (MDA); the calibration curve was prepared with
176 malonyldialdehyde bis-diacetal (Placer et al., 1968); hydroxyl radical by the inhibition of
177 deoxyribose degradation and expressed by nmol $OH^{\bullet}$ in the model system (Cheesman et al., 1988).
178 The quantity of GSH was determined with Ellman reagent (Sedlak & Lindsay, 1968) and
179 protein content according to Bradford (1976).
180 Radical scavenging capacity (RSC) was determined using 1, 1-diphenyl-2-picrylhydrazyl
181 radical ($DPPH^{\bullet}$). Reduction of DPPH radical was determined measuring disappearance of DPPH at
182 515 nm. RSC is expressed by percents compared to the control (Abe & Hirota, 1998). The percent
183 inhibition of the DPPH radical (RSC) by the samples was calculated using the formula:
184 $$RSC= [(A_c-A_x)/A_c] \times 100\ \%$$
185 where A_c is absorbance of the control and A_x is absorbance of the sample after 30 min of incubation.
186 Total antioxidant capacity was estimated according to the FRAP (Ferric Reducing
187 Antioxidant Power) assay (Benzie & Strain, 1999). Total reducing power is expressed as FRAP
188 units. FRAP unit is equal with 100 μmol/dm^3 Fe^{2+}. FRAP value was calculated using formula:
189 $$FRAP\ value=\Delta A_{sample}/\Delta A_{standard}$$
190

191 2.6. *Polyphenol determination in plant extracts*

192 The total phenolic content was determined by a modification of the Folin-Ciocalteu method
193 and the results expressed as mg gallic acid/g extract (Vinson et al., 2003).

194 Ethanolic extracts of the investigated plants (bearberry, parsley and corn silk leaves) were
195 used for HPLC analysis. HPLC analysis was performed by using a liquid chromatograph (Agilent
196 1200 series; Agilent Technologies, Palo Alto, CA, USA), equipped with a diode array detector
197 (DAD), Chemstation Software (Agilent Technologies), a binary pump, an online vacuum degasser,
198 an autosampler and a thermostated column compartment, on an Agilent, Eclipse XDB-C18, 1.8 μm,
199 4.6×50 mm column, at a flow-rate of 1 mL min-1. Solvent gradient was performed by varying the
200 proportion of solvent A (methanol) to solvent B (1% formic acid in water (v/v)) as follows: initial
201 10% A; 0-10 min, 10 -25% A; 10-20 min, 25 - 60% A; 20-30 min, 60-70% A. The total running
202 time and post-running time were 45 and 10 min, respectively. The column temperature was 30ºC.
203 The injected volume of samples and standards was 5 μL and it was done automatically using
204 autosampler. The spectra were acquired in the range 210–400 nm and chromatograms plotted at 280,
205 330 and 350 nm with a bandwidth of 4 nm, and with reference wavelength/ bandwidth of 500/100
206 nm. Content of the investigated plant phenolic compounds in crude extracts is expressed as mg g^{-1}
207 extract (Mišan et al., 2011).

208 *2.7. Histological procedure*

209 Kidney specimens were taken and fixed in 10 % neutral formalin solution. The fixed
210 specimens were dehydrated in ascending grades of alcohol, cleared in xylene. They were embedded
211 in paraffin boxes, sectioned at 4-6 microns thickness, stained with Hematoxylen and Eosin (H&E)
212 and examined microscopically according to Carleton (1976).

213

2.8. Statistical evaluation

All determinations were performed in triplicate and 10 experimental animals. Data were expressed as mean ± standard error (SE). Values were means for ten mice. Statistical comparisons between samples were performed with Duncan t–test (Snedecor & Cohran, 1986), for independent observations were done using STATISTICA 9.1. Differences were considered significant at $p < 0.05$.

3. Results and discussion

The kidneys function is to excrete toxins through the urine and balance out our electrolytes (sodium, potassium, calcium, magnesium, chloride and phosphorous) and amino acid levels. It is especially important for effective elimination of ammonia, which is highly toxic and destructive to brain tissue.

3.1. Pharmacodynamic investigations

Table 1- presents the results concerning urine pharmacodynamic investigations. The effects of 5% water extracts of *Maydis stigma, Petroselinum crispum* and *Uvae ursi folium* on diuresis, K^+ and Na^+ quantities.

Table 1.

On the basis of pharmacodinamic results presented in Table 1, it is possible to observe that the best diuretic effect was achieved on the second day of experiment under parsley influence. It is well known that parsley has high antioxidant ability and also provokes an osmotic water flow into the lumen, and diuresis (Kreydiyyeh & Usta, 2002). The corn silk extract induced maximum diuresis on the first day of the experiment. The lowest diuretic effect was observed under bearberry influence where maximum was also reached on the first day of experiment (Table1).

240 Comparing the results from all three experimental groups after seven days of administration,
241 parsley extract induced the strongest diuretic effect. The best effect on K^+ quantity in urine was
242 observed under the action of parsley and corn silk. After seventh day of experiment, the decrease in
243 K^+ quantity was observed under the action of parsley and corn silk extracts. Bearberry extract
244 induced K^+ quantity increase effect at the first day of administration but smaller comparing to the
245 corn silk and parsley. After seventh days of administration, K^+ quantity induced by bearberry was
246 under the control values. Na^+ quantity reached a maximum at seventh day (parsley extract) or first
247 two days (corn silk). The lowest Na^+ quantity was observed under bearberry action at the first day of
248 administration. After the first day of bearberry extracts administration significant decrease of Na^+
249 quantity comparing to the control group was observed (Table1).
250 The nephroprotective effect of *Petroselinum crispum* herb, reported in the present study, was
251 similar to that reported by Afzal et al., 2004, who found that a polyherbal formulation containing
252 *Petroselinum crispum* (parsley) produced a nephroprotective effect in mice. This effect of
253 *Petroselinum crispum* was attributed to its *in vitro* antioxidant activity as free radical scavenger or
254 due to its high content of flavonoids (Fejes et al., 1998). The diuretic effect of *Petroselinum*
255 *crispum* was reported by Kreydiyyeh and Usta (2002), who found that parsley aqueous seeds extract
256 produced a diuretic effect in rats. The previous authors concluded that the mechanism of action of
257 parsley seems to be mediated through an inhibition of the Na^+/K^+ pump that would lead to a
258 reduction in Na^+ and K^+ reabsorption thus leading to an osmotic water flow into the lumen, and
259 diuresis. Urinary potassium and sodium levels could be changed as the consequence of longer
260 administration of different agents (Ali, 2010), as it happen also under influence of *Maydis stigma*
261 *and Petroselinum crispum* extracts during seven days of administration.
262 In Table 2, the results concerning the effects of extracts of *Maydis stigma, Petroselinum*
263 *crispum* and *Uvae ursi* folium on BUN (blood urea nitrogen) and serum creatinine are presented.

Comparing control values, the best effect in urea and creatinine reduction was observed under the extracts of corn silk and parsley. Bearberry extract reduced urea content comparing to the control, but slightly increased the content of creatinine.

Table 2.

Results presented in Table 2. Supported the results from Table 1. They connected and explained pharmacodynamic effects, with urea and creatinine reduction under the influences of *Maydis stigma* and *Petroselinum crispum* extracts.

3.2. Prooxidant / antioxidant activity

The effects of bearberry, parsley and corn silk extracts, on SOD, CAT, GPx and GSH-Px activities in mice kidneys are presented in Fig. 1. SOD activity was higher in kidneys of mice treated with corn silk extract (16.03 U/mg protein) and lowest in kidneys of mice treated with parsley extract (12.33 U/mg protein). In kidneys of animals which drank bearberry extract (14,05 U/mg protein) SOD was slightly smaller from the control value (14.05 U/mg protein). Statistically significant differences concerning the control value were observed under parsley and corn silk influence. Bearberry extract did not provoke significant effect. Our results implicated that corn silk provoked the most favorable effect to SOD activity what is in agreement with results of other authors (Hua et al., 2010), which proved that *Maydis stigma* had antioxidative and protective role in kidneys and liver of experimental animals (Fig 1A).

Fig 1B. presents the effects of bearberry, parsley and corn silk extracts on CAT activity of mice kidneys . CAT activity was high in kidneys of animals that consumed parsley extract (67.21 nmol H_2O_2/mg protein) and in the control sample (66.72 nmol H_2O_2/mg protein) but lowest in kidneys of animals drank with bearberry extract, 46.85 U/mg protein. Kidneys of animal which drank corn silk extract, exhibited low CAT activity of 49.11 nmol H_2O_2/mg protein. (Fig.1B.).

289 Statistically significant differences concerning control value were observed under bearberry and
290 corn silk influence, though parsley did not provoke significant effect.

291 Fig. 1

292
293 Presented results (Fig.1A. and Fig 1B.) are in the agreement with results that mice kidneys exhibited
294 substantial activities of antioxidant enzymes (Lenzen et al., 1996).

295 The effects of bearberry, parsley and corn silk extracts, on GPx activity of mice kidneys are
296 presented in Fig. 1C. GP_X activity was higher in kidneys of animals which drank bearberry extract
297 (151,01 nmol guajacol/mg protein). GPx activities were substantially lower in kidneys of animal
298 which drank the extracts of other two medicinal plants. The lowest was in kidneys of mice treated
299 with parsley extract, 48.58 nmol guajacol/mg protein. Statistically significant differences concerning
300 the control value were observed under the influence of all used plants extracts. (Fig 1C.). High GPx
301 in kidneys and other mice organs play important role in defense from different inflammatory
302 processes (Duget et al., 2001).

303 GSH-Px activity was high in kidneys of animals which drank corn silk (75.18 nmol GSH/mg
304 protein) and bearberry (69.85 nmol GSH/mg protein) extracts as presented in Fig.1D. Control
305 sample exhibited the smallest GSH-Px activity of 28.93 nmol GSH/mg protein. Statistically
306 significant differences concerning the control value occurred under the treatments with bearberry
307 and corn silk. Our results pointed to high GSH-Px activity in kidneys, what is in connection with
308 enzymic functioning of detoxification (Klivenyi et al., 2000). It was also proved that the use of
309 plants extracts increased activities of antioxidant enzymes in mice (Caia & Weib 1996), and
310 therefore improved abilities to reduce the consequences induced by different types of oxidative
311 stress (Güven & Gülmez 2003), what is in agreement with our results presented in Fig. 1.

312 Effects of *Maydis stigma, Petroselinum crispum* and *Uvae ursi* folium extracts, on reduced
313 glutathione quantity, ferric reducing antioxidant power and soluble protein content in mice kidneys
314 are presented in Fig.2.

315 Fig.2.

316 Quantity of GSH in mice kidneys was affected by the action of all examined medicinal
317 plants Fig. 2A. GSH quantity was high in kidneys of animals which drank corn silk extract (187.14
318 nmol/mg protein) and in the control sample (179.50 nmol/mg protein) and lower in kidneys of
319 animals treated with parsley extract, 143.02 nmol/mg protein. Statistically significant differences
320 concerning the control value occurred under treatment with corn silk. It was proved that high
321 quantities of GSH in kidneys are connected with induced tolerance of mice to oxidative stress (Saito
322 et al., 2010). Oral administration of *Petroselinum crispum* expressed nephroprotective and diuretic
323 effects, as it reversed the biochemical and antioxidant activity as evident by decreasing lipid
324 peroxidation, increasing content of reduced glutathione and restoring activities of antioxidant
325 (SOD, GPx and CAT) enzymes in renal tissue (Shalby et al., 2014).

326 Considerable influence of parsley and corn silk extracts on FRAP values in kidneys of the
327 experimental animals was observed (Fig. 2B). Bearberry extract induced the highest FRAP value
328 (24.17 μmol Fe^{2+}/g), and parsley extract, the lowest 20.51 μmol Fe^{2+}/g. All used medicinal plants
329 increased FRAP values comparing to the control (7.33 μmol Fe^{2+}/g). Statistically significant changes
330 were observed under the influence of all three used medicinal plants. Due to our results presented in
331 Fig. 2B., it was obvious that all FRAP values in kidneys of mice increased under influence of
332 investigated medicinal plants. Similar effects induced antioxidants from red vines (Rodrigo et al.,
333 2002).

334 Results concerning the effect of bearberry, parsley and corn silk extracts on soluble proteins
335 content in kidneys of experimental animals were shown in the Fig.2C. The content of soluble

proteins was pretty equal, but still the highest under the influence of parsley extract (62.82 mg/g). Significant changes were observed only under parsley influence.

The effects of bearberry, parsley and corn silk extracts on DPPH radical scavenging capacity (RSC) in kidneys of mice are presented in Table 3. DDPH RSC was improved by the action of all three medicinal plants. IC_{50} values were lower in samples treated with medicinal plants than in the control sample (4.64 mg/cm^3). The highest RSC was observed in kidneys of mice treated with parsley extract (2.62 mg/cm^3) and lowest in kidneys of mice treated with corn silk extracts (3.48 mg/cm^3). DPPH scavenging is often used to assess the antioxidative effects of different plants in tissues of experimental animals in order to prevent different diseases (Alvares- Gonzales et al., 2014).

Table 3.

The effects of bearberry, parsley and corn silk extracts on •OH and lipid peroxidation are presented in Fig.3. •OH quantity in kidneys changed under the influence of medicinal plants (Fig.3A). It was the highest in control sample (3.10 U nmol/mg protein), but in samples treated with medicinal plants it was almost at the same lower level. Statistically significant changes were observed under the influence of bearberry and parsley extracts. Control sample accumulated higher quantity of •OH than after the treatment with medicinal plants what proves that they diminished •OH generation and acted protectively (Li et al., 1999; Ueno et al., 2007). Statistically significant changes were observed under the influences of all investigated medicinal plants.

Fig.3.

Bearberry, parsley and corn silk influenced LP in kidneys of experimental animals (Fig 3B). Lipid peroxidation was reduced under the influence of all three medicinal plants and ranged from 3.64 nmol MDA equivalents/mg protein in kidneys of animals which drank bearberry extract to 4.20 nmol MDA equivalents/mg protein in kidneys of animals which drank corn silk extract. The highest

LP was observed in the control group of animals, 4.80 nmol MDA equivalents/mg protein. Statistically significant changes were observed under the influences of all investigated medicinal plants. Lipids containing polyunsaturated fatty acids are readily oxidized by molecular oxygen and such oxidation proceeds by a free radical chain mechanism causing tissue damages (De Groot, & Noll, 1987). Oral administration of *Petroselinum crispum* expressed nephroprotective and diuretic effects, as it reversed the biochemical and antioxidant activity as evident by decreasing lipid peroxidation, increasing content of reduced glutathione and restoring activities of antioxidant (SOD, GPx and CAT) enzymes in renal tissue (Shalaby et al., 2014). Other authors suggested that corn silk extract induces reduction of LP and increase of catalase and SOD activities and therefore could be used in repairing renal damages (Sukandar et. al.,2013) what is in agreement with our results presented in Figures 1-3. Antioxidants also significantly controlled the speed of lipid peroxidation and the reduction of the activites of SOD, GSH-Px, and CAT (Wu et al., 2013). Similar effects were observed in kidney tissues under influence of *Maydis stigma and Petroselinum crispum.*

3.3. *Polyphenol characterization of plant extracts*

In Table 4, the results concerning polyphenol constitution of bearberry, parsley and corn silk extracts are presented.

Table 4.

Total content of phenol compounds determined by Folin-Ciocaltey method was: 95 mg/g bearberry extract; 119 mg/g parsley extract and 110 mg/g corn silk extract. Sum of phenolics determined by HPLC was 34.8 mg/g bearberry extract; 10.39 mg/g parsley extract and 4.21 mg/g corn silk extract. Regardless to the highest content of phenolic compounds found in bearberry by HPLC, parsley extract showed the greatest diversity of present phenolic structures including different phenolic acids, but the dominant component of parsley extract were kaempferol and kaempferol derivates

384 (5.84 mg/g). Dominant compounds in bearberry extract were rutin and its derivates (13.74mg/g),
385 catechin and its derivates (12.23 mg/g) and gallic acid (7.75 mg/g). The dominant phenlic
386 compounds in corn silk were orientin derivates (3.24 mg/g). Presented results are in the accordance
387 with previous polyphenol characterization of bearberry, parsley and corn silk extracts (Panusa et al.,
388 2015; Yidiz et al., 2015 and Liu et al., 2012). The best diuretic effect, the effect on electrolyte
389 excretion and DPPH antiradical activity showed parsley extract which could be attributed to the
390 presence of wide range of phenolic compounds, especially kaempferol and it´s derivates. As
391 phenolic compounds are classical antioxidant agents, this effect could increase the bioavailability of
392 endothelial nitric oxide leading to dilatation of the renal afferent arterioles and increasing glomerular
393 filtration rate (Gasparotto Junior et al., 2012). Although corn silk contained the poorest profile of
394 polyphenols in crude extract, it showed significant positive effect on SOD and GSH-Px activity in
395 mice kidneys, probably because of the orientin derivates presence. Bearberry extract, full of rutin
396 and catechin derivates could be probable cause of peroxidase induction in mice kidneys. These
397 initial observations and findings suggest that the ethanolic extracts of parsley, bearberry and corn
398 silk leaves contain different chemical compounds which biological potential activity deserves
399 further investigation.
400
401 3.4. *Histopatological examinations*
402 Histological examination of kidneys of healthy mice showed normal histological structure of
403 renal parenchyma (glomerulli and tubules) as illustrated in Fig. 4A.
404 Fig.4.
405
406 Histotopathological studies showed that bearberry extract induced significant changes on
407 kidney parenchyma (Figs 4B. and 4C.). *Uvae ursi* folium extract caused microcystic changes with

visible protein hyalin cylinder, focal bleeding, peritubular exudation and pertubular nephritis especially in the regions with cystic changes. Under influence of *Maydis stigma* and *Petroselinum crispum* extracts, no harmful changes were observed comparing to the control kidney tissue.

4. *Conclusion*

The results obtained in this work demonstrated that all three tested plants differently influenced physiological function of kidney, as well as their antioxidant capacity and histophatological features. The best diuretic effect which was maintained for the longest time, was observed under the influence of parsley extract full of polyphenols including phenolic acids and flavonols. Furthermore, parsley extract showed the best results concerning electrolyte excretion. Most pronounced reduction in BUN (blood urea nitrogen) and creatinine was observed by the action of corn silk extract, and then parsley extract. Overall, high antioxidant capacity was observed in kidneys by the action of all examined medicinal plants, which indicated their high antioxidant capacity. Histopathological examination pointed that kidney parenchyma was significantly influenced by the action of bearberry extract. Other extracts did not change kidney tissue. On the basis of the overall presented results (diuretic, excretion of Na^+, K^+, antioxidant, histopathological investigations), parsley extract can be proposed for further investigations as a new therapeutic approach for functional nutraceuticals to be used in the prevention and treatment of kidney disease. The results reported the principle pre-clinical data for application of *Petroselinum crispum* and *Maydis stigma* to attenuate kidney diseases. That also means that they could have huge potential in preventing various kidneys disorders immediately as it appears. As such, further hypothesis testing and mechanistic studies are warranted.

Conflict of interest

None declared.

Acknowledgement

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Table 1. The effects of corn silk, parsley and bearberry extracts on diuresis, excreted K^+ and Na^+ quantities.

Day	0	1	7	14	21	28
Corn silk extract						
Diuresis (ml)	3.52^{ab}	4.1^{d}	3.8^{c}	3.58^{bc}	3.47^{a}	3.45^{a}
K^+(mmol/l)	202.48^{b}	233.3^{d}	219.17^{c}	200.7^{b}	194.7^{a}	201.61^{b}
Na^+(mmol/l)	317.68^{b}	334.10^{c}	318.09^{b}	312.09^{a}	310.98^{a}	316.71^{b}
Parsley extract						
Diuresis (ml)	3.59^{b}	3.98^{c}	4.18^{d}	3.49^{a}	3.5^{a}	3.57^{b}
K^+(mmol/l)	196.87^{b}	239.05^{e}	218.3^{d}	192.46^{b}	184.39^{a}	203.66^{c}
Na^+(mmol/l)	347.98^{b}	397.01^{a}	450.0^{d}	361.93^{c}	346.69^{b}	354.98^{b}
Bearberry extract						
Diuresis (ml)	3.32^{b}	3.41^{c}	3.35^{b}	3.25^{a}	3.28^{a}	3.30^{ab}
K^+(mmol/l)	185.98^{c}	211.71^{d}	209.19^{d}	179.69^{b}	181.91^{bc}	166.79^{a}
Na^+(mmol/l)	330.72^{d}	353.93^{e}	334.24^{d}	300.92^{c}	270.85^{a}	290.93^{b}
Control (water)						
Diuresis (ml)	3.48^{bc}	3.45^{a}	3.51^{c}	3.49^{ab}	3.46^{a}	3.50^{c}
K^+(mmol/l)	200.86^{c}	198.49^{bc}	195.30^{a}	201.0^{c}	197.93^{ab}	200.0^{c}
Na^+(mmol/l)	333.39^{bc}	$326.\ 0^{a}$	335.73^{c}	329.19^{ab}	$331.\ 9^{b}$	333.32^{bc}

Values were means for ten mice. Values with the same letter, in each colon, are not significantly different according to Duncan test ($p < 0.05$).

Table 2. Effects of extracts of *Maydis stigma, Petroselinum crispum* and *Uvae ursi folium* on blood urea nitrogen and serum creatinine contents.

Investigated extract	Urea (mg/l)	Creatinine(mg/l)
Corn silk	$8{,}50^{c}$	$19{,}00^{c}$
Parsley	$10{,}40^{b}$	$30{,}00^{b}$
Bearberry	$11{,}40^{ab}$	$68{,}0^{a}$
Control (water)	$12{,}6^{a}$	$67{,}5^{a}$

Values were means for ten mice. Values with the same letter, in each colon, are not significantly different according to Duncan test ($p < 0.05$).

Table 3. Effects of extracts of *Maydis stigma, Petroselinum crispum* and *Uvae ursi folium* extracts on DPPH radical scavenging capacity of mice kidneys.

Extract	Kidney DPPH (IC_{50}; mg/cm^3)
Control	4.64^a
Bearberry	3.19^b
Parsley	2.62^c
Corn silk	3.48^b

Values were means for ten mice. Statistical comparisons between samples were performed with Duncan t–test. Differences were considered significant at $p < 0.05$.

Table 4. Content of plant phenolics in crude parsley, bearberry and corn silk extracts are expressed as mg g^{-1} extract

Phenolic compound	parsley	bearberry	corn silk
gallic acid	0.16^a	7.75^b	0.15^a
protocatechuic acid	0.56	-	-
catechin and catechin derivates	0.16^a	12.23^b	-
caffeic acid	0.46	-	-
vanillic acid	0.55^a	-	0.50^a
chlorogenic acid	0.11	-	-
epicatechin	0.40	-	-
ferulic acid	-	-	0.32
rutin and rutin derivates		13.74	
quercetin and quercetin derivates	1.86^b	0.47^a	-
kaempferol and kaempherol derivates	5.84	0.26	-
apigenin and apigenin derivates	0.30	-	-
luteolin and luteolin derivates	-	0.23	-
myricetin and myricetin derivates	-	0.12	-
orientin derivates	-	-	3.24
total	10.39^b	34.80^c	4.21^a

All samples were extracted and analyzed in triplicate. Values with the same letter, in each row, are not significantly different according to Duncan test ($p < 0.05$).

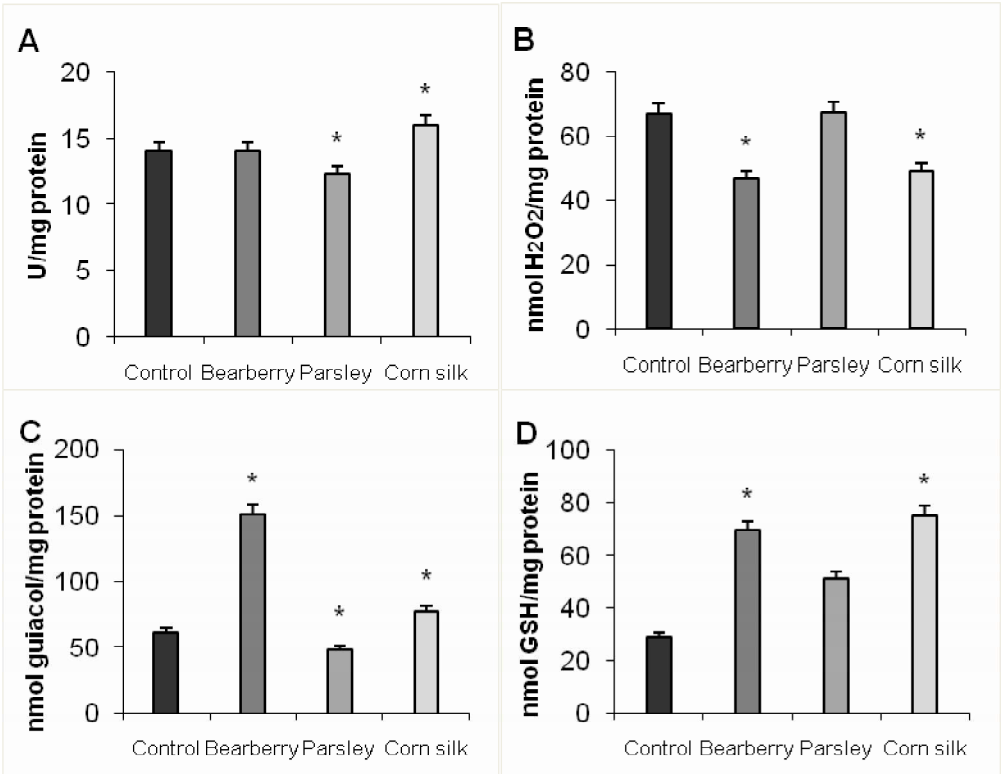
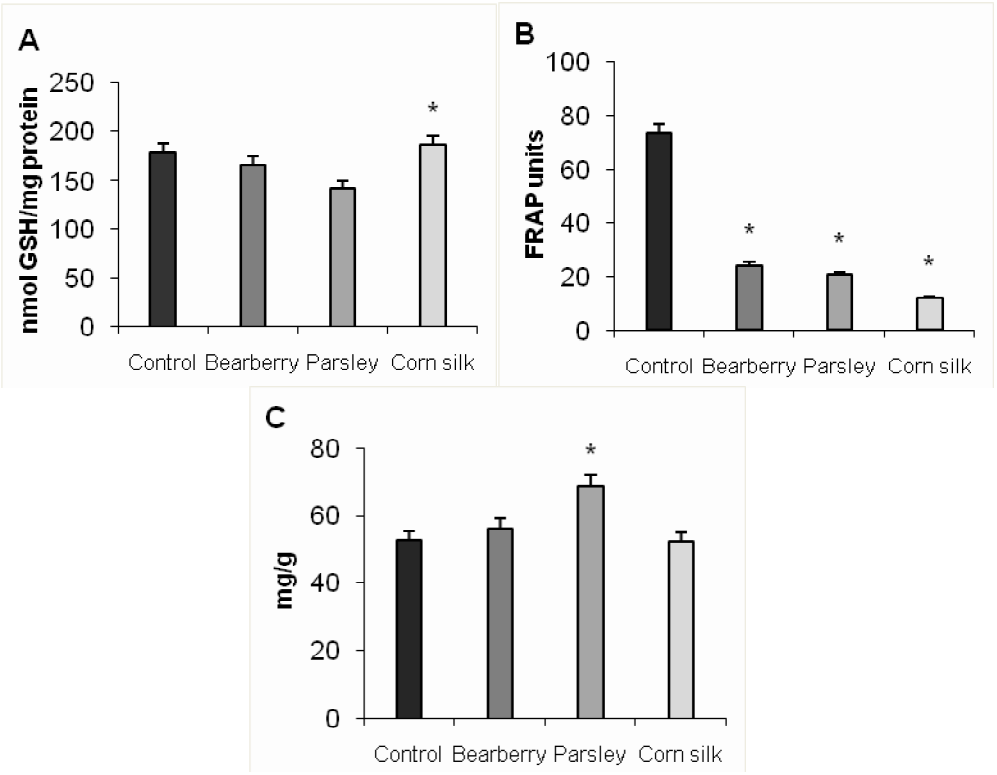


Fig. 1 - Effects of extracts of *Maydis stigma, Petroselinum crispum* and *Uvae ursi folium* extracts, on antioxidant enzymes activities in mice kidneys: A) Superoxide dismutase, SOD; B) Catalase, CAT; C) Guiacole peroxidase, GPx and D) Glutathione peroxidase, GSH-Px

Values were means for ten mice. Statistical comparisons between samples were performed with Duncan t–test.

* Marked differences were considered as significant at $p < 0.05$ from control group.



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765 **Fig. 2.** Effects of extracts of *Maydis stigma, Petroselinum crispum* and *Uvae ursi folium* extracts on
766 glutathione quantity (A), ferric reducing antioxidant power (B) and soluble protein content (C) in
767 mice kidneys.
768 Values were means for ten mice. Statistical comparisons between samples were performed with Duncan t–test.
769 * Marked differences were considered as significant at $p < 0.05$ from control group.
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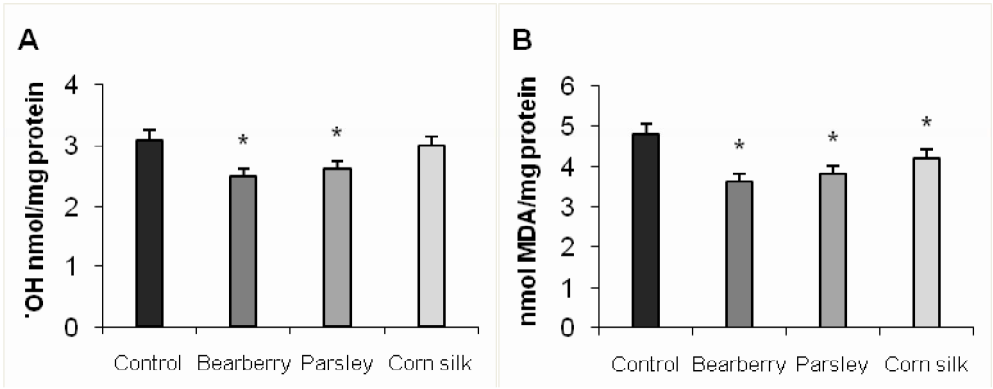

773
774

775 **Fig. 3.** Effects of extracts of *Maydis stigma, Petroselinum crispum* and *Uvae ursi folium* extracts on
776 hydroxyl radical quantity (A) and lipid peroxidation (B) in mice kidneys.
777 Values were means for ten mice. Statistical comparisons between samples were performed with Duncan t–test.
778 * Marked differences were considered as significant at $p < 0.05$ from control group.
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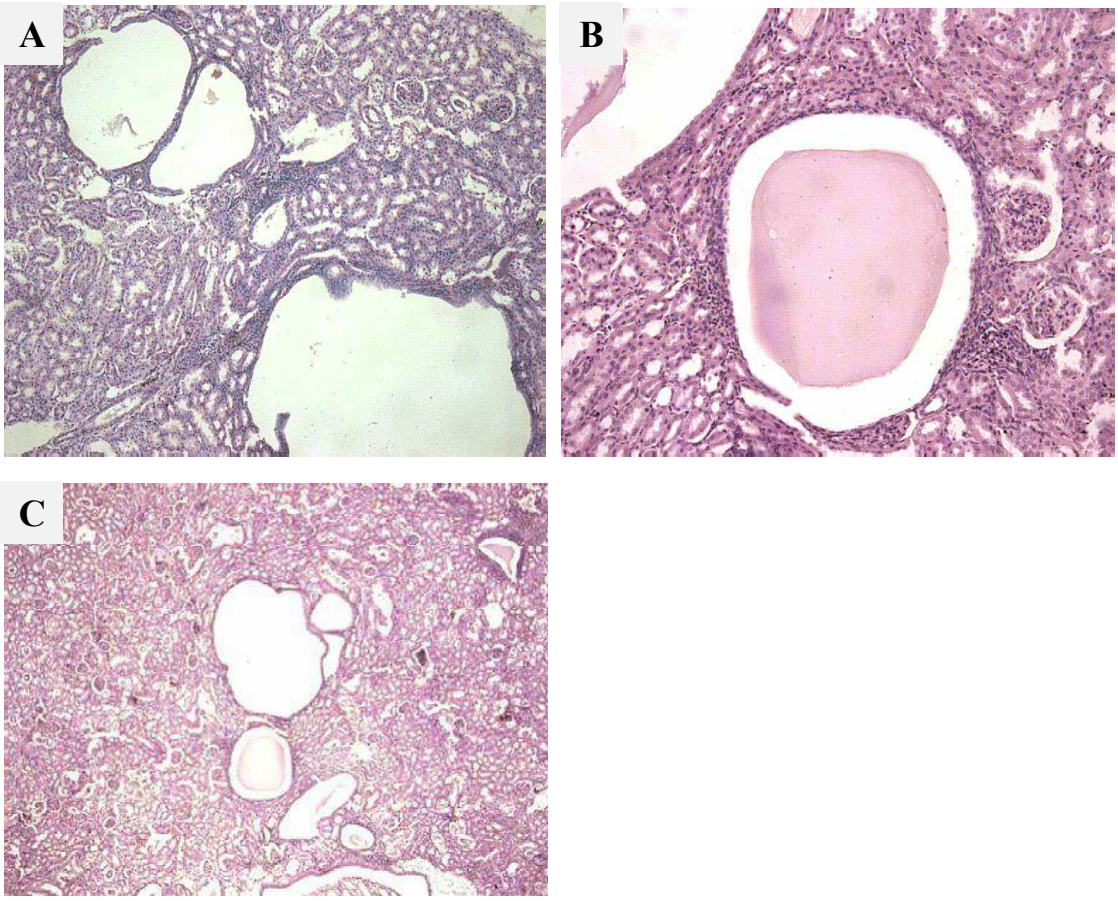

Fig.4. Histopathological features of kidney tissue after *Uva ursi* extract administration
A- microcystic changes in cortical region, 100x, H&E ; B - hyalic cylinders in cystic formation 200x, H&E; C- microcystic changes in cortical region, 50x, H&E.