Xanthine Oxidase-Inhibitory Activity of Extracts Prepared from Polygonaceae Species

Short title: Xanthine Oxidase-Inhibitory Activity of Polygonaceae Species

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Abstract

The xanthine oxidase (XO)-inhibitory activity of aqueous and organic extracts of 27 selected species belonging in five genera (*Fallopia*, *Oxyria*, *Persicaria*, *Polygonum* and *Rumex*) of the family Polygonaceae occurring in the Carpathian Basin were tested *in vitro*. From different plant parts (aerial parts, leaves, flowers, fruits and roots) a total of 196 extracts were prepared by subsequent extraction with methanol and hot H_2O, and solvent–solvent partition of the MeOH extract yielding *n*-hexane, chloroform and 50% MeOH subextracts. It was found that the chloroform subextracts and/or the remaining 50% MeOH extracts of *Fallopia* species (*F. bohemica*, *F. japonica* and *F. sachalinensis*), *Rumex* species (*R. acetosa*, *R. acetosella*, *R. alpinus*, *R. conglomeratus*, *R. crispus*, *R. hydrolapathus*, *R. pulcher*, *R. stenophyllus*, *R. thyrsiflorus*, *R. obtusifolius* subsp. *subalpinus*, *R. patientia*) and *Polygonum bistorta*, *P. hydropiper P. lapathifolium* and *P. viviparum* demonstrated the highest XO-inhibitory activity (> 85% inhibition) at 400 μg/mL. The IC_{50} values of the active extracts were also determined. On the basis of the results, these plants, and especially *P. hydropiper* and *R. acetosella*, are considered worthy of activity-guided phytochemical investigations.

Keywords: xanthine oxidase-inhibitory activity, Polygonaceae, *Fallopia*, *Rumex*, *Polygonum*, *Persicaria* species

INTRODUCTION

Xanthine oxidase (XO), an enzyme present in significant concentrations in the gastrointestinal tract and liver, is responsible for the metabolism of hypoxanthine and xanthine to uric acid in the purine catabolic pathway, yielding superoxide radicals. XO is an important biological source of $O_2^{\bullet-}$ and has been reported in various pathological processes; it plays a crucial role in various forms of ischaemic and other types of tissue and vascular injuries, inflammatory diseases, stroke, diabetes mellitus, rheumatic disease, liver disorders, renal failure and chronic heart failure (Parks *et al*., 1983; Harrison, 2002). Moreover, excessive levels of uric acid in the blood, i.e. hyperuricaemia, cause gout. XO inhibitors are able to hinder the synthesis of uric acid in the organism and, as anti-inflammatory agents, can alleviate the symptoms of inflammatory associated diseases (Liu *et al*., 2008). In clinical practice, only allopurinol is used to inhibit the enzyme, in the treatment and prevention of gout, but it can cause a number of adverse side-effects, such as allergic and hypersensitivity reactions, gastrointestinal distress, nephropathy, skin rash and enhancement of 6-mercaptopurine toxicity (Pacher *et al*., 2006). The development of new hypouricaemic agents with greater effectiveness and a better safety profile is therefore highly desirable.

In recent years, a number of research groups have commenced explorations of potencial XO inhibitors from a wide variety of traditional folk medicines. Numerous studies have dealt with investigations of the XO-inhibitory activities of plant extracts used in certain countries for the treatment of hyperuricaemia, and especially gout (Owen *et al*., 1999; Kong *et al*., 2000). Among the natural compounds that have been tested,

the flavonoids have attracted considerable attention, and structure-activity relationship studies have been performed (Cos *et al*., 1998).

As a continuation of our screening programme relating to plants and bioactive natural compounds in the flora of the Carpathian Basin (Réthy *et al*., 2007; Csupor-Löffler *et al*., 2009; Lajter *et al*., 2013,Ványolós *et al*., 2014), the present work describes for the first time a systematic *in vitro* XO-inhibitory assay of extracts prepared from 27 species belonging in the *Fallopia*, *Rumex*, *Polygonum*, *Persicaria* and *Oxyria* genera of the Polygonaceae family occurring in the Carpathian Basin.

Polygonaceae species are distributed worldwide; this family comprises approximately 1200 species in 50 genera. The largest genera are *Calligonum*, *Coccoloba*, *Persicaria Polygonum*, *Rheum* and *Rumex*. There are 32 species that are native or naturalized to Hungary and 45 in the Carpathian Basin (Jávorka and Csapody 1991). Numerous species are traditionally used as vegetables (e.g. *Rheum rhabarbarum* and *Rumex acetosa*) and in folk medicine. Rhubarb (*Rheum palmatum*) is a well-known medicinal plant of the family; it has been used as a laxative for centuries. *P. cuspidatum* has been utilized in traditional Asian medicine as a diuretic agent (Mazid *et al*., 2009). The leaves of *R. nepalensis* have found use in traditional medicine for the treatment of injury caused by stinging nettles and against colic and syphilic ulcers (Gautam *et al*., 2010). Its roots are used to cure dysentery. *Fallopia* species are traditionally utilized in Asia for the treatment of inflammation, hepatitis, osteomyelitis, gallstone and skin burn (Hromádková *et al*., 2010). *Polygonum cognatum*, *Rheum ribes* and *Rumex acetosella* have been applied for the treatment of urinary inflammation and as diuretic agents in Turkish ethnomedicine (Cakilcioglu *et al*., 2010).

The H_2O extract of *P. cuspidatum* rhizome has been reported to display noteworthy XO-inhibitory activity (IC_{50} = 38 μg/mL). In analogous tests, neither the MeOH nor the H_2O extract of *P. aviculare* (whole plant) or *R. palmatum* (rhizome) exerted any activity (Kong *et al*., 2000).

The most characteristic compounds identified in the species of the Polygonaceae family are phenolic compounds (e.g. anthraquinones, flavonoids, naphthalenes, stilbenes and tannins), terpenoids and polysaccharides (Hegnauer, 1990; Demirezer *et al*., 2001). In some cases, drimane sesqui- and norsesquiterpenoids and sulfated flavonoids have also been isolated from *Polygonum* species (Fukuyama *et al*., 1982; Yagi *et al*., 1994).

Polygonaceae species are rich sources of bioactive constituents which contribute to a wide range of medicinal properties. Stilbene derivatives (e.g. resveratrol and piceid) with antimicrobial and antioxidant activities have been isolated from Polygonaceae species (Shan *et al*., 2008). Flavonoids and chalcones of *P. hydropiper* exhibit strong antioxidant effects, and play an important role against pathologic processes that give rise to oxidative stress, such as arteriosclerosis or cancer (Yagi *et al*., 1994). The widely used medicinal plant *R. palmatum* contains anthranoids (e.g. emodin, aloe-emodin and physcion) as main active constituents. Their pharmacological activities have been investigated in many assays. Aloe-emodin has been reported to induce the apoptosis of hepatocellular carcinoma cells (Jeon *et al*., 2012). Emodin has been investigated for its antidiabetic and antitumour activity (Xue *et al*., 2010; Hsu *et al*., 2012), and for its lipid-lowering and neuroprotective effects in rat cortical neurons (Liu *et al*., 2010; Mishra *et al*., 2014). Quercetin and its derivatives, isolated from many plants of this

family, are used widely as standards in XO-inhibitory assays because of their strong activities (Lee *et al*., 2008).

MATERIALS AND METHODS

Plant materials. Plants were collected in several regions of the Carpathian Basin (Croatia, Hungary and Romania) in the flowering period between June and September 2010. Botanical identifications were performed by Gusztáv Jakab (Institute of Environmental Sciences, Faculty of Water and Environmental Management, Szent István University, H-5540 Szarvas, Hungary) and Lajos Balogh (Natural History Collection, Savaria Museum, H-9700 Szombathely, Hungary). Voucher specimens for each plant (Nos 777-803) have been deposited at the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

Preparation of the extracts. Extracts were prepared from 10 g of air-dried, powdered plant material with 100 mL of MeOH with the use of an ultrasonic bath (3 × 15 min). After filtration, the solutions were evaporated to dryness under reduced pressure. The residues were dissolved in 50 mL of 50% aqueous MeOH and were subjected to solvent–solvent partition between *n*-hexane (3 × 50 mL) (extracts A) and $CHCl_3$ (3 × 50 mL) (extracts B); and the residual 50% MeOH extracts were named extracts C. After the extraction with MeOH, the residual plant materials were dried and extracted with 30 mL of boiling H_2O for 15 min. The filtered extracts were freeze-dried, affording extracts D. The yields (w/w) of the extracts are shown in Table 1.

Xanthine oxidase assay. The method is based on a modified protocol of Sigma, a continuous spectrophotometric rate determination: the absorbance of XO-induced uric acid production from xanthine was measured at 290 nm for 3 min in a 96-well plate, using the plate reader FluoSTAR OPTIMA (BMG LABTECH). The XO-inhibitory effect was determined via the decreased production of uric acid. Reagents: 50 mM potassium phosphate buffer, pH 7.5 with 1 M KOH, 0.15 mM xanthine solution (pH 7.5), and XO solution (0.2 units/mL). XO, isolated from bovine milk (lyophilized powder) and xanthine powder were purchased from Sigma-Aldrich Co. The different plant extracts (12 mg/mL) were prepared in DMSO. For enzyme-activity control, the final reaction mixture comprised of 100 μL of xanthine, 150 μL of buffer and 50 μL of XO in a 300 μL well. The reaction mixture for inhibition was made with 100 μL of xanthine, 140 μL of buffer, 10 μL of sample and 50 μL of XO. Allopurinol served as positive control. Extracts were added in appropriate volumes so that the final concentration of DMSO in the assay did not exceed 3.3% of the total volume. All the experiments were conducted in triplicate. The reaction was initiated by the automatic addition of 0.050 mL of XO solution to a final concentration of 0.006 units/mL. The IC_{50} values were calculated by analysing the inhibition (%) of each concentration, by using GraphPad Prism 5.04 software (GraphPad Software Inc.) with non-linear regression.

RESULTS AND DISCUSSION

In the course of our screening study, the XO-inhibitory activities of 27 species of the Polygonaceae family [*Fallopia* (3), *Oxyria* (1), *Persicaria* (2), *Polygonum* (8) and *Rumex* (13)] occurring in the Carpathian Basin, were evaluated. The results of the assays are listed in Table 1. The extracts were prepared with methanol from selected plant organs and solvent–solvent partition was performed with *n*-hexane (A) and $CHCl_3$ (B). The remaining aqueous MeOH (C) and the H_2O (D) extracts (altogether 196 extracts) were also tested. Although there was no generally accepted threshold for efficacy in our experiment, XO-inhibitory effects of < 10% were considered irrelevant, and are therefore not presented in Table 1. At 400 μg/mL, a total of 69 extracts demonstrated substantial XO-inhibitory activity (≥50% inhibition), while 32 among them exhibited a >80% inhibitory effect. For these extracts, IC_{50} values were also determined (Table 1).

Among the fractions with different polarities, fractions B (containing $CHCl_3$-soluble lipophilic constituents) and some fractions C (aqueous MeOH extracts) proved to be active. The *n*-hexane and residual aqueous extracts (fractions A and D, respectively) demonstrated pronounced XO-inhibitory effects (>50% inhibition) in only a few cases [*F. japonica* roots (A), *P. arenarium* whole plant (A), *P. bistorta* roots (A) and *R. crispus* herbs (D)]. Moreover, the extracts B and/or C of aerial parts and roots of different plants were equally active.

33 $CHCl_3$-soluble subfractions (16.8% of the total) and 28 aqueous MeOH subextracts (14.3%) exerted XO-inhibitory activity at 400 μg/mL. Among the active subextracts, 48% of the $CHCl_3$-soluble fractions and 55% of the aqueous MeOH

extracts possessed an inhibition rate > 80%. Under the conditions of the assay, the IC_{50} of allopurinol, used clinically as a XO-inhibitory drug, was 1.019 ± 0.04 μg/mL.

As concerns the *Fallopia* species, all of the investigated plants (*F. bohemica*, *F. japonica* and *F. sachalinensis*) displayed significant XO inhibitory activities. These results were in agreement with data published by Kong *et al*. (in 2000) on *F. japonica* (syn. *P. cuspidatum*; IC_{50} = 38 μg/mL). This plant has been used in traditional Chinese medicine for the treatment of gout.

As regards the *Polygonum* species, the roots of *P. bistorta* (90.7% C) and *P. hydropiper* (100.3% B and 96.6% C), the aerial parts of *P. lapathifolium* (101.7% C) and the whole plant of *P. viviparum* (80.9% B) proved to exert marked efficacy against XO enzyme. The XO inhibitory activity of five fractions with different polarity of methanolic extract of *P. hydropiper* was investigated by Hashim et al., and it was observed that the *n*-butanol and ethyl acetate fractions showed marked inhibitory effect with IC_{50}s = 28.72 and 165.25 μg/mL (Hashim *et al*., 2013). Previous phytochemical investigations have revealed the presence of flavonoids (e.g. quercetin, quercitrin, rutin, etc.) and sulfated flavonoids (isorhamnetin-3,7-disulfate) in *P. hydropiper* (syn. *Persicaria hydropiper*) (Yagi *et al*., 1994); it can be supposed that such compounds are responsible for the XO inhibitory activity.

A comparison of the measured activities with the ethnomedicinal uses of the plants led to the conclusion that the present screening results for several *Rumex* species are in accordance with the traditional uses of the plants against gout, inflammatory diseases and chronic heart failure. Especially the $CHCl_3$ extracts (B) of the whole plant of *R. acetosella* (IC_{50} = 19.3 μg/mL), the $CHCl_3$ extract (B) prepared from the flowers and fruits of *R. alpinus* (IC_{50} = 23.4 μg/mL), the herb extract (B) of *R. conglomeratus*

(IC_{50} = 23.4 μg/mL), the root extract (C) of *R. hydrolapathum* (IC_{50} = 25.4 μg/mL), and the flowers and fruits extracts of *R. patientia* (B) and *R. stenophyllus* (C) (IC_{50} = 27.6 and 27.3 μg/mL) exhibited high activity against XO. Some of them are applied traditionally as an anti-inflammatory, diuretic and anticancer agent (Rao *et al.*, 2011).

In view of the pronounced XO-inhibitory activity of the appreciable number of investigated species, they are worthy of further investigations, including bioassay-guided isolation and identification of the active substances. Earlier publications on the highly active *R. acetosella* indicated the presence of phenolic compounds and its antioxidant capacity, but its XO-inhibitory activity has not been investigated previously (Özen, 2010). However, the ability of inhibiting XO is strongly connected with the antioxidant capacity, since reactive oxygen species are produced during the formation of uric acid in the presence of XO. Hence, the results of the investigations of the radical-scavenging activity or the reducing power of the plants suggest the XO-inhibition potency of the plants and their compounds (Maksimovic *et al.*, 2011).

In summary, the present paper reports for the first time a systematic comprehensive study on the XO-inhibitory activity of Polygonaceae species native to the Carpathian-basin, including 27 species of *Fallopia*, *Oxyria*, *Persicaria*, *Polygonum* and *Rumex* genera. The *in vitro* screen has provided important preliminary data promoting the selection of Polygonaceae species (native to the Carpathian Basin) and their different extracts with potential anti-hyperuricaemic properties for future work. These species, and especially *P. hydropiper*, *R. acetosella*, *R. alpinus*, *R. conglomeratus*, *R. hydrolapathum*, *R. patientia* and *R. stenophyllus*, are promising candidates for further activity-guided fractionation in the search for new active XO-inhibitory natural compounds.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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Table 1. The yield and XO inhibitory effects of the prepared extracts

Species	Plant parts	Solvent	Yield (w/w%)	Xanthine oxidase enzyme inhibition	
				400 µg/ml (% ± SD)	IC_{50} (µg/ml ± SD)
Fallopia × *bohemica* (Chrtek et Chrtková) J. P. Bailey	leaves	A	6.6	39.22 ± 7.91	.
		B	0.9	79.91 ± 7.82	.
		C	7.4	35.29 ± 3.89	.
		D	4.6	17.69 ± 5.80	.
	roots	A	1.3	24.23 ± 9.45	.
		C	19.2	**86.59 ± 5.25**	91.29 ± 1.28
		D	4.3	21.90 ± 5.88	.
Fallopia japonica (Houtt.) Ronse Decr.	leaves	B	2.2	53.17 ± 6.34	.
		C	7.3	45.31 ± 1.09	.
		D	5.6	11.92 ± 2.10	.
	roots	A	1.1	65.67 ± 14.02	.
		B	4.7	**90.22 ± 9.57**	112.37 ± 9.24
		C	19.2	**92.39 ± 3.30**	55.71 ± 6.98
		D	2.1	37.27 ± 7.55	.
Fallopia sachalinensis (F. Schmidt) Ronse Decr.	leaves	B	2.5	**104.37 ± 30.23**	65.49 ± 14.19
		C	13.2	75.43 ± 15.19	.
		D	4.3	23.90 ± 2.42	.
	roots	A	2.0	25.71 ± 4.91	.
		B	2.5	**101.14 ± 38.51**	147.93 ± 9.40
		C	12.2	56.49 ± 16.23	.
Oxyria digyna (L.) Hill.	whole plant	B	3.0	36.01 ± 8.08	.
		C	9.8	56.61 ± 4.06	.
Persicaria amphibia f. *terrestris* (L.) Gray	whole plant	A	2.0	26.15 ± 13.06	.
		B	0.6	41.31 ± 17.14	.
		C	2.92	43.19 ± 21.13	.
Persicaria maculosa Gray	herbs	A	2.6	39.38 ± 2.50	.
		B	3.3	**92.98 ± 7.94**	68.83 ± 5.75
	roots	B	8.6	69.12 ± 7.98	.
		C	5.9	10.58 ± 3.41	.
Polygonum arenarium W. et K.	whole plant	A	7.7	63.80 ± 2.11	.
		B	1.67	24.05 ± 12.30	.
		C	8.3	52.03 ± 6.59	.
Polygonum aviculare L.	whole plant	B	2.2	61.61 ± 0.74	.
		C	9.1	39.15 ± 5.09	.
Polygonum bellardii All.	whole plant	A	1.5	22.64 ± 7.52	.
		B	4.1	65.49 ± 11.22	.
		C	12.2	54.01 ± 4.40	.
		D	4.9	22.52 ± 7.20	.
Polygonum bistorta L.	leaves	B	8.7	48.37 ± 14.85	.
		C	12.0	33.63 ± 5.85	.
		D	1.8	28.51 ± 1.71	.
	roots	A	1.7	78.61 ± 11.82	.
		B	0.6	66.13 ± 6.86	.
		C	26.8	**90.72 ± 2.81**	33.40 ± 0.87

Species	Plant parts	Solvent	Yield (w/w%)	Xanthine oxidase enzyme inhibition	
				400 µg/ml (% ± SD)	IC_{50} (µg/ml ± SD)
		D	9.0	28.38 ± 1.90	.
Polygonum hydropiper L.	herbs	A	4.0	12.77 ± 1.11	.
		B	4.1	53.46 ± 3.43	.
		C	8.3	43.25 ± 8.85	.
		D	5.3	11.44 ± 4.22	.
	roots	B	1.1	**100.25** ± **32.19**	85.49 ± 11.17
		C	4.7	**96.56** ± **4.90**	16.41 ± 3.36
		D	1.4	12.53 ± 3.63	.
Polygonum lapathifolium L.	herbs	A	4.1	20.55 ± 7.52	.
		B	1.1	78.77 ± 10.57	.
		C	9.5	**101.70** ± **5.80**	74.86 ± 8.33
	roots	B	1.5	31.97 ± 11.02	.
		C	15.2	54.40 ± 9.62	.
		D	4.0	12.36 ± 2.48	.
Polygonum rurivagum Jord. ex. Boreau	whole plant pll	A	1.5	24.22 ± 4.77	.
		B	3.5	49.50 ± 10.52	.
		C	11.6	46.46 ± 4.37	.
		D	4.9	19.27 ± 3.20	.
Polygonum viviparum L.	whole plant	A	6.2	25.66 ± 5.87	.
		B	2.3	**80.90** ± **11.46**	42.33 ± 2.42
		C	8.9	48.15 ± 10.67	.
Rumex acetosa L.	herbs	A	5.0	28.83 ± 16.51	.
		B	3.4	**90.29** ± **9.84**	91.08 ± 9.78
		C	8.2	37.96 ± 8.39	.
	roots	A	1.8	40.88 ± 10.87	.
		B	3.3	54.08 ± 2.39	.
Rumex acetosella L.	whole plant	A	2.9	21.45 ± 4.02	.
		B	2.9	**83.29** ± **2.01**	19.32 ± 3.11
		C	3.9	61.03 ± 2.15	.
		D	3.6	33.13 ± 1.75	.
Rumex alpinus L.	flowers/ fruits	A	1.9	40.69 ± 11.94	.
		B	2.4	**93.95** ± **10.01**	23.40 ± 3.04
		C	12.6	63.04 ± 7.29	.
	leaves	A	3.7	18.05 ± 13.21	.
		B	2.2	**96.98** ± **2.82**	49.34 ± 6.73
		C	13.4	60.85 ± 3.87	.
		D	4.3	12.26 ± 1.77	.
	roots	A	2.4	47.86 ± 0.78	
		B	3.9	**90.89** ± **13.22**	146.60 ± 25.76
		C	21.7	49.75 ± 5.64	
		D	3.4	51.53 ± 6.45	
Rumex aquaticus L.	leaves	A	3.3	30.68 ± 15.61	
		B	1.4	55.75 ± 5.78	
		C	6.3	19.28 ± 9.77	
	roots	A	1.4	24.71 ± 9.55	
		B	2.1	63.89 ± 22.86	
		C	18.4	77.91 ± 15.72	

Species	Plant parts	Solvent	Yield (w/w%)	Xanthine oxidase enzyme inhibition	
				400 μg/ml (% ± SD)	IC_{50} (μg/ml ± SD)
Rumex conglomeratus Murr.	herbs	A	3.3	35.88 ± 14.89	
		B	6.3	**98.92** ± **4.92**	23.38 ± 3.97
		C	10.6	**80.86** ± **15.94**	51.49 ± 9.41
		D	1.5	31.38 ± 3.34	
Rumex crispus L.	herbs	A	2.0	15.03 ± 3.17	
		B	3.1	68.48 ± 18.62	
		C	7.8	**81.72** ± **0.01**	37.34 ± 3.07
		D	3.7	73.41 ± 6.85	
	roots	A	2.4	19.12 ± 2.59	
		C	16.6	35.85 ± 1.52	
		D	1.7	47.04 ± 4.59	
Rumex hydrolapathum Huds.	leaves	A	2.1	28.08 ± 5.39	
		B	2.9	57.25 ± 11.99	
		C	14.5	**94.23** ± **9.84**	73.83 ± 7.09
		D	2.5	49.89 ± 9.90	
	roots	C	25.0	**90.93** ± **13.68**	25.40 ± 2.23
		D	0.7	55.34 ± 5.16	
Rumex obtusifolius subsp. *obtusifolius* L.	herbs	A	7.5	30.57 ± 0.91	
		B	3.8	46.74 ± 2.32	
		C	7.1	61.93 ± 7.10	
		D	6.6	28.76 ± 4.05	
	roots	B	3.3	52.95 ± 3.18	
		C	14.3	70.36 ± 7.66	
		D	2.3	43.47 ± 2.84	
Rumex obtusifolius subsp. *subalpinus* (Schur) Rech. fil.	herbs	A	3.8	51.99 ± 21.73	
		B	1.2	**91.52** ± **6.29**	112.90 ± 7.80
		C	6.2	**92.11** ± **7.68**	33.62 ± 5.60
	roots	A	0.9	14.97 ± 4.79	
		B	1.4	**95.86** ± **6.88**	134.00 ± 23.82
		C	31.7	42.48 ± 6.16	
		D	7.9	19.52 ± 6.78	
Rumex patientia L.	flowers	A	3.9	12.42 ± 1.78	
		B	4.0	**112.20** ± **5.04**	27.63 ± 3.29
		C	6.0	**104.24** ± **1.50**	18.87 ± 1.23
		D	0.7	17.37 ± 0.52	
	roots	A	2.4	42.57 ± 5.70	
		B	3.0	**87.33** ± **11.35**	97.61 ± 8.25
		C	22.4	**110.04** ± **7.06**	60.02 ± 11.67
		D	2.7	13.93 ± 1.55	
Rumex pulcher L.	whole plant	A	3.5	44.04 ± 4.16	
		B	1.6	75.43 ± 9.23	
		C	8.8	**96.24** ± **1.56**	51.72 ± 4.80
Rumex scutatus L.	whole plant	B	3.4	49.30 ± 16.96	
		C	7.0	36.27 ± 6.17	
		D	4.7	10.09 ± 6.63	
Rumex stenophyllus	flowers/ fruits	A	3.7	35.71 ± 0.45	
		B	5.5	21.02 ± 10.61	

Species	Plant parts	Solvent	Yield (w/w%)	Xanthine oxidase enzyme inhibition	
				400 µg/ml (% ± SD)	IC_{50} (µg/ml ± SD)
Ledeb.		C	12.6	**99.94** ± **8.56**	27.28 ± 0.41
		D	1.8	31.93± 5.44	
	leaves	A	2.0	30.46 ± 4.03	
		B	0.6	77.52 ± 15.69	
		C	8.8	42.69 ± 2.21	
		D	2.9	12.77 ± 2.48	
	roots	B	5.4	46.00 ± 3.91	
		C	17.3	76.07 ± 3.41	
		D	2.8	41.60 ± 9.64	
Rumex thyrsiflorus Fingerh.	herbs	A	3.6	35.80 ± 28.81	
		B	2.6	56.53 ± 11.95	
		C	7.1	**99.67** ± **5.77**	78.45 ± 18.81
		D	4.6	49.26 ± 8.48	
	roots	C	6.3	**97.79** ± **7.25**	39.25 ± 4.11
		D	1.2	11.87 ± 1.46	