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Application of isobolographic analysis for the evaluation of interactions between bioactive constituents from dandelion (Taraxaci flos) and lime (Tiliae flos)

Zastosowanie analizy izobolograficznej w celu określenia interakcji bioaktywnych składników mniszka (*Taraxaci flos*) i lipy (*Tiliae flos*)

INTRODUCTION

Reactive oxygen species (ROS) are various forms of activated oxygen. ROS have been implicated in more then 100 diseases, including heart disease, stroke, arteriosclerosis, diabetes and cancer. In living organisms various ROS can be formed by different ways, eg. normal aerobic respiration and some metabolic processes [15]. Creating reactive ROS is connected; *inter alia*, with lipoxygenase (LOX) activity. LOX catalyzes oxygenation of polyunsaturated fatty acids containing a *cist, cis*-1,4- pentadiene system to hydroperoxides. The lipoxygenase pathway of arachidonic metabolism produces reactive oxygen species and these reactive forms of oxygen and other arachidonic acid metabolites may play a role in inflammation and tumor promotion [5]. In the more recent studies, dandelion (*Taraxacum officinale* L.) flower extracts, scavenged ROS and prevented DNA from ROS-induced damage *in vitro*. The suppression of oxidative stress was attributed to luteolin and luteolin 7-O-glucoside [4]. Apart from being used as a pharmaceutical, dandelion inflorescences, leaves and roots are processed into different food products [7]. Lime or linden flowers, *Tiliae flos*, are of prominent importance in phytotherapy. They are stated to possess expectorant, diuretic, diaphoretic, antispasmodic, anti-inflammatory agent in respiratory tract diseases, stomachic and sedative activities and have been used for the treatment of flu, cough, migraine, nervous tension, ingestion problems, various types of spasms and liver and gall bladder disorders [11]. The interaction of plant secondary metabolites in intact plants and their action in the vital functions of organisms raise the bar for synergistic effects of metabolites *in vitro* at the target site. This idea has been adopted by pharmacologists, in order to explore combinations of several metabolites in multi-target therapy [12]. In fact the mechanism of action of many phytochemicals is still unknown. Speculation as to the reason for this, whether it involves synergy, enhanced bioavailability, cumulative effects or simply the additive properties of the constituents requires further research [14].

Dandelion and lime flowers seem to be good components of mixture with potentially beneficial anti-inflammatory effect. Thus, the aim of this study was to evaluate the interactions occurring between LOX inhibitors from lime and dandelion flowers.

MATERIALS AND METHODS

Dried *Taraxaci flos* (TXF) and *Tiliae flos* (TLF) were purchased in local herbal market. Ethanolic extracts (tinctures) were prepared as follows: 100 ml of 70% ethanol was added to 2 g of plant material and left in darkness for 2 weeks. The amount of total phenolics was determined using Folin-Ciocalteau reagent [10] and was expressed as gallic acid equivalents (GAE) in mg per g dry weight [mg/g d.w]. Qualitative-quantitative analysis of phenolic compounds was performed using a Varian ProStar HPLC System separation module (Varian, Palo Alto, USA) equipped with Varian ChromSpher C18 reverse phase column (25mm x 4.6 mm) column and ProStar 325 UV-Vis detector according to Gawlik-Dziki and Świeca [3]. Inhibition of LOX activity was determined based on method described by Axelroad et al. [2] with modifications and expressed as IC_{50} value [μg d.w./ml]. All experimental results were mean ± S.D. of three parallel measurements and the data were evaluated by using one-way analysis of variance (Tukey test). P values < 0.05 were regarded as significant.

RESULTS

As Table 1 shows, dandelion flowers tincture was a good source of phenolic acids, especially gallic, caffeic and benzoic acid, while flavonoid aglycones were represented by quercetin and kaempferol. Better source of flavonoids - especially quercetin, kaempferol and apigenin were lime flowers tinctures. Beside of this in lime flowers tincture significant amounts of phenolic acids (especially chlorogenic acid) were determined.

Table 1. HPLC analysis and total phenols content in *Taraxacum officinale* and *Tilia platyphyllos* tinctures

Compounds [μg/g d.w.]	*Traxacum officinale* tincture	*Tilia platyphyllos* tincture
Gallic acid	42.34±4.50	ND**
Protocatechuic acid	24.76±0.90	2.854±0.42
p-hydroxybenzoic acid	4.54±0.21	32.79±5.43
Chlorogenic acid	24.76±0.00	150.89±22.79
Caffeic acid	61.97±5.71	23.13±8.27
Vanilic acid	4.85±0.18	ND
Syryngic acid	3.42±4.48	ND
p-coumaric acid	3.63± 0.32	20.86±12.07
Synapinic acid	0.64±0.44	2.67±1.44
Ferulic acid	ND	2.41±1.89
Rutin	10.95±1.97	70.80±1.59
t-cinnamic acid	16.98±0.31	ND
Bezoic acid	90.60±0.86	6.39±3.66
o-coumaric acid	1.99±0.14	ND
Quercetin	1.75±0.23	43.54±11.42
Kaempferol	8.87±0.50	46.71±26.76
Luteolin	ND	6.37±2.78
Apigenin	ND	28.81±4.47
Total phenols* [mg/g d.w.]	6.04±1.25	4.52±0.85

*total phenols content was measured with Folin-Ciocalteau reagent and expressed as gallic acid equivalents [GAE mg/g.d.w]; **ND – not detected

LOX activity was effectively inhibited by both lime and dandelion flowers tinctures (Fig.1 and 2). Inhibitory capacity of individual extracts showed a dose-response relationship at various concentrations which enabled the theoretical calculations of IC_{50}. Higher inhibitory activity was obtained in the case of dandelion tincture (IC_{50} about 36.42 μg d.w./ml) whereas for lime tincture IC_{50} average was about 46.41 μg d.w./ml.

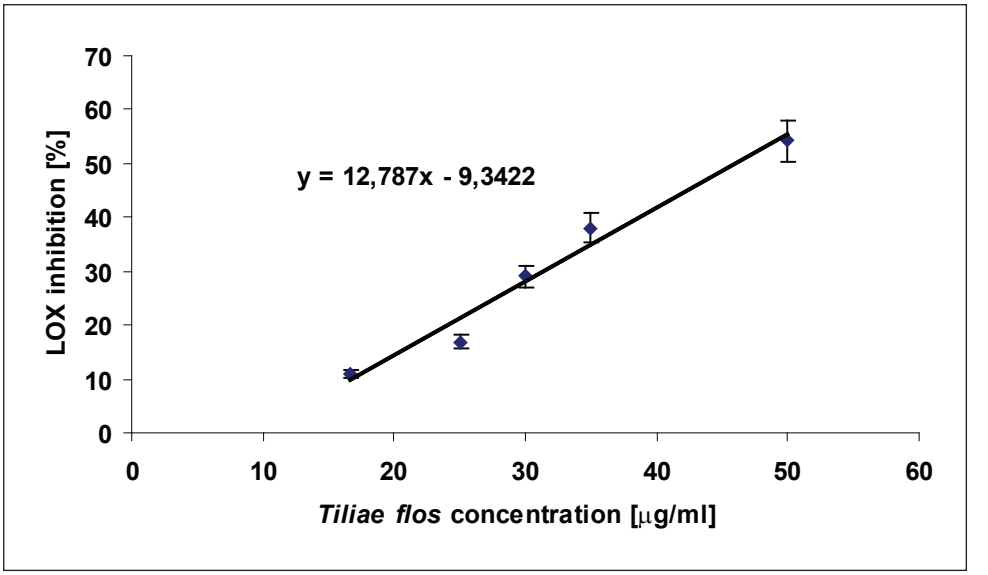

Fig. 1. Relationships between *Tiliae flos* concentration and LOX inhibitory activity

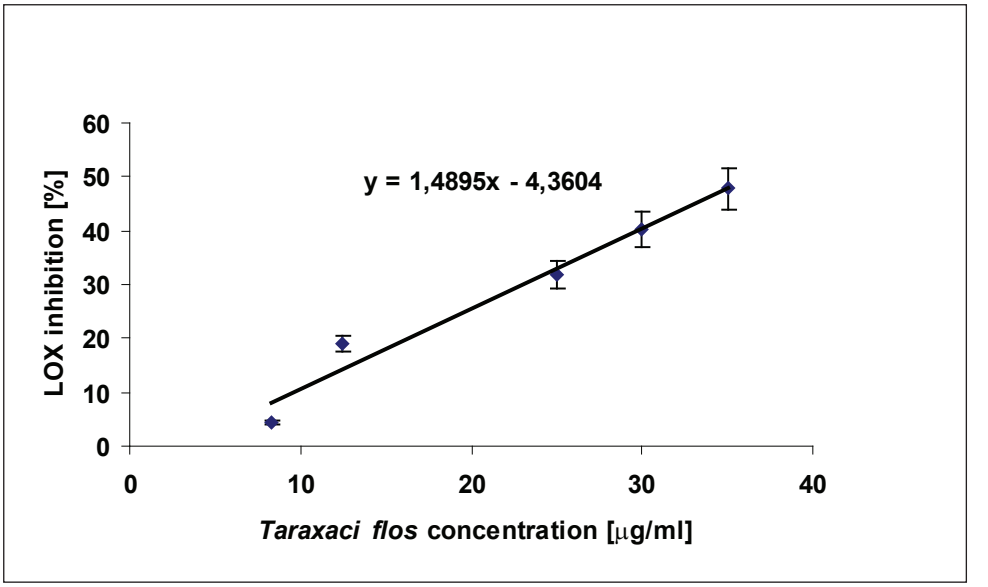

Fig. 2. Relationship between *Taraxaci flos* concentration and LOX inhibitory activity

For interaction assay the mixtures of both tinctures were prepared and IC_{50} values were calculated. The data presented in Table 2 indicates that the inhibitory activity depended on TLF: TXF ratio. Based on these results, an isobole was prepared (Fig.3).

Table 2. The IC_{50} values of various TLF+ TXF mixtures obtained by an in vitro LOX inhibition assay

Mixture TLF: TXF (v:v)	IC50 [mg d.w./ml]	Taraxaci flos concentration [mg d.w./ml]	Tiliae flos concentration [mg d.w./ml]
4:1	56.92 c	45.53 d	5.69 a
3:2	54.55 b	34.20 c	17.2 b
2:3	51.44 a	20.57 b	29.50 c
1:4	72.73 c	14.54 a	31.71 d

*a,b,c,d, – the same letters in columns represent statistically not significant differences ($p<0.05$)

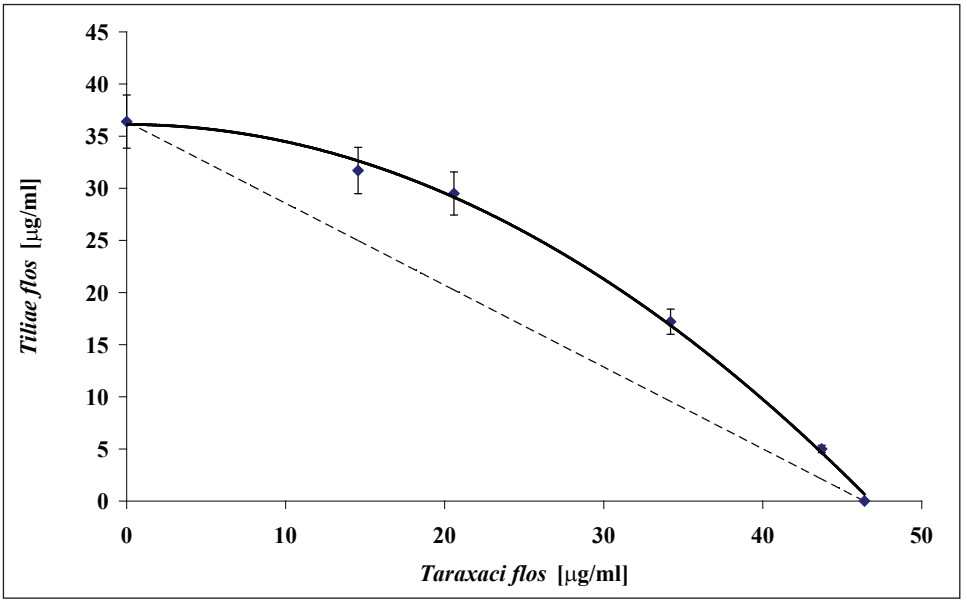

Fig.3. Isobole curve for 50% inhibition of TLF : TXF mixture

As being present in Fig. 3, isobole took the convex form. This result indicates that LOX inhibitors included in dandelion and lime tinctures acted antagonistically.

DISCUSSION

The most abundant phenolic compounds in leaves and flowers are hydroxycinnamic acid derivatives, in particular caffeic acid esters such as chlorogenic, dicaffeoyltartaric (chicoric acid) and monocaffeoyltartaric acids. Various flavonoid glycosides such as luteolin 7-O-glucoside, luteolin 7-O-rutinoside, isorhamnetin 3-O-glucoside, quercetin 7-O-glucoside and apigenin 7-O-glucoside were identified in dandelion leaves and in a combined leaf and dandelion flower extract [13]. Phytochemical studies of European Tilias have demonstrated that several flavonoids such as rutin (quercetin-3-O-rutinoside), hyperoside (quercetin-3-Ogalactoside), isoquercitrin (quercetin-3-O-glucoside), quercitrin

(quercetin-3-O-rhamnoside), kaempferol-3-O-rhamnoside, astragalin (kaempferol-3-O-glucoside), tiliroside (kaempferol-3-O-(6-p-coumaryl)-glucoside), quercetin-3.7-dirhamnoside, kaempferol-3.7-dirhamnoside, quercetin-3-O-glucoside-7-Orhamnoside and kaempferol-3-O-glucoside-7-O-rhamnoside have been isolated from *Tilia argentea*. *Tilia cordata*. *Tilia phatyphyllos*. and *Tilia rubra* [1]. These results were partially confirmed by results obtained in our study. In dandelion flowers tincture significant amounts of phenolic acids (especially cinammic acid derivatives) were determined, whereas lime flowers tincture contained flavonoids – mainly quercetin, kaempferol and apigenin. It is worth nothing, that in this study only aglycones were determined.

The medicinal properties of lime flowers claimed for the drug have been attributed to its flavonoids, volatile oil and mucilage components [11]. The interaction of flavonoids with mammalian 15-lipoxygenase-1 draws particular attention, since this enzyme is a potential target for the health-preserving effect of flavonoids [9]. Isobole method is independent of the mechanism of action and applies under most conditions. An isobole is an "iso-effect" curve in which a combination of constituents (d_a.d_b) is represented on a graph, the axes of which are the dose axes of the single agents (d_a and d_b). If the agents do not interact, the isobole (the line joining the points representing the combination to those on the dose axes representing the individual doses with the same effect as the combination) will be a straight line. If synergy is occurring the curve is said to be "concave". The opposite applies for antagonism, in which the dose of combination is greater than expected and produces a "convex" isobole [14]. Isobolic analysis of LOX inhibition by combination of *Taraxacum officinale* and *Tilia platyphyllos* tinctures showed that bioactive constituents acted antagonistically (Fig. 3). The results indicate that simultaneous using of drugs or/and other preparation containing studied plant could not give expected effects.

CONCLUSION

In spite of the fact that antioxidant and anti-inflammatory activities of single plants predispose them for simultaneous use in prevention and therapy of inflammatory diseases, tinctures combinations demonstrated lower activity than single agents and acted antagonistically. It seems reasonable to assume that in new diet, potential negative interactions between components of supplements/plant drugs should be taken into account.

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SUMMARY

Reactive oxygen species (ROS) have been implicated in more than 100 diseases. In living organism ROS can be formed eg.with lipoxygenase (LOX) action. In this study the LOX-inhibitory activity and phenolics profiles of dandelion and lime flowers tinctures were compared and the interactions between them were evaluated using an isobolographic analysis. Dandelion flowers tinctures were a good source of phenolic acids, whereas lime flowers tinctures were better source of quercetin, kaempferol and apigenin. Higher LOX inhibitory activity was obtained for dandelion tincture (IC_{50} about 36.4 μg d.m. /ml) whereas for lime tincture IC_{50} average about 46.4 μg d.m./ml. Isobole curve for 50% inhibition of dandelion : lime flowers mixture took the convex form. This result indicates that LOX inhibitors of studied preparation acted antagonistically.

Keywords: Isobolic analysis, dandelion, lime, phenolic compounds, lipoxygenase

STRESZCZENIE

Etiologia ponad 100 stanów chorobowych jest związana z występowaniem reaktywnych form tlenu (ROS). W żywych organizmach ROS powstają miedzy innymi w wyniku działania lipooxygenazy (LOX). Celem niniejszej pracy była analiza zawartości polifenoli, porównanie zdolności do inhibicji LOX oraz określenie wzajemnych interakcji składników biologicznie aktywnych przy użyciu analizy izobolograficznej. Nalewki z kwiatów mniszka lekarskiego były dobrym źródłem kwasów fenolowych, podczas gdy nalewki z kwiatów lipy zawierały większe ilości flawonoidów, w tym

kwercetyny, kaempferolu i apigeniny. Wyższą zdolnością do inhibicji LOX charakteryzowały się nalewki z kwiatów mniszka (IC_{50} około 36.4 μg s.m./ml), natomiast wartości IC50 oznaczone dla nalewek lipowych wynosiły około 46.4 μg s.m./ml. Izobola wykonana dla mieszaniny nalewek mniszka i lipy przybrała kształt wypukły. Wyniki te wskazują na fakt, że inhibitory LOX zawarte w badanych preparatach oddziaływają antagonistycznie.

Słowa kluczowe: analiza izobolograficzna, mniszek lekarski, lipa, związki fenolowe, lipoksygenaza