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Antioxidant activity of taxifolin derived from larch: synergistic studies

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The mechanisms of interactions (synergistic, antagonistic or additive) between synthetic or natural antioxidants used as active ingredients or excipients in a multicomponent mixtures is of great interest for the standardization and optimization of pharmaceutical formulations.

The current study aims to evaluate the interactions between the natural antioxidants taxifolin and fucoidan used as excipients in pharmacy.

Taxifolin was isolated and purified from Dahurian Larch (Larix Gmelinii). Fucoidan was isolated from brown algae. The antioxidant capacity of pure taxifolin, fucoidan, and their combinations was determined using ABTS radical-cation decolorization assay. The type of interaction between the tested antioxidants was defined using isobole methodology. When tested alone, taxifolin revealed higher antioxidant activity than fucoidan. The concentration (1.035%) at which it caused 50% effect was almost three times lower than that of fucoidan (3.2%). Further taxifolin reached its highest ABTS-radical scavenging activity at concentration 0.03%, while fucoidan revealed maximum activity at 1.0%.

The type and the strength of interactions between the tested antioxidants with respect to their ABTS radical scavenging activity were evaluated using the combination indexes (CI), calculated for each of the tested taxifolin/fucoidan mixtures. All CI values were less than 1, which indicated a synergistic effect of the tested mixtures. The most pronounced ones was taxifolin/fucoidan = 1:3 (CI = 0.55), followed by taxifolin/fucoidan = 1:1 (CI = 0.66), and taxifolin/fucoidan = 3:1 (CI=0.80).

The synergistic antioxidant effect of taxifolin/fucoidan mixtures is important for optimization of pharmaceutical formulations for prevention and treatment of various pathological conditions caused by oxidative damage.

Key words: taxifolin, fucoidan, antioxidant activity, isobole analysis

INTRODUCTION

Taxifolin (3,5,7,3,4-pentahydroxyflavanon or dihydroquercetin) belongs to the group of flavonoids. Good sources of taxifolin are evergreen coniferous species, such as *Pinus roxburghii*, *Cedrus deodara*, *Larix sibirica*, and *Taxus chinensis var. mairei* [1, 2]. The compound can also be found in the silymarin extract from milk thistle seeds, vinegars aged in cherry wood, fruits, vegetables, wine, tea, and cocoa. [3].

There is growing amount of data supporting the health benefits of taxifolin. It acts as an effective antioxidant inhibiting the inducible NO-synthase and the pro-inflammatory cyclooxygenase in rat models [4]. In cell culture models, taxifolin stimulates the expression of antioxidant enzymes via Nrf 2 dependent pathway, acts as a critical defense molecule against DNA oxidative damage [5], and preserves human keratinocytes from the damaging effects of UVB irradiation [6]. Administration of taxifolin to human colorectal cancer cells leads to cell growth arrest and apoptosis in a concentration dependent manner, to decreased gene expression of β-catenin, of AKT family serine-threonine protein kinases and of survivin [7].

Due to its health benefits, taxifolin may be appropriate for use as a food supplement and functional food ingredient. Very recently taxifolin-rich water/ethanol extract from Dahurian Larch wood was approved by EFSA Panel on Dietetic Products, Nutrition and Allergies as a supplement in non-alcoholic beverages, yogurt, and chocolate confectionery [8].

Fucoidan is a water-soluble sulfated polysaccharide belonging to a group of fucose-containing sulfated polysaccharides. The brown algae *Laminaria digitata*, *Ascophyllum nodosum*, and *Fucus vesiculosus* are one of the best sources of fucoidans [9]. Recently, fucoidans are subject to numerous research studies examining their various health benefits. Fucoidan has been found to have antidiabetic, anticancer, anticoagulant, antiviral, antibacterial, and antioxidant activity [10, 11].

It is suggested that mixtures of natural antioxidants and their by-products in plant extracts may have synergistic effects. A prerequisite for synergistic interactions is the optimization of the composition in a given preparation based on different synthetic or natural antioxidants. The

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synergistic antioxidant activity of the optimized composition may be more effective at lower doses, with greater efficacy in the treatment of various diseases caused by oxidative damage, and thereby it may reduce possible adverse effects caused by the excessive use of a single antioxidant.

The current study aims to evaluate the interactions between the natural antioxidant taxifolin derived from larch and the natural polysaccharide fucoidan as potential food supplements and excipients in pharmaceutical products.

EXPERIMENTAL

Taxifolin and fucoidan preparations

A commercial preparation "Lavitol", Ametis JSC, Russia (License № 00207-ЛС) was used as a source of taxifolin (99%). The taxifolin in "Lavitol" was derived from the stumps of Dahurian Larch (Gmelinii Larch) and purified with ethanol extraction. Taxifolin was dissolved in 70% ethylic alcohol via 15 min. ultrasound mixing. Further 1% solution was prepared and used for testing the ABTS antioxidant activity.

A commercial preparation "Fukolam-S sirjo ", (patent № 2315487) developed by the Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of Russian Academy of Science, Russia was used as a source of fucoidan. A 1% solution was used for testing the ABTS antioxidant activity.

The aforementioned solutions were used for the preparation of taxifolin/fucoidan mixtures in different ratios (1:1, 3:1, and 1:3). The mixtures in these ratios were subsequently tested for ABTS antioxidant activity.

Determination of antioxidant activity

Antioxidant activity was determined using 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radical monocation decolorization assay [12]. The method was based on the ability of antioxidants to quench the long-lived ABTS radical cation, a blue/green chromophore with characteristic absorption at 734 nm. External calibration was done using uric acid (UA) as a standard antioxidant. The results are expressed in mM uric acid equivalents (UAE). The percentage decrease of the absorbance at 734 nm is calculated using the formula:

$A = [(A_{sample\ t=0min.} A_{sample\ t=6min.}) - [(A_{blank\ t=0min.} A_{blank\ t=6min.})$,

where *A* is the absorption.

Statistical analysis

GraphPad PRIZM v.6.0 was used for statistical analysis. Results are expressed as mean±standard deviation (SD). Nonlinear regression analysis was used to determine the "concentration - effect" relationships and to calculate the concentration of each one of the tested substances that had caused 50% inhibitory effect (IC_{50}).

Isobole method of analysis

Isobole method of analysis was used to determine the type of interaction between the tested antioxidants [13]. The relationship "concentration - antioxidant effect" was studied in a range 0.03% - 0.009% for fucoidan, and 0.2% - 1.0% for taxifolin. Combinations between the tested antioxidants were used at fixed ratios taxifolin/fucoidan 1:1, 1:3 and 3:1. The following concentration ranges of both compounds were tested at various ratios as follows:

- taxifolin/fucoidan ratio 1:1, concentration range 0.0045 – 0.025% for both substances;
- taxifolin/fucoidan ratio 1:3, concentration range 0.0068 – 0.075% for fucoidan and 0.0023-0.025% for taxifolin;
- taxifolin/fucoidan ratio 3:1, concentration range 0.0023-0.0083% for fucoidan and 0.0068-0.025% for taxifolin.

Results giving IC_{50} were extrapolated and used to obtain isobole diagrams illustrating the interactive effects. The combination index (CI) is calculated using the equation [14]:

$CI = [C_{fucoidan}/IC_{fucoidan} + C_{taxifolin}/IC_{taxifolin}]$, where

$C_{fucoidan}$ and $C_{taxifolin}$ are the concentrations that produce a certain effect if applied together; $IC_{fucoidan}$ and $IC_{taxifolin}$ are the concentrations that produce the same effect when applied individually.

RESULTS AND DISCUSSION

Figure 1 presents the "concentration – effect" curves for taxifolin and fucoidan when applied alone.

Taxifolin revealed higher antioxidant activity when tested alone. Its concentration causing 50% effect (IC_{50}) was almost three times lower than that of fucoidan (1.035% vs 3.2%, respectively). It showed highest ABTS- radical scavenging activity at concentration 0.03%, while fucoidan revealed a maximum activity at 1.0%.

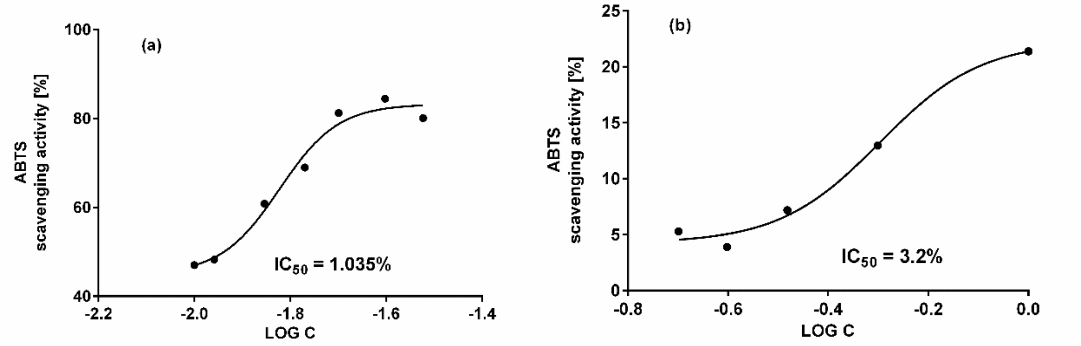

Fig. 1. Concentration – effect curves of fucoidan and taxifolin ethanolic solutions. The antioxidant effect was evaluated as percentage ABTS radical cation scavenging activity. (a) – taxifolin, (b) – fucoidan.

Taxifolin revealed higher antioxidant activity when tested alone. Its concentration causing 50% effect (IC_{50}) was almost three times lower than that of fucoidan (1.035% vs 3.2%, respectively). It showed highest ABTS- radical scavenging activity at concentration 0.03%, while fucoidan revealed a maximum activity at 1.0%.

The effect on ABTS scavenging activity of three different combinations between both antioxidants is presented in Figure 2.

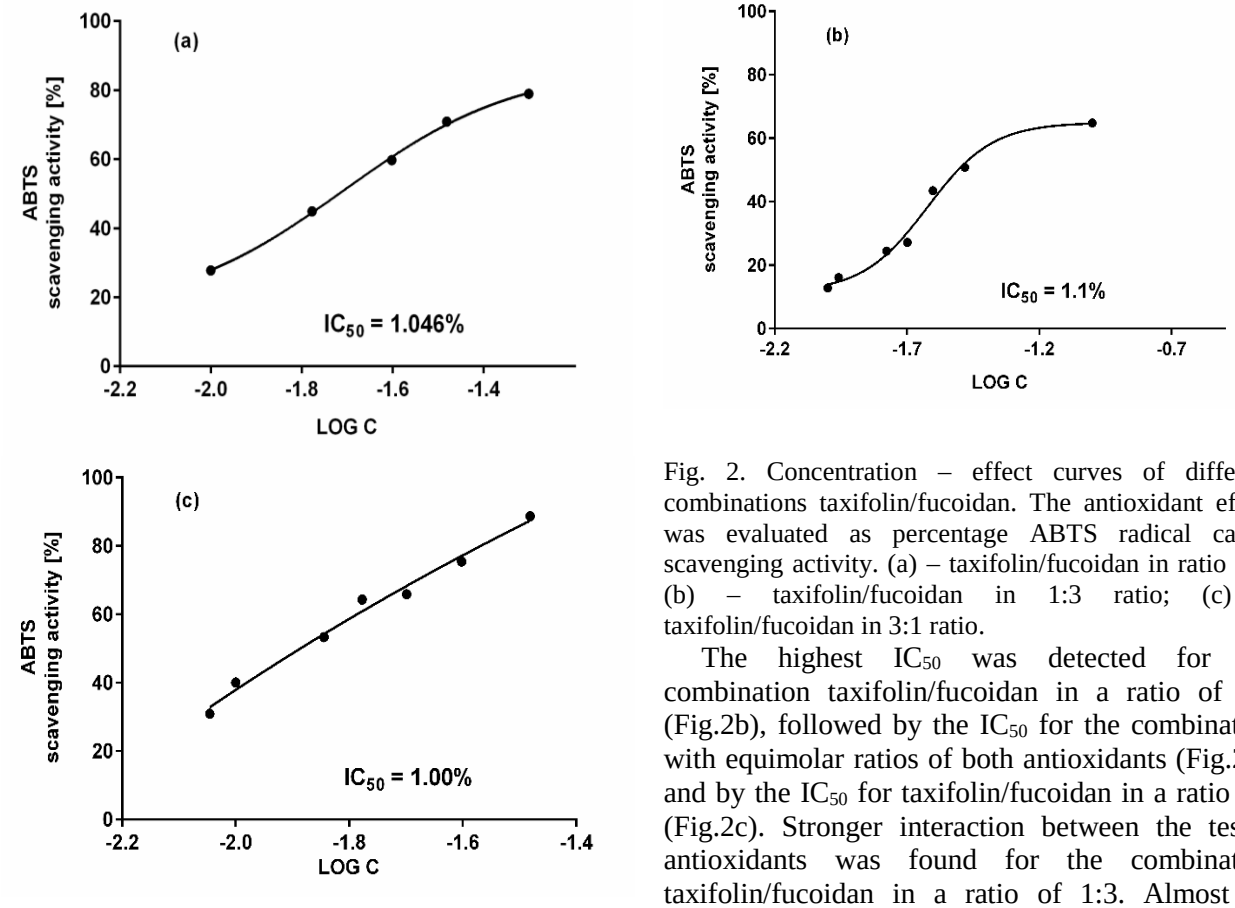

Fig. 2. Concentration – effect curves of different combinations taxifolin/fucoidan. The antioxidant effect was evaluated as percentage ABTS radical cation scavenging activity. (a) – taxifolin/fucoidan in ratio 1:1; (b) – taxifolin/fucoidan in 1:3 ratio; (c) – taxifolin/fucoidan in 3:1 ratio.

The highest IC_{50} was detected for the combination taxifolin/fucoidan in a ratio of 1:3 (Fig.2b), followed by the IC_{50} for the combination with equimolar ratios of both antioxidants (Fig.2a), and by the IC_{50} for taxifolin/fucoidan in a ratio 3:1 (Fig.2c). Stronger interaction between the tested antioxidants was found for the combination taxifolin/fucoidan in a ratio of 1:3. Almost no interactive effect was indicated for the other tested combinations.

Figure 3 is an isobologram of the tested antioxidant combinations.

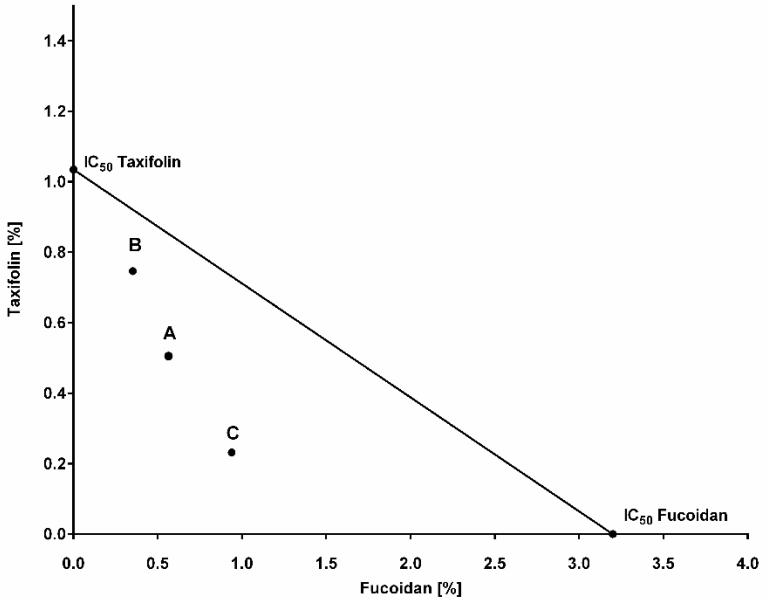

Fig. 3. Isobologram of the tested antioxidant combinations.
The points A, B, C represent the effects of taxifolin/fucoidan combinations on ABTS radical scavenging activity. A – taxifolin/fucoidan = 1:1; B – taxifolin/fucoidan = 3:1; C – taxifolin/fucoidan = 1:3.

The CI values were calculated for each of the tested taxifolin/fucoidan combinations. All CI values less than 1 indicated synergistic effect of the tested combinations regarding their ABTS scavenging activities. The effect was strongest for taxifolin/fucoidan = 1:3 (CI = 0.55), intermediate for taxifolin/fucoidan = 1:1 (CI = 0.66), and weakest for taxifolin/fucoidan = 3:1 (CI=0.80).

Natural products like plant extracts are good source of native antioxidants. Mixtures of different plant extracts, containing various combinations of natural antioxidants may show synergistic, antagonistic or additive effects. The plant antioxidants alone are usually effective in relatively higher concentrations, which may lead to some unwanted side effects. This may be overcome by using suitable combinations of various natural/synthetic antioxidants, which can interact synergistically. The synergistic interactions between the antioxidants in a mixture may increase the antioxidant activity and reduce the adverse effects of a single natural antioxidant used in higher concentrations and could also be very important for the standardization of multi-extract preparations and food supplement formulations featuring different natural and synthetic antioxidants [15].

Taxifolin is a natural antioxidant possessing better antioxidant capacity than BHT, BHA, alfa-tocopherol and Trolox [16, 17, 18]. Taxifolin was reported to be an efficient ABTS radical scavenger in a concentration-dependent manner [19]. This was also confirmed in the present study (Fig.1a).

Fucoidan is a natural antioxidant derived from marine resources which exhibits highest antioxidant and free radical scavenging activity among different polysaccharides derived from brown and red seaweeds [20, 21]. Our results showed that in comparison with taxifolin, fucoidan possessed weaker antioxidant activity (IC_{50} 1.035% vs IC_{50} 3.2%, respectively).

There is a number of studies demonstrating that mixtures of antioxidants from various natural products possess synergistic effect. Synergistic effects were reported for binary mixtures of rosmarinic acid and quercetin, or rosmarinic acid and caffeic acid [15]. Unfortunately, we did not find any information about potential synergistic or antagonistic interactions between taxifolin and fucoidan in different mixtures. In our study we demonstrated synergistic antioxidant effects (evaluated by ABTS-radical decolorization assay) for all tested taxifolin/fucoidan mixtures. A recent study demonstrated that a mixture of green tea polyphenols, alfa-tocopherol and L-ascorbic acid possessed markedly enhanced antioxidative efficacy as compared to the additive efficacy of individual antioxidants [22]. Other studies have also shown that polyherbal combination of individual plants rich in phenolic, flavanoids and green tea revealed higher antioxidant activity (synergistic effect) compared to individual herbs [23].

Another factor influencing the synergistic antioxidant activity is the concentration and the type of antioxidant. In our study we found stronger synergistic effect at a ratio of 1:3 taxifolin/fucoidan mixture (CI 0.55) and weaker at 3:1 taxifolin/fucoidan combination (CI 0.80). Other studies reported that lycopene interacted synergistically with vitamin E at a specific

concentration and ratio to inhibit 2,2-azobis (2,4-dimethylvaleronitrile) induced oxidation of linoleic acid methyl ester, whereas β-carotene showed no synergistic effect with vitamin E at the same concentration [24].

CONCLUSIONS

Taxifolin possessed better antioxidant activity than fucoidan as evaluated by ABTS-radical cation assay. Isobolographic analysis indicated that the combination taxifolin/fucoidan showed best synergistic activity in an ABTS assay at the ratio of 1:3. The synergistic antioxidant effect of taxifolin mixtures can be used for optimizing the composition of formulations for prevention or treatment of various pathological conditions caused by oxidative damage.

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