

CONTRIBUTIONS TO THE PHARMACOGNOSTICAL AND PHYTOBIOLOGICAL STUDY ON *SOJAE SEMEN*

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Abstract

The purpose of our study was the pharmacognostical and phytobiological analysis of soybean (*Sojae semen*) and soy textured protein. In the microscopic examination of *Sojae semen* the following anatomical elements were observed: endosperm and embryo fragments, drops of oil, small xylem vessels and cellulose fibers. The chemical analysis established the presence of isoflavonoids (genistin), sterols and triterpenes (ursolic acid/ oleanolic acid), hydroxycinnamic acid derivatives, flavonoids and mucilages in both products. Soybeans have a higher amount of flavonoids and hydroxycinnamic derivatives compared to soy textured protein. The *Triticum* bioassay revealed a mitoinhibitory effect of both aqueous extracts only at high concentrations (5-10%).

Rezumat

Scopul acestei lucrări constă în studiul comparativ (farmacognostic și fitobiologic) al semințelor de soia (*Sojae semen*) și al produsului de prelucrare denumit „soia texturată“. În *Sojae semen* s-au identificat următoarele elemente anatomice: fragmente de endosperm și embrion, ulei gras, fibre celulozice, vase de lemn mici. Prin examenul chimic s-a stabilit prezența de izoflavonoide (genistin), steroli, triterpene (acid ursolic/acid oleanolic), derivați ai acidului hidroxicinamic, flavonoide și mucilagii, în ambele materii prime. Semințele de soia au un conținut mai ridicat în flavone și derivați ai acidului hidroxicinamic, comparativ cu produsul de prelucrare. Testul *Triticum* a relevat efectul mitoinhibitor al soluțiilor apoase de concentrații mari (5-10%), obținute din cele două produse.

Keywords: *Sojae semen*, genistin, mitoinhibitory action

Introduction

According to scientific literature, soybean has: hormonal effects due to *isoflavones* (genistein, daidzein, glycitein), which are selective estrogen receptor modulators (like raloxifen), and are used in therapy in order to treat climacteric symptoms [9, 10]; isoflavones are estrogen receptor β -agonists, so they are also used in the treatment of osteoporosis; protective effects against different types of cancer (breast cancer, prostate cancer) due to *genistein* (estrogen receptor α antagonist, tyrosine kinases

inhibitor, matrix metalloproteinase inhibitor), inositolhexaphosphate, peptide compounds (Birk-Bowman factor and lunasin), nitrogen compounds (canavanine), polyphenols (gossypol), saponins and their aglicons (lupeol), bikunin (protease inhibitor) [6-8, 13]; cardiovascular benefits due to flavonoids (isoliquiritigenin), *p*-coumaric acid and fatty unsaturated acids, which inhibit lipid peroxidation and platelet aggregation; genistein protects against pro-inflammatory factors that induce vascular endothelial barrier dysfunction and inhibit leukocyte endothelium interactions, thereby modulating vascular inflammation, a major event in the pathogenesis of atherosclerosis; genistein regulates the vascular function by activating the nitric oxide synthase and producing nitric oxide in the endothelial cells [12]; liver protective effects due to phospholipids and polyphenol carboxylic acids [17]; antioxidant properties due to vitamins (A, E), oligoelements (Zn, Cu, Mn, Mg, Ca), equol (daidzin's metabolite) and polyphenols derivatives [11, 17]; antibacterial effects – genistein is active on all *Staphylococcus* species, even the ones that are resistant to methicillin [14]; benefits in lack of concentration due to lecithin [17].

The purpose of our study was the pharmacognostical and phytobiological study of soybean (*Sojae semen*) and textured soy protein (*Sojae product*).

Material and methods

The raw material consists of *Sojae semen* (the mature beans from *Glycine max* (L.) Munch., soybean, *Fabaceae*) and *Sojae product* (textured soy protein) produced in Romania.

For the microscopic study, clarified preparations with a chloral hydrate solution 800 g/L (Zeiss microscope; ob. 10x and 40x) were used. For the qualitative analysis the raw material was successively extracted with different solvents (ethyl ether, methanol, water). Half of the above alcoholic and aqueous solutions were hydrolyzed. Specific reactions were carried out on the initial and hydrolyzed solutions, in order to identify the active principles [3, 4]. For triterpenes thin-layer chromatography (TLC) was applied [3]. For isoflavonoids (genistin) high performance liquid chromatography (HPLC) was applied.

Parameters of TLC for triterpenes

Test solution: 1g of both products was powdered and extracted with 10 mL methanol (R) for 10–15 min., then filtered and concentrated; silica gel GF₂₅₄ (Merck) TLC plates were used; solvent system (mobile phase) chloroform : acetone / 8:2 (v:v); reference substances (0.1% in methanol): ursolic acid and oleanolic acid (Sigma); detection: spraying with acetic

anhydride and ethanolic sulphuric acid mixture (1:1), heating at 100 °C for 10 min. and examination in UV light - 366 nm (using a Camag UV lamp).

Parameters of HPLC for genistin

A Varian Prostar equipment was used, consisting of a quaternary pump (Prostar 210), a diode-array detector (Prostar 330 PDA), manual injector (Rheodyne 7125 with a 20 µL loop) and a Prostar 510 column oven. An Inertsil 5 ODS-2 column was used (250 x 4.6 mm ID, 5µm particles), with a guard-column C18 (10 x 4.6 mm ID). The mobile phase consisted of phosphate buffer solution pH 2.5 (solvent A) and acetonitrile (solvent B). Determinations were performed using a mobile phase gradient as described in table I.

Table I
Gradient programme

Time (min)	Flow (mL/min)	Solvent A (phosphate buffer solution pH 2.5)	Solvent B (acetonitrile)
0	1.0	80	20
35	1.0	63	37
45	1.0	0	100
50	1.0	0	100
51	1.0	80	20
61	1.0	80	20

Detection was performed at a wavelength of 254 nm, with full spectra recorded between 220-400 nm. Determinations were performed at room temperature (23- 24 °C). The samples were prepared by refluxing the two products with ethanol. Genistin (Fluka) was used as a reference.

In order to evaluate the quality of the herbal product the loss on drying was determined (according to the European Pharmacopoeia 5-th edition), the flavonoids were quantified (using a spectrophotometric method based on the reaction with aluminium chloride, according to the Romanian Pharmacopoeia 10-th edition, *Cynarae folium* monograph, calibration curve of rutin) and the total hydroxycinnamic acid derivatives were assayed (according to European Pharmacopoeia 5-th edition, Ash leaf monograph), using a spectrophotometric method based on the reaction with sodium nitrite and sodium molybdate [15, 16]. The spectrophotometric determinations were carried out using a spectrophotometer *Cecil series 2000*.

A plant bioassay (Constantinescu method, *Triticum* bioassay) used embryonic roots of wheat karyopses (*Triticum vulgare* Mill.) as a biological material [1, 2]. The wheat karyopses were germinated in laboratory conditions (the main root must have 1 cm length). The aqueous extracts from both products were tested. From *Sojae semen*, five samples were

prepared: SS1 -10%; SS2 -5%; SS3 -3.33%; SS4 -1.66%; SS5 -0.66% (concentration is expressed as g of plant product per mL of solution). In the same way five samples were prepared from *Sojae product*: ST1, ST2, ST3, ST4, ST5 (the same concentrations as for the *Sojae semen*). A control sample (M) was prepared using distilled water. The root elongation was evaluated at the same time of the day for 5 days. For the microscopic study of the embryonic root, the acetic orcein squash method was used, the squash being examined by immersion in cedar oil (ob. 100 x) [2].

Results and discussion

In the microscopic examination of *Sojae semen* the following anatomical elements were observed: endosperm cells and embrion fragments, drops of oil, cellulose fibers, small xylem vessels (fig. 1).

Sterols, triterpenes (aglycones and glycosides), hydroxycinnamic acid derivatives, flavonoids, and mucilages were identified in both products by specific chemical reactions. These compounds are mentioned in the scientific literature regarding *Sojae semen* [5, 17].

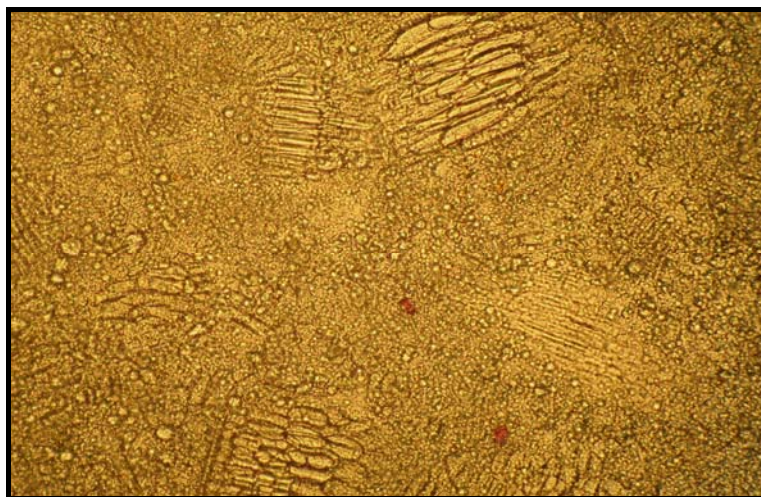


Figure 1

Microphotograph showing cellulose fibers and oil droplets in *Sojae semen* (magnification 10x)

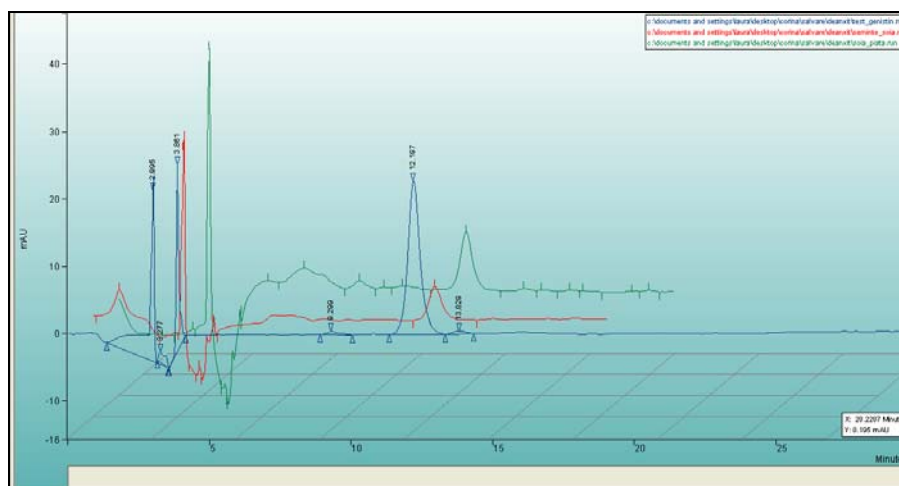
Ursolic acid/ oleanolic acid ($R_f=0.64$, violet colour in visible and yellow fluorescence in UV, after spraying with acetic anhydride and H_2SO_4 /ethanol) was identified by TLC in both samples (fig. 2).

**Figure 2**

TLC chromatogram of triterpenes
(viewed with a UV lamp at 366 nm)

- 1 – methanolic solution 10% of *Sojae semen*;
 - 2 – methanolic solution 10% of Soy textured protein;
 - 3 – oleanolic acid (r.s., methanolic solution 0.1%);
 - 4 – ursolic acid (r.s., methanolic solution 0.1%).
- r.s. = reference substance

The alcoholic extracts of both products (*Sojae semen* and soybean textured protein) were analysed and the HPLC chromatograms were compared with the one of genistin and other chromatograms from literature. The retention time for genistin was 12.197 min. (12.056 min., 12.120 min. respectively for the extracts) and the content of genistin in the samples was 0.055 g% in *Sojae semen* and 0.049 g% in soybean textured protein (compared with an external standard).

**Figure 3**

The HPLC chromatogram for isoflavones

The results confirm the presence of genistin in both products and that is why they should be used carefully during pregnancy, because genistin's aglicon (genistein) inhibits tyrosine kinases signaling cascade (important enzymes in the biosynthesis of thyroid hormones, which become active from the third month of pregnancy and ensure a normal growth of the fetus). On the other hand, due to its remarkable composition soy could be used with excellent results in the diet of patients that suffer from cardiovascular diseases (due to omega 3 fatty acids, fibers and antioxidants such as equol, vitamins), osteoporosis (due to isoflavonoids) and climacteric symptoms (isoflavonoids).

The results of the quantitative chemical analysis (calculated on a dry basis) are presented in table II. It was observed that soybeans have a low concentration of polyphenols (flavonoids and hydroxycinnamic acid derivatives), but higher compared to the soy textured protein.

Table II
The results of quantitative chemical analysis

Parameter	Results (g %)	
	<i>Sojae semen</i>	<i>Soy textured protein</i>
Flavonoids (expressed as rutin)	$7.1-7.8 \times 10^{-3}$	$2.2-2.9 \times 10^{-3}$
Total hydroxycinnamic acid derivatives (expressed as chlorogenic acid)	2.64×10^{-3}	1.35×10^{-3}

The results of the *Triticum* bioassay - Constantinescu method (the root elongation values for each tested sample, in the five days) are presented in figure 4. The *Triticum* bioassay established a moderate inhibitory effect of the extracts on the root elongation. The microscopic examination of the embryonic wheat roots revealed for the extracts of *Sojae semen* and soybean textured protein at the higher concentration (5-10%) a statmokinetic effect (cells with deckled walls, nuclei with 1-2 hypertrophied nucleoli, often 2 nucleoli of unequal sizes). At lower concentrations (0.66-3.33%), generally, a moderate mito-depressive effect could be observed (numerous interphases and prophases; frequent metaphases; anaphases and telophases in tropokinesis).

The inhibitory effect can be correlated with the presence of saponins and other active water soluble principles, that are mentioned in the scientific literature (polypeptides lunasin and Birk-Bowman factor) [7, 8].

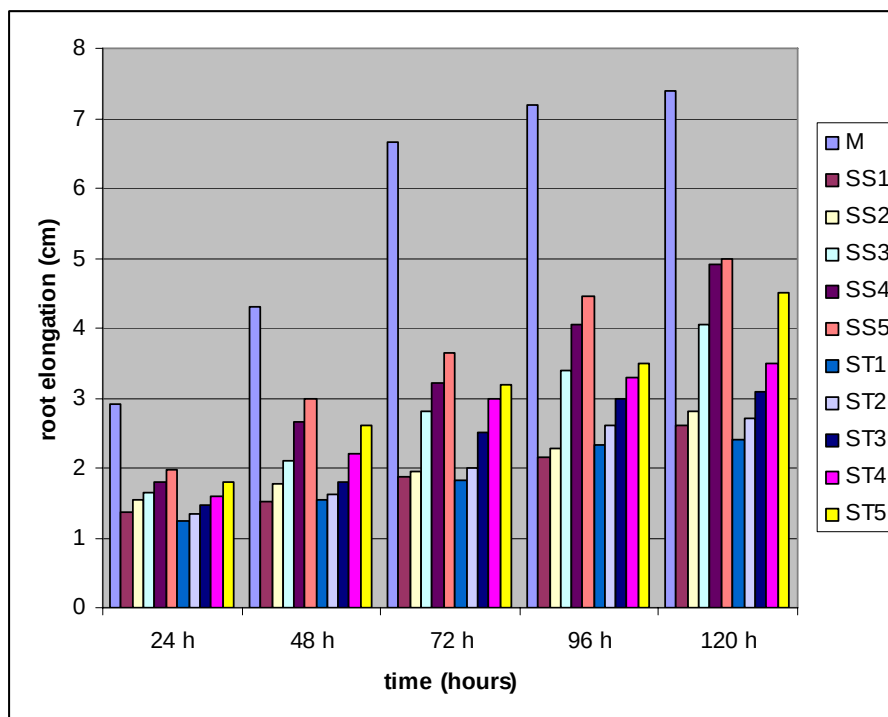


Figure 4

The influence of aqueous extracts from *Sojae semen* (SS) and soybean textured protein (ST) on *Triticum* embryonic root elongation

Conclusions

Soy textured protein has a similar chemical composition with *Sojae semen*. Genistin's presence in both products imposes caution in its use during pregnancy.

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