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УДК 633:88. 577.1

BIOLOGICALLY ACTIVE SUBSTANCES OF ISATIS TINCTORIA AND COMMUNICATION CAPABILITIES IN THE STUDY OF PLANT CHEMICALS

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Abstract: *This review examines of the development of communication on the phytoproducts of Woad in geographically distant cultures: China and Europe. The growing interest in the beneficial properties of Woad has mainly focused on the roots of this plant as a promising source of complex metabolites. Important bioactive substances derived from the root of Woad include alkaloids, flavonoids. These bioactive compounds of Woad are believed to possess antiviral properties [Yu, B; Lin, F; Ning, H; Ling, B, 2021].*

Keywords: *Isatis tinctoria L., communication, phytoproducts, bioactive substances, methods for determining biological activity.*

Key findings: Through the analysis of international databases, scientists from Europe and China can recognize and conduct complete studies on biologically active plant substances. A review of sources reveals commonality in its use in diseases accompanied by inflammatory processes in China and Europe.

Over the last decade, more than seventy different chemical constituents such as alkaloids, lignans, ceramides, flavonoids and sulfur-containing metabolites have been identified, which subsequently showed remarkable biological efficacy, summarized biological properties such as antiviral activity, neuroprotective properties, nitric oxide inhibition and other pharmacological properties [1].

Analysis of experimental data on the root of Woad in terms of their bioactive activities has shown a relevant role at present.

Introduction. Woad (Latin. Isatis tinctoria L.) (from the Greek word isadso - to cure) is a biennial herbaceous plant of the Cruciferous family 40-120 cm tall with a multi-headed root [4].

Woad was well used according to historical descriptions both as a temperate dye plant and as a medicinal plant. The original habitat of woad is probably in the grasslands of southeastern Russia, however, the plant was introduced early to Europe and Eastern Asia including China and Japan. The first written references in Europe to the medicinal use of Woad were made by Galen and Pliny. During the Middle Ages and up to the 18th century, Woad was the most important blue dye in European rope making. Woad was cultivated in Germany (Thuringia, Julich), France (Languedoc, Somme, Normandy), England (Somerset, Lincoln-shire) and Italy (Tuscany), but with the advent of other less expensive sources for obtaining the dye, Woad lost its importance as a medicinal use as well.

Henning Danz, Stefka Stoyanova, et al. [2001] reports that in Europe the medicinal properties of the plant were described in detail in a number of Renaissance and Baroque herbalists. Woad has been recommended for the treatment of wounds, ulcers and tumors, hemorrhoids, snakebites and various inflammatory diseases; in China, the plant has an equally rich history as a dye and medicinal plant. The roots of the Woad plant are used in traditional Chinese medicine to make a medicine known as banlangen, which has potential antiviral properties [8; 6]. Banlangen is used in China as an herbal medicinal tea for cold and tonsillar diseases. Used as a tea, it is brownish in color and (unlike most Chinese medicines) slightly sweet to the taste.

More recently, the National Health Commission of the PRC has proposed the use of Isatis Radix granula in the treatment of COVID-19 [1].

This is particularly relevant in studying the levels of bioactive substances of *Isatis tinctoria*. Since it is known that under field conditions, bioactive substances in the root of *Isatis tinctoria* always fluctuate due to geographical, environmental and seasonal factors [3].

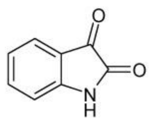
The main chemicals in the root of Woad.

Woad is an important source of two known indole alkaloids called indigo and indirubin. The former is blue in color and the latter is red, and both are widely used for dyeing textiles, cosmetics, foods, and pharmaceuticals. The plant is not able to synthesize indigoid pigments directly, but it produces several precursors: indican, isatan A, isatan B, and isatan C (Figure 1).

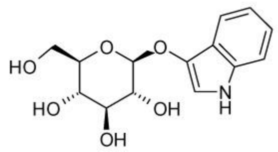
Wood and other plants that produce blue pigment contain precursors to the indigo dye, not indigo itself.

Woad plants contain two indigo (or indoxyl) precursors, indican (25%) and isatan B (75%), with indican being colorless, water-soluble, and stable, and isatan B being relatively unstable.

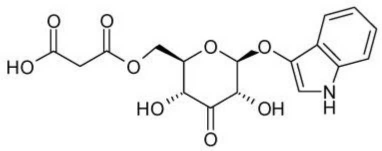
Hydrolysis of isatan B and indican yields the intermediate compound indoxyl, from which indigo or indigotine is subsequently formed.



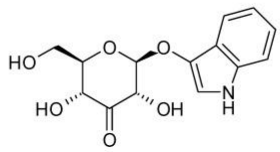
Isatin



Indican



Isatan A



Isatan B

Figure 1. Chemical structure of indigo precursors

When exposed to air, the precursors undergo enzymatic hydrolysis by β-d-glucosidase and β-glucuronidase. After cleavage of the O-glycosidic bond, indoxyl is released, giving in turn indigo (blue indigoid pigments) during oxidation and producing isatin as a side reaction due to the oxygen-rich environment, Isatin is produced from indoxyl in an oxygen-rich environment. Condensation of indoxyl with isatin leads to the formation of indirubin (red indigoid pigment) in Isatis tinctoria [8].

Isatindosulfonic acid glycosides A 3-O-b-d-glucopyranoside, indole-3-acetonitrile 6-O-b-d-glucopyranoside, isatindigobisindoloside A, isatindigobisindoloside B, and isatindigobisindoloside F were detected in roots by comparing spectroscopic and optical rotation data [7].

Flavonoids identified in the root of Woad: rutoside, neohesperidin, budleoside, liquiritigenin, quercetin, isoramnetin, kaempferol and isoliquiritigenin [2] by a method based on reversed-phase high-performance liquid chromatography with chemiluminescence detection. The procedure was based on the enhancement of chemiluminescence by flavonols of the cerium(IV)-rhodamine 6G system in sulfuric acid medium. The effect of several parameters on HPLC resolution and CL emission was systematically studied. Good separation was achieved by isocratic elution using a mixture of methanol and 1.0% aqueous acetic acid (37:63 by volume) for 25 min.

Biological activity of substances isolated from root cells.

Supplemental blue LED light can simultaneously promote root growth, the formation of additional buds, and the accumulation of medically important flavonoids in hairy roots of *Isatis tinctoria*. Increased root biomass productivity in general in *Isatis tinctoria* (up to 1.86-fold increase) together with higher yields of multiple hydroxy-substituted flavonoids such as rutin (up to 4.15-fold increase), quercetin (up to 9.31-fold increase), kaempferol (up to 9.09-fold increase), and isoramnetin (up to 2.88-fold increase) [3]. Classical in vivo models in mice and modern in vitro assay technologies exploring multiple targets associated with inflammation provide increased sensitivity and selectivity and thus allow for clearer endpoints for decision making to study pharmacologic activity. For example, indirubin has potent activity against influenza virus A/NWS/33- and B/Lee/40-infected H292 bronchial epithelial cells by reducing the expression and production of the chemokine RANTES.

Methods for the production of bioactive substances from root cells.

During the last four years, a group of two researchers, Gai and Jiao from Northeast Forestry University in Harbin, China, have published a series of research papers [Speranza, Miceli, Taviano, etal., 2020] on hairy root cultures of Woad. The cultures were successfully initiated from in-vitro petiole explants co-cultured with Agrobacterium rhizogenes supplemented with acetosyringone and arginine. High production of alkaloids and flavonoids was confirmed in the resulting hairy root cultures.

The alkaloids epigoitrin, isatin, indole-3-carboxaldehyde, tryptantrine, indigo and indirubin were qualitatively and quantitatively determined by LC-MS/MS method. The total alkaloid content (521.77 μg/g dry weight) was 1.12 times higher than that of biennial roots of field plants (464.69 μg/g dry weight) HPLC/MS method was used to investigate the flavonoid composition. The effective solvents for the determination of total flavonoid levels in roots were ethyl acetate, acetone and n-hexane-ethanol.

Under optimum conditions, the total flavonoid content (438.10 μg/g dry matter) was 1.28 times higher than that of two-year-old roots grown under field conditions (341.73 μg/g dry matter), which were analyzed for comparison. In addition, in-vitro antioxidant assays showed that hairy root extracts had higher activity than root extracts from field-grown plants [2].

Table 1

Chemical composition and methods for the determination of biologically active substances of the root of Woad (according to Speranza, Miceli, Taviano, et al., 2020).

Biological Activity	Experimental Model	Site of Collection	Plant Part/ Extract or Compound	Mode of Administration and Doses
Antiviral activity	Production of RANTES by Human bronchial epithelial cells H292 infected with influenza virus A/NWS/33 and		Indirubin	100–200 μM

	B/Lee/40 – ELISA			
Antioxidant activity	1,1-diphenyl-2-picrylhydrazyl (DPPH) test		Dried *I. tinctoria* (plant part not specified) 95% Ethanol	IC_{50} = 1583.45 ± 23.69 mg/mL
	Trolox Equivalent Antioxidant Capacity (TEAC)			mMTrolox/g = 589 ± 0.51
	Reducingpowerassay			Abs700 = 0.32 ± 0.004
	1,1-diphenyl-2-picrylhydrazyl (DPPH) test		Indigo Indirubin	EC50 = > 0.26 mg/mL > 0.26 mg/mL
	Superoxide anion radical scavenging activity		Indigo Indirubin	EC50 = 0.61 mg/mL 0.74 mg/mL
	Hydroxyl radical scavenging activity		Indigo Indirubin	Notactive Notactive
	Reducingpower		Indigo Indirubin	Notactive Notactive

Currently, in collaboration with groups from the Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina (Messina, Italy) and the Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum (Krakow, Poland), shoot cultures of I. tinctoria invitro were successfully obtained. Tinctoria in-vitro, the aim of which was to investigate their use as a potential source of biologically active compounds. The cultures were successfully initiated from seeds and preliminary optimization of the medium composition was carried out. Variants of MS medium with BA and KIN as cytokinins and NAA and IBA (indole-3-butyric acid) as auxins at different concentrations (ranging from 0 to 2 mg/L) were tested. The best conditions for shoot agar cultures were MS medium containing 1 mg/L BA and 1 mg/L NAA, on which the biomass growth was 3.58-fold (by live weight).

In terms of crude extract amount, all plant parts showed significant positive correlation [5].

Conclusion. Communication studies by researchers on the bioactive substances of Woad from growing sites in China and Europe have revealed new prospects for sustainable information to gather more information on this plant. Both Chinese authors and European scientists recognize information on the biological activity of Woad and refer to international experts.

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УДК 37.013.46

ADVANTAGES OF USING ARTIFICIAL INTELLIGENCE IN ADAPTIVE LEARNING

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Abstract: *The article is devoted to the application of artificial intelligence in the framework of adaptive learning. Its positive aspects have been analyzed.*