

Isatis tinctoria L. (Woad)

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Isatis tinctoria L. (Brassicaceae), which is commonly known as woad, is a species with an ancient and well-documented history as an indigo dye and medicinal plant. Currently, *I. tinctoria* is utilized more often as medicinal remedy and also as a cosmetic ingredient. In 2011, *I. tinctoria* root was accepted in the official European phytotherapy by introducing its monograph in the European Pharmacopoeia. The biological properties of raw material have been known from Traditional Chinese Medicine (TCM). Over recent decades, *I. tinctoria* has been investigated both from a phytochemical and a biological point of view. The modern in vitro and in vivo scientific studies proved anti-inflammatory, anti-tumour, antimicrobial, antiviral, analgesic, and antioxidant activities. The phytochemical composition of *I. tinctoria* has been thoroughly investigated and the plant was proven to contain many valuable biologically active compounds, including several alkaloids, among which tryptanthrin, indirubin, indolinone, phenolic compounds, and polysaccharides as well as glucosinolates, carotenoids, volatile constituents, and fatty acids. This article provides a general botanical and ethnobotanical overview that summarizes the up-to-date knowledge on the phytochemistry and biological properties of this valuable plant in order to support its therapeutic potential. Moreover, the biotechnological studies on *I. tinctoria*, which mainly focused on hairy root cultures for the enhanced production of flavonoids and alkaloids as well as on the establishment of shoot cultures and micropropagation protocols, were reviewed. They provide input for future research prospects.

alkaloids and flavonoids

anti-inflammatory

anti-tumour

antiviral

antioxidant

Brassicaceae

hairy roots

woad

1. Introduction

The genus *Isatis* L., which belongs to the Brassicaceae family, comprises about 80 herbaceous annual to perennial species diffused in the Middle East and central Asia and extending to the Mediterranean region ^{[1][2][3]}.

Isatis tinctoria L., which is commonly known as woad, is an herbaceous biennial or short-lived perennial species ^{[1][2]}. This species is thought to be native of Central Asia, as confirmed by genetic analyses ^{[4][5]}, even though other authors report it as native to South-eastern Russia to South-west Asia and, perhaps, to some parts of South-eastern Europe ^{[2][6]}.

Currently, it is a common ruderal plant of dry and sunny locations and it grows spontaneously in the rocky substrates, abandoned crops, open woods, clearings, and along the roads. It is very widespread from the sea level up to the maximum altitude of 1900 meters and it usually prefers hot spots and nitrogen-rich limestone soils [7][8]. It is considered a noxious weed in most of the Western United States [9].

2. History

Isatis tinctoria has a long and well-documented history for its medicinal properties and its indigo blue colour. This double use of woad is shown by its name. On one hand, the term “Isatis” that derives from the latin word “Isazein” and the greek word “Isadso” is linked to the its ancient use to treat wounds [10]. On the other hand, the term “tinctoria” refers to the use that was done in the dye works [8].

This species was introduced in Europe in late Prehistory and Protohistory periods, as confirmed by the discovery of textile remains dyed in blue and preserved from the Neolithic, Bronze, and Iron Ages [11]. The ancient Egyptians used *I. tinctoria* as an indigo source to dye the cloth wrappings applied for the mummies [12].

Historical accounts about the use of indigo in Europe date back to Roman times. Historical sources report that Celtic and Germanic people used woad to paint their body and hair for prophylactic or ritual purposes.

Pliny the Elder often mentioned woad in its writings, and reported the custom of female Britons covering their bodies with indigo blue for religious ceremonies [13]. Julius Caesar reported in his book *De Bello Gallico* that the Celtic populations used woad indigo to colour themselves to generate a fearsome appearance [14]. They pricked their skin and rubbed woad on to form a blue tattoo. The Romans called these people *Picti*, which means “painted people” [13]. This suggests that the *I. tinctoria* dye may have been used both for textile and body art [11].

From the 12th up to the 17th century, *I. tinctoria* has been widely cultivated in Europe (Germany, France, England, and Italy), and extensively used as indigo dye and medicinal plant. In the early 17th century, *I. tinctoria* was intentionally taken from Europe into North America by early colonists as a textile dye crop [6][15]. In the late 17th century, the decline of the woad industry in Europe was initiated due to the import of indigo blue from *Indigofera tinctoria* cultivated in Asia (India, Bangladesh) and, afterward, from other *Indigofera* species in the Caribbean and the American colonies, which was easier and more economical to extract. The crop was definitively abandoned in the late 19th century, when the production of synthetic dyes completely replaced natural indigo production [5][14][16]. With the declining importance as a dye and the disappearance of woad cultures, the plant also fell into oblivion as a medicinal plant [6][17].

3. Applications

Currently, *I. tinctoria* is widely utilized for medicinal purposes in the Traditional Chinese Medicine (TCM) in three forms, namely: *Bǎn Lán Gēn* (*Isatidis radix*), *Dà Qīng Yè* (*Isatidis folium*), and *Qīng Dai* (*Indigo naturalis*) [18]. The TCM recommends *I. tinctoria* in treatment various diseases, including inflammation, leukemia and solid tumors,

and viral infections. From the 2011 year the European Pharmacopoeia reports the monograph of *I. tinctoria* root (*Isatidis radix*) for use in official European phytotherapy [19]. Nowadays, the National Health Commission of P.R. China proposed *Isatidis radix* granula as treatments against COVID-19 [20].

Currently, *I. tinctoria* is utilized in cosmetic industries for the production of soaps and body creams. The seed oil and the leaves (powder/extract) are cosmetic ingredients for skin and hair conditioning due to their emollient and moisturising properties [5]. The roots (powder/extract) have astringent and skin protecting properties. The CosIng Database elaborated by the European Commission gives positivity to these previously mentioned raw materials for the production of cosmetics in Europe [21].

In recent years, renewed interest in natural dyes showed by the dyestuff industry and its potential use for medicinal and cosmetic products has encouraged growers to reintroduce *I. tinctoria* crops in the European agricultural system [5][22]. Horticulturists have become interested in utilizing *I. tinctoria* as an ornamental plant because of its tolerance to heat and water stresses, a long flowering period, and attractive inflorescences [10].

Although this species is not considered an edible vegetable worldwide, rural people living in Sicily (Italy) around Vulcan Etna consume boiled flower buds of this plant as ingredients for salads and omelettes [10][23].

Due to the relevance of *I. tinctoria*, over recent decades, this species has been studied both from a phytochemical and a biological point of view. The phytochemical composition of *I. tinctoria* has been thoroughly investigated and the plant was proven to contain many valuable biologically active compounds, including several alkaloids, among which tryptanthrin, indirubin, indolinone, phenolic compounds, polysaccharides, glucosinolates, carotenoids, volatile constituents, and fatty acids. The modern *in vitro* and *in vivo* scientific studies proved anti-inflammatory, anti-tumour, antimicrobial, antiviral, analgesic, and antioxidant activities.

A previous review article by Hamburger [6] reported the studies published from 2000 to 2002 on the anti-inflammatory activity of *I. tinctoria* extract and its active component tryptanthrin.

The publication "*Isatis tinctoria* L. (Woad): A review of its botany, ethnobotanical uses, phytochemistry, biological activities, and biotechnological studies" provides a general botanical and ethnobotanical overview and summarizes the up-to-date knowledge on the phytochemistry and biological properties demonstrated for this valuable species in order to support its therapeutic potential and to offer input for future research prospects. The article also focuses on all attempts in plant biotechnology studies for the enhanced production of flavonoids and alkaloids from *I. tinctoria* hairy root and shoot cultures as an alternative to plant raw materials.

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