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The Genus *Isatis* L.: A Review on its Flavonoid and Phenolic Compound Profile

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Abstract: The Brassicaceae family is one of the world's top ten economically significant plant families. The genus *Isatis* belongs to the family Brassicaceae. *Isatis* species have antibacterial, anticancer and anti-viral properties. Flavonoids and phenolics are bioactive secondary metabolites commonly found in medicinal plants. The present review summarises the flavonoid and phenolic compounds of the genus *Isatis* and aims to highlight the recent advances in current knowledge of *Isatis* species as a source of flavonoids and phenolics. Many known flavonoids and phenolic compounds from *Isatis* species, such as quercetin, rutin, caffeic acid, kaempferol, ferulic acid, gallic acid, p-coumaric acid, p-hydroxybenzoic acid, rosmarinic acid, sinapic acid, neohesperidin, buddleoside, liquiritigenin, isorhamnetin have been isolated.

Keywords: *Isatis*; *I. tinctoria*; *I. indigotica*; *I. microcarpa*; flavonoid; phenolic compound. © 2022 ACG Publications. All rights reserved.

1. Introduction

The Brassicaceae family, one of the ten most economically significant plant families in the world, contains 338 genera and 3350 species distributed worldwide [1, 2]. Vegetables of the Brassicaceae family are also a great source of nutrients with phenolics, antioxidants such as vitamins (like ascorbic acid), nutritional minerals and flavonoids [3]. In addition, the species of the family are used as antidiabetic, antibacterial [4], antifungal, anticancer [5] and antirheumatic [6] treatments and exhibit a potent insecticidic effect [7].

The Brassicaceae (syn. Cruciferae) include numerous plant species, including many familiar vegetables. Due to their economic and agricultural importance, Brassicaceae plants have been the subject of considerable scientific interest, especially Brassica oleracea, as they are the predominant food source of glucosinolates but also with high levels of polyphenols, minerals, vitamins and other antioxidant compounds. All these phytochemical compounds have additive and synergistic effects, which may contribute to anticancer, antioxidant, anti-inflammatory and cardioprotective activities recently associated with consuming cruciferous vegetables [8].

In recent years, with the increasing recognition of their bioactivity values, phenolic compounds have been found to exert various effects such as antioxidant [9], antimicrobial [10], anti-carcinogenic [11], anti-inflammatory [12] and the prevention of cardiovascular disease, cancer, diabetes and oxidative

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stress-related diseases [13]. Flavonoid is a significant class of phenolic compounds. Flavonoids are associated with a wide range of health benefits and are an indispensable component in a variety of nutraceutical, pharmaceutical, medical and cosmetic applications. This is due to their antioxidant, anti-inflammatory, antimutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzymatic functions [14].

The genus *Isatis* is a biennial, herbaceous shrub of the Brassicaceae family, with about 79 species worldwide [15]. It is represented by 40 taxa, of which 24 are endemic to Türkiye [16, 17]. The general characteristics of the genus *Isatis* are single, two or more years; 50-100 cm in length, with yellow flowers, and leaves are described as hairy or hairless [18]. The chemical compounds found in the leaves of the *Isatis* species have antibacterial, anticancer, antiviral, astringent and febrifuge properties. These species are also used to treat a wide range of disorders, including meningitis, encephalitis, mumps, influenza, erysipelas, heat rash etc. In a similar way, compounds isolated from the roots of these plants are rich in antibacterial and anticancer properties [19].

Isatis tinctoria has been widely cultivated in Europe and widely used as an indigo dye and medicinal plant from the 12th to the 17th century. In the early 17th century, *I.* tinctoria was intentionally taken from Europe to North America by early colonists as a textile dye [20, 21]. The crop was abandoned at the end of the 19th century when the production of synthetic dyes completely replaced the natural production of indigo [21, 22]. Nowadays, *I. tinctoria* is widely used for medicinal purposes in traditional Chinese medicine [20, 23, 24], and since 2011, it has been recognised as a pharmacopoeia plant in Europe [25]. Although this species is not considered an edible vegetable, people living in rural areas of Italy around Vulcan Etna consume boiled flower buds of this plant as ingredients for salads and omelettes [26, 27]. It has traditionally been used to treat injuries, ulcers and tumours, haemorrhoids and inflammatory illnesses [20]. In China, its leaves were mainly used for the treatment of infections. In particular, upper respiratory tract infections and gastroenteritis [28]. In addition, *I. tinctoria* is used in the cosmetic industry to manufacture soaps and body creams. Seed oil and leaves (powder/extract) are cosmetic ingredients for treating skin and hair, thanks to their emollient and moisturising properties [29]. In addition, the roots (powder/extract) are astringent and protect the skin. The CosIng database developed by the European Commission gives a positive value to these previously mentioned raw materials in the production of cosmetics in Europe [30].

However, traditional Chinese medicine, Banlangen (*Isatis* roots extract) and Daqingye (*Isatis* leaves extract), derived from the taxonomically closely related *I. indigotica* Fort., was still popular in the treatment of inflammatory diseases [31]. *I. indigotica* was widely used in the traditional medicine of China from ancient times. The root of *I. indigotica*, an important herbal medicine to treat influenza, has been used in China for over 2000 years [32]. *I. indigotica* leaves are a traditional Chinese medicine widely used with a long history as an antibacterial [33], antiviral [34], antiendotoxin [35], immunoregulator [36] and antitumor [37]. The various key bioactive compounds of *I. indigotica* that exhibit anti-inflammatory, antimicrobial, anticancer, and antiallergic properties and protective effects against neural injury and bone fractures were examined [38].

This review aims to provide an up-to-date and comprehensive overview of the ethnobotany and phytochemistry demonstrated for valuable *Isatis* species to support its therapeutic potential and provide input for future research prospects.

2. The Flavonoids and Phenolic Profile of *Isatis* Species

Taviano et al. [39] characterised the phenolic profile of *I. tinctoria* using HPLC-PDA-ESI-MS analysis and correlated phenolic content with observed biological effects. The HPLC analysis of leaf extracts of *I. tinctoria* showed a similar polyphenolic fingerprint, while they differed slightly in quantitative content (neo-chlorogenic acid, caffeic acid, sinapic acid, ferulic acid). Vicenin-2 proved to be the most abundant flavonoid detected in *I. tinctoria* cauline leaves (It-CL), whereas luteolin-glucuronide and stellarin-2 were the main flavonoids for *I. tinctoria* basal leaves (It-BL) and *I. tinctoria* flowers (It-F). The extract cauline leaf of *I. tinctoria* showed higher levels of phenolics (23.68 mg/g extract) than the basal leaf extract of *I. tinctoria* (10.13 mg/g extract). For the flower extracts of *I. tinctoria*, the total content of the identified phenolics was 19.95 mg/g extract. Concerning all extracts, flavonoids were the main class of compounds, representing approximately 91.77, 72.95 and 92.98% of the entire polyphenolic content of the analysed extracts, It-CL, It-BL and It-F, respectively.

Nguyen et al. [40] profiled *I. tinctoria* fresh leaf extracts' methanol extracts by liquid chromatography-mass spectrometry (LC-MS) and focused on the phenyl propanoid derivatives. They reported the structural characterisation of 99 compounds, including 18 flavonoids, 21 oligolignols, two benzoid and 58 hydroxycinnamic acid esters (Table 1).

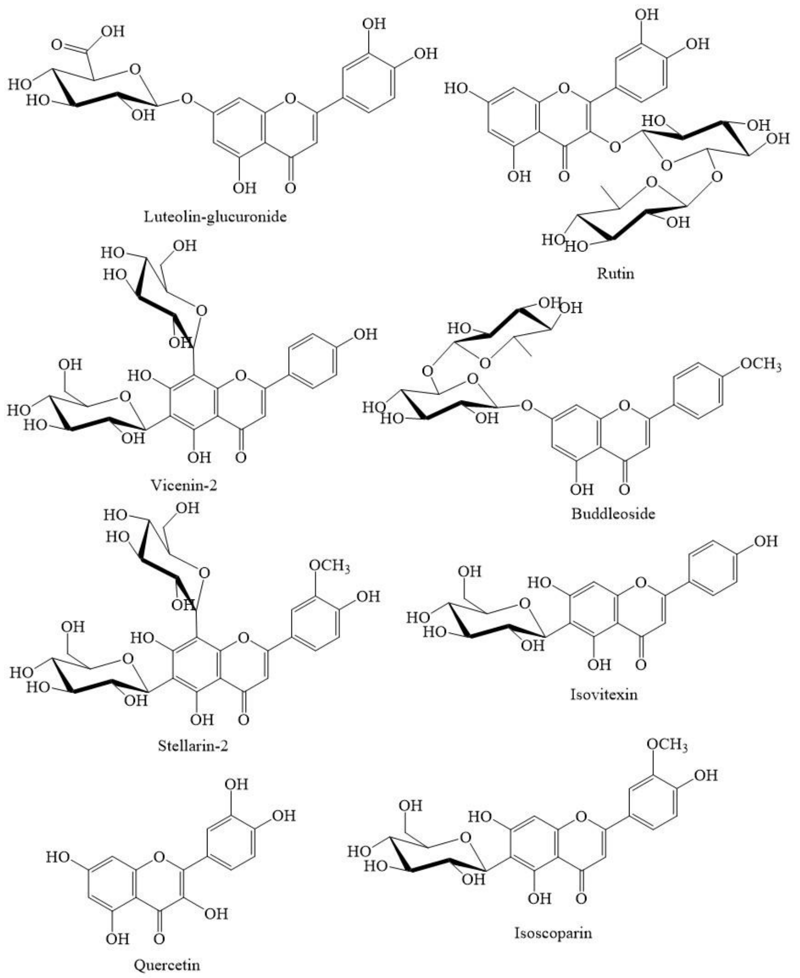

Figure 1. Most abundant flavonoid identified in *I. tinctoria* leaves

In another study, the total phenolic, flavonoid, and condensed tannin contents in the fraction were determined by spectrophotometry, while phenolic was assessed by HPLC-PDA-ESI-MS methods. A total of 20 phenolic/flavonoid compounds have been positively identified, and 12 out of these have never been reported previously in *I. tinctoria* leaves. Among flavones, vicenin-2 was the most abundant flavonoid identified, followed by flavone diglucoside, isovitexin, and apigenin glucosides (Figure 1). Micelli et al. [41] have determined the chemical characterisation and biological activities of phenolic-rich fractions from cauline leaves of *I. tinctoria* growing in Sicily, Italy.

For the first time, *I. tinctoria* hairy root cultures (ITHRCs) were co-cultured with two immobilized live GRAS (Generally Recognised as Safe) fungi, i.e. *Aspergillus niger* and *Aspergillus oryzae*, for high-yield of pharmacologically active flavonoids produced by Jiao et al. [42]. Determining eight flavonoid derivatives (rutin, neohesperidin, buddleoside, liquiritigenin, quercetin, isorhamnetin, kaempferol and isoliquiritigenin) in sample solutions was carried out using an Agilent 1100 series HPLC (Agilent 1100 series) coupled to an API 3000 triple tandem quadrupole MS system.

Table 1. Characterization of flavonoids in *I. tinctoria* leaf by LC-MS

No	Flavonoids
1	Luteolin-6-C-glucoside-7-O-glucoside
2	Vicenin-2
3	Isovitexin-3″-O-glucoside-7-O-glucoside
4	Stellarin-2
5	Isoscoparin-3″-O-glucoside-7-O-glucoside
6	Isoorientin
7	Isoorientin-3″-O-glucoside
8	Isovitexin
9	Isovitexin-3″-O-glucoside
10	Isoscoparin
11	Isoscoparin-3″-O-glucoside
12	4′-O-feruloyl isoscoparin-3″-O-glucoside-7-O-glucoside ***or*** 4′-O-sinapoyl Isovitexin-3″-O-glucoside-7-O-glucoside
13	2″-O-feruloyl isoscoparin-3″-O-glucoside-7-O-glucoside
14	Isoscoparin-3″-O-glucoside-7-O-feruloylglucoside
15	Chrysoeriol-7-O-glucoside
16	Isoscoparin-3″-O-p-coumaroylglucoside
17	Isoscoparin-3″-O-sinapoylglucoside
18	Isoscoparin-3″-O-feruloylglucoside

Mohn et al. [31] have reported on a broad-based characterization of the pharmacologically active extract from *I. tinctoria* leaves, using multiple detection systems to ensure a wide coverage of secondary metabolites with highly divergent analytical properties. Diode array detector, evaporative light scattering detector, atmospheric pressure chemical ionization, and electrospray ionization mass spectrometry, and electrospray ionization time-of-flight mass spectrometry detectors have been used in parallel to ensure wide coverage of secondary metabolites with very diverging analytical properties. Off-line microprobe nuclear magnetic resonance spectroscopy after peak purification by semi-preparative high-pressure liquid chromatography was used to elucidate the structure elucidation of some minor constituents. In total, 65 compounds belonging to various structural classes were identified, including alkaloids, flavone glycosides, glucosinolates and indigo precursors, fatty acids, porphyrins, carotenoids, lignans, cyclohexenones, and phenylpropanoids.

In the other study, the distribution of phytochemicals in the dried root of *I. tinctoria* was revealed by microscopic mass spectrometry imaging, with the application of atmospheric pressure–matrix-assisted laser desorption/ionisation (AP-MALDI) and ion trap–time-of-flight mass spectrometry (IT-TOF/ MS) by Nie et al. [43]. According to the result obtained, it has been detected phytochemicals belong to a wide range of classes of chemical compounds such as alkaloids, sulfur-containing compounds, phenylpropanoids, amino acids, nucleotides, organic acids, flavonoids, terpenes, saccharides, aromatics, peptides, and sphingolipids.

Gao et al. [44] obtained eight flavone C-glucosides from *I. indigotica* (*Folium Isatidis*). The compounds produced were specified using mass spectrometry and NMR. The separated compounds were identified as isoscoparin, isoscoparin-3″-O glucopyranoside, isovitexin, and isovitexin-3″-O-glucopyranoside. According to the results, compounds isoscoparin, isoscoparin-3″-O glucopyranoside, and isovitexin-3″-O-glucopyranoside are isolated from *Isatis indigotica* Fort. for the first time.

The leaves of *I. indigotica* are recorded as "Daqingye" in the current Chinese Pharmacopeia [45]. The UPLC-PDA-ESI-MS/MS-based analysis of flavonoids in Daqingye was described by Deng et al. [46]. Separation was performed on an Acquity UPLC BEH C_{18} column with 0.1% formic acid and methanol as a mobile phase under gradient conditions. Eight flavone C-glucosides (saponarin; Isovitexin 6″-O-glucopyranoside; Isoorientin; Isoorientin 3″-O-glucopyranoside; Isoscoparin; Isoscoparin 3″-O-glucopyranoside) were identified, and their mass spectrometric fragmentation patterns were studied.

Jinlianhua Decoction (JD), composed of *Flos Trollii*, *Herba Taraxaci*, *I. indigotica*, *Radix Puerariae Lobatae* and *Folium Perillae* in a ratio of 6:15:10:10:6, is a prescription for Fengwen which is a group of febrile diseases due to the wind in Chinese medicine. It was originally used for the prevention and treatment of Severe Acute Respiratory Syndrome (SARS) and could also be used to treat influenza because of its common pathomechanism. To elucidate the unclear pharmacodynamic basis of JD, the LC-QExactive-MS system was used to qualitatively analyze its main components in another study [47]. As a result, based on the qualitative and quantitative results in combination with the reported bioactivities, 16 compounds, including orientin, 2"-O-β-L-galactopyranosylorientin, puerarin, trollisin I, rosmarinic acid, 2"-O-(2'"-methylbutanoyl) isoswertisin, daidzin, scutellarin, 3'-methoxy puerarin, vitexin, 3'-hydroxy puerarin, 2"-O-(2'"-methylbutanoyl) vitexin, kaempferol, caffeic acid, 3,4-methoxy benzoic acid, and cynaroside were identified as the major components of JD.

A Chromatographic study of the flavonoid constituents of *I. microcarpa* revealed the presence of eight flavonoids (apigenin-7-O-rutinoside, apigenin-7-O-glucoside, luteolin, luteolin-7-O-glucoside, kaempferol, kaempferol-7-O-glucoside, kaempferol-3-O-glucoside and naringenin) [48]. These compounds were identified through R_f values, acid hydrolysis, enzymatic hydrolysis, UV, ^{1}H-NMR and Mass spectral data.

However, Emam and El-Moaty [49] have indicated the phenolic acid compounds of aqueous ethanol extracts of *I. microcarpa* Boiss. As a result of this study identified the phenolic acid compounds of *I. microcarpa* as ferulic acid, gallic acid, and caffeic acid (Figure 2).

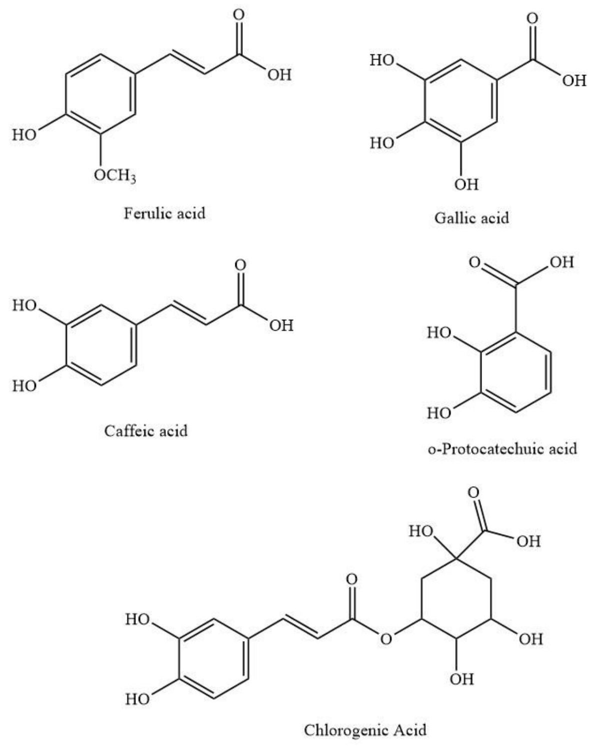

Figure 2. The phenolic acid compounds identified in *I. microcarpa* Boiss

Wu et al. [50] isolated ten compounds from the roots of *I. indigotica*. One of them (4-(4′-hydroxy-3′,5′-dimethoxy phenyl)-3-buten-2-one) was a phenolic compound isolated for the first time from natural products (Figure 3).

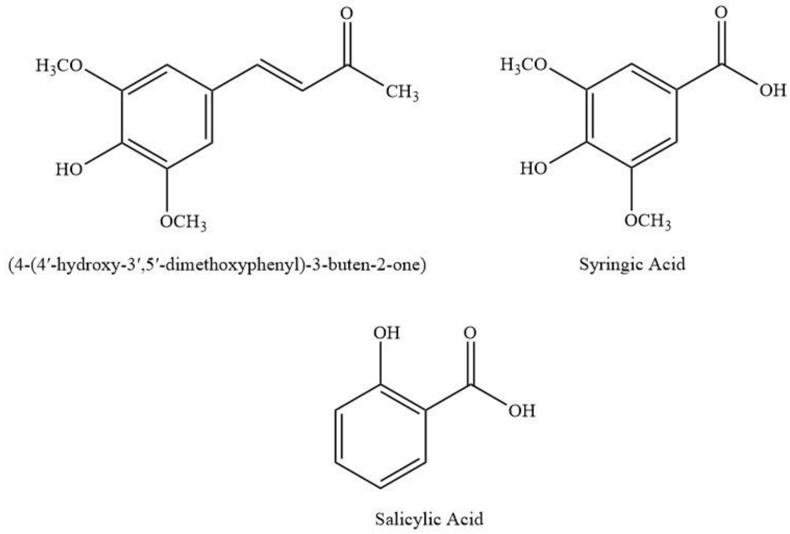

Figure 3. The phenolic acid compounds identified in *I. indigotica*

Karakoca et al. [51] determined the phenolic composition of *I. floribunda* root methanolic extract by high-performance liquid chromatography. Ten components were analyzed in the methanolic root extract, and eight phenolic components were determined. The results of the HPLC analysis indicated that chlorogenic acid was the major component, followed by quercetin, p-coumaric acid, caffeic acid, and p-hydroxybenzoic acid (Figure 4**)**.

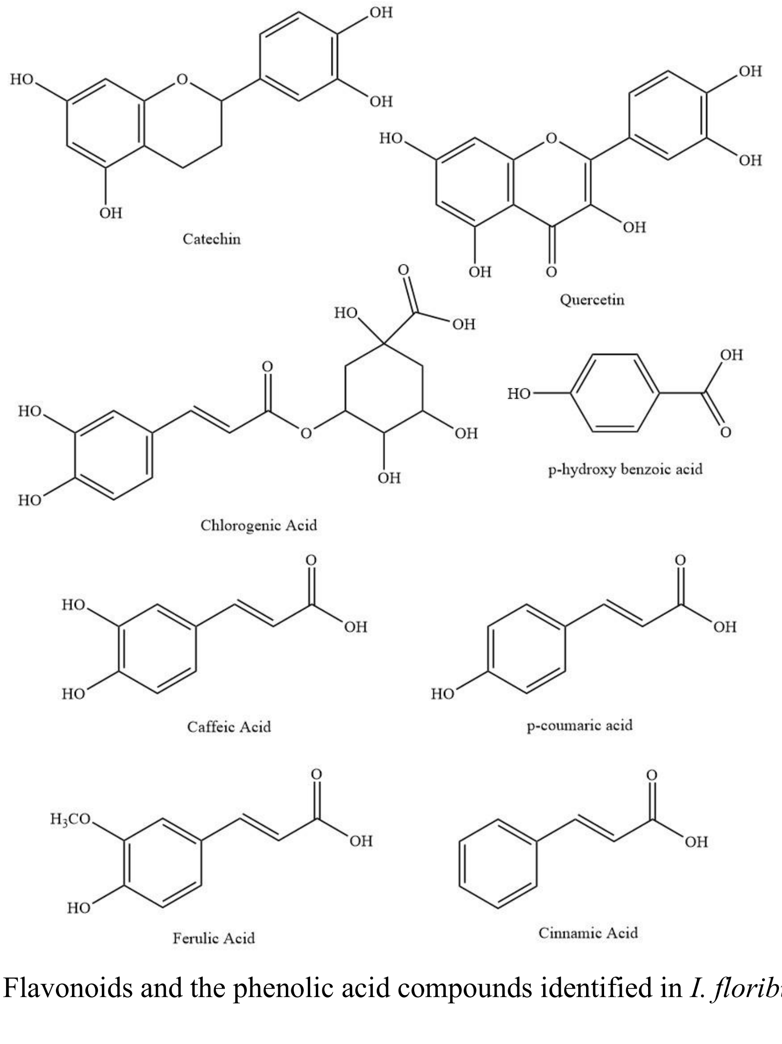

Figure 4. Flavonoids and the phenolic acid compounds identified in *I. floribunda* root

The methanol extract of *I. tinctoria* was analyzed by Mohn et al. [31]. In the dried rosette leaves, they found vicenin-2, stellarin-2, isoorientin, isovitexin, isoscoparin, and some of their glucosides. Simultaneously, the isocoparin, sinapic, and ferulic acids resulted from the analysis of dichloromethane extract from the *I. tinctoria*.

Karakas et al. determined the flavonoid and phenolic contents in the *I. demiriziana Mısırdalı* by LC-MS/MS. Among the 27 compounds studied, malic acid appeared to be the most abundant compound in the methanol extracts of samples. Moreover, a considerable amount of salicylic acids and p-coumaric were determined in the root extract [52].

3. Conclusions

Phenolic compounds play an essential role in the natural antioxidant, antimicrobial, and anti-inflammatory effects, as well as in the treatment of diseases such as obesity, cancer, and diabetes [53]. Flavonoids, a class of phenolic compounds, have received a great deal of attention in the literature over the last decade, and a variety of potential beneficial effects have been elucidated. However, more and more researches are necessary to discover new flavonoids from nature's bounty. The study of the biological potential of many natural phenolic plants remains a hot topic in the scientific community.

Looking at the literature, it is seen that the flavonoid content of the *Isatis* species is composed primarily of flavone and then flavonols. Especially in the flavones found in these species, a C-glucoside molecule is attached to the 6^{th} carbon atom. Moreover, flavonones with glycoside bonds at carbons 3'' and 7th are present in *Isatis* species. When the flavonols of *Isatis* species were examined, flavonol-3-glycoside derivatives were obtained, and sugar structures were not found in other flavonol compounds. Furthermore, phenolic compounds include chlorogenic acid, caffeic acid, ferulic acid, syringic acid, salicylic acid, gallic acid, cinnamic acid, *p*-coumaric acid, and *p*-hydroxybenzoic acid constitute the phenolic acid content of the *Isatis* species.

Overall, this review summarised the flavonoids and phenolic compounds of the genus *Isatis* L., including all *Isatis* species. This study showed that *Isatis* is a source of flavonoids and phenolics with a variety of promising. This article provides an overview of the phytochemical profile of *Isatis* species containing phenolic and flavonoid compounds.

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