International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2023; 11(5): 144-151
Received: 18-07-2023
Accepted: 01-09-2023

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# *Fumaria officinalis* L. active compounds and biological activities: A review

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**DOI:** https://doi.org/10.22271/flora.2023.v11.i5b.900

**Abstract**
*Fumaria officinalis* L. is a medicinal plant traditionally used in Europe and Asia on hepato-biliary problems, hypertension, gastrointestinal colic and skin problems. In this review, previous investigations on the phytochemistry and pharmacological properties of *F. officinalis* were critically summarized. *F. officinalis* is rich in alkaloids and phenolic compounds including flavonoids, to which are imputed its important pharmacological activities. It has shown high antioxidant activity and cytoprotective effect on the liver, kidney and testicles. It has shown anti-diabetic, analgesic and anti-inflammatory activities. It has potential against Alzheimer's disease. Some extracts can have high cytotoxic activity against some cancer lines, and antimicrobial activity against some microbes. It has also demonstrated diuretic and skin protective effects. This set of activities gives it potential usefulness in a range of pathologies. Further studies are required to better understand these effects and underlying mechanisms.

**Keywords:** *Fumaria officinalis*, Pharmacognosy, alkaloids, antioxidant, hepatoprotective, cytoprotective

## Introduction
The genus Fumaria, of the Papaveraceae family, comprises 56 species [1], distributed in Europe, Asia and Africa. The different Fumaria species, commonly known as fumitory, look similar and are hard to differentiate due to inter-specific hybridization [2]. Several Fumaria species have long been used in folk medicine, the most popular being *F. indica*, *F. officinalis*, and *F. parviflora* [3]. One major ethnomedicinal uses of Fumaria species is purifying blood in liver obstruction [3]. Romanian traditional medicine describes the use of aerial parts of Fumaria species as diuretic agents as well as to treat hepatobiliary diseases [4]. Extracts of different Fumaria species have been used traditionally for treatment of rheumatism, stomach ache, abdominal cramps, fever, diarrhea, skin rashes, conjunctivitis, syphilis and leprosy [5]. *Fumaria officinalis* L. is an herbaceous plant, annual or biennial, erect or diffuse, rarely climbing, with an erect stem of 30 to 70 cm, strongly rowed. The leaves, alternate, divided, green or glaucous, are finely pinnate and segmented. Purple or pink flowers, very even, are arranged in clusters on the terminal part of the stem, the upper spread extending in spur. The sepals are oval and lanceolate, irregularly dented, wider than the pedicle and narrower than the corolla. The fruit is an indehiscent globose silicle containing a single seed, truncate and notched at the top. The plant, polymorphic, contains latex and has bitter taste. This plant is very common in Europe and Asia, on the edges of paths and wastelands, along old walls, up to 1500 m altitude [6]. Fumitory is known in Europe since antiquity and used since the middle Ages. Back in the early 13th century, the plant was already known by the name fumus terrae, "smoke of the earth." Around two thousand years ago, Dioscorides mentioned in his work, *De Materia Medica*, that if you rub the sap or latex of this plant on your eyes, it will cause tears similar to the stinging sensation caused by acrid smoke [7]. Infusion of the flowering parts are used against hepatic and gallbladder diseases in Northern Portugal [8] In Italy, it has been used as cholagogue, hypertensive, antispasmodic, respiratory stimulant, and anti-arteriosclerotic. In Cyprus, for hypertension, constipation, liver detoxification, and as spasmolytic. In Morocco, in hypertension and cardiac disease [9]. A questionnaire study among indigenous communities in Pakistan found that the plant is used for blood purification, skin problems and allergy, and as laxative [10]. The European Pharmacopoeia determines the use of dried aerial parts, harvested in full bloom [11].

## 2. Materials and methods
Scientific search engines including PubMed, ScienceDirect and Google Scholar were scanned for works on *F. officinalis*. The phytochemical and pharmacological data was analyzed and synthetized.

## 3. Results and Discussion

### 3.1 Phytochemical compounds

In a phytochemical evaluation of the powder of the dried plant using different assays, *F. officinalis* tested positive for the presence of alkaloids, glycosides, tannins/phenols, organic acids, saponins, steroids/terpenes, besides the primary metabolites [12]. The plant contains some aliphatic acids, like fumaric acid, which has been reported to have hepatoprotective, antipruritic, antioxidative, and anti-inflammatory activities. Fumarates may also modulate the central nervous system function [3]. The analysis of the apolar fractions showed that 1-Stearoyl-glycerol, at 73.7 % TIC (Total Ion Current) and palmitic acid, at 20.3 % TIC were the most abundant lipids [5]. The plant also contains bitter principles, mucilage, resin and potassium salts [11]. A study about the use of plants as forage quantified the main compound groups in *F. officinalis* as 12.211 g/kg DM flavonoids, 3.961 g/kg DM phenolic acids and 6.015 g/kg DM alkaloids. The main individual active compounds in the plant were identified as quercetin-OHex-Hex (2.384 g/kg DM), quercetin O-Pen-Hex (3.5 g/kg DM), fumariline (1.728 g/kg DM) and caffeoylmalic acid (1.212 g/kg DM) [13]. In another study, the most abundant compounds overall were investigated using HPLC-DAD, showing the richness of 6 components in *F. officinalis*, in decreasing order: naringenin (5.78 mg/g extract), ferulic acid, quercetin, kaempferol, caffeic acid and rosmarinic acid (2.17 mg/g) [14].

#### 3.1.1 Phenolic compounds

Several studies have shown that the plant contains significant amount of phenolic compounds, to which some of the antioxidant properties are imputed. Sengul et al., 2009, studied several plants, measuring the total phenolic content of *F. officinalis* at 10.50 mgGAE/g DW [15]. Another study quantified it as 0.64 mg GAE/100 g of methanolic extract, with 18 mg QE/100 g of total flavonoids [16]. In a comparative study of five Fumaria species from Bulgaria, *F. officinalis* presented the highest amount of total phenolic compounds (30.03 mg GAE/g DW) and total flavonoids (15.70 mg QE/g DW) [5].

Several phenolic acids and derivatives have been identified in its extracts: cynarin and chlorogenic, isochlorogenic and ferulic acids [17], p-Coumaric acid and ferulic acid [4] and sinapic acid [18]. Caffeic acid and its derivatives, like cholorogenic and caffeoylmalic acids, have also been reported [11].

##### 3.1.1.1 Flavonoids

The major class of phenolic compounds in this species are flavonoids, some of which have already been mentioned. Using an LC-MS method, they have been quantified: rutin (854.40 mg/Kg DW), isoquercitrin (506.2 mg/Kg DW), quercetin (348 mg/Kg DW), quercitrin (48.4 mg/Kg DW) and kaempferol (44.14 mg/Kg DW) [4]. In a study with plants from Luxembourg and Belgium, the flavonoids isovitexin, rutin, isoquercitrin and quercitrin were found in all the samples [17]. In a study with plants from Bulgaria, myricetin, kaempferol, quercetin, rutin, hyperoside, and apigenin were identified [18].

#### 3.1.2 Alkaloids

The Fumaria genus is known for its richness in alkaloids derived from isoquinoline, which is a structure made up of a benzene ring fused to a pyridine ring. According to the European Pharmacopoeia, the dried aerial parts contain a minimum of 0.40% of total alkaloids, expressed as protopine [11]. In a HPLC-DAD analysis of extracts of both commercially available and spontaneous samples of the plant, protopine was the major alkaloid (123.38 - 258.3 mg/100 g vegetal product) and was identified in all extracts, along with sanguinarine (1.41 – 5.03 mg/100 g). Chelidonine was found in high quantity (94.13 mg/100 g), but only in some samples. This study also reported and quantified the presence of bicuculline (tr – 8.3 mg/100 g) and stylopine (tr – 4.12 mg/100 g). The concentration of isoquinoline alkaloids expressed in chelidonine was between 0.69 and 0.76 g/100 g vegetal product in all samples [17]. Another study performing phytochemical investigations on the plant found that the alkaloid-rich fraction contained two major alkaloids, stylopine (48.3%) and sanguinarine (51.6%) [19]. The study about plants as forage identified fumariline (1.728 g/kg DM), protopine and protopine type (1.240) parfumine (0.884), and stylopine (0.785) as the main alkaloids in the samples [13]. The different conclusions found by different studies about the most abundant alkaloids in the plant, may be attributed to variations due to growing factors, intra-species variation, and the inter-conversion among compounds across phenological stages.

Zhang et al. made a detailed inventory of all the alkaloids isolated from Fumaria species until 2019, which they organized into the following groups [3]:

##### 3.1.2.1 Protoberbines and berberines

These compounds are important intermediates for the biosynthesis of alkaloids in the genus *Fumaria*. The compounds reported for *F. officinalis* falling in this group are coptisine, (S)-cis-N-methylstylopine and N-methylsinactine [3]. Chlebek. *et al* had also found (-)-stylopine, (-)-sinactine and (-)-cheilanthifoline [20].

##### 3.1.2.2 Benzylisoquinolines

Although none was reported in *F. officinalis*, benzylisoquinolines are the basic skeleton of the alkaloids from *Fumaria* species [3].

##### 3.1.2.3 Spirobenzylisoquinolines (SBIs)

These compounds are almost exclusively found in the genera *Fumaria* and *Corydalis*. The following have been reported in *F. officinalis*, being very characteristic of this species: (+)-fumariline, dihydrofumariline, (+)-parfumine (fumarilicine), (+)-parfumidine, (−)-fumaritine, (−)-fumaricine, (–)-fumarophycine, (−)-O-methylfumarophycine and (−)-fumaritine N-oxide [3]. (+)-fumariline has shown potential central nervous system depression effects in rats, producing dose-dependent anticonvulsant and antinociceptive effects and an increase in pentobarbital-induced hypnosis [3].

##### 3.1.2.4 Classical phthalideisoquinolines (PTIs)

Phthalideisoquinolines (PTIs) also play an important role in the biosynthesis of isoquinoline alkaloids. (+)-bicuculline, (+)-adlumine, (−)-α-Hydrastine [20], as well as (+)-corlumine [21] have been identified in the plant. (+)-Bicuculline is a light-sensitive competitive antagonist of gamma-aminobutyric acid receptors in the central nervous system [3].

##### 3.1.2.5 Secophthalideisoquinolines

Secophthalideisoquinoline alkaloids from the genus *Fumaria*, having a seco-ring B, include enol lactones, keto acids, diketo acids and ene-lactams subgroups. Inspection of the structure of some of these compounds in *Fumaria* species suggest that they are essential intermediates in the metabolic sequence. N-methylhydrastine, adlumidiceine, adlumiceine, N-

methylhydrasteine, N-methyloxohydrasteine and corydamine have been identified in *F. officinalis* [3].

#### 3.1.2.6 Indenobentazepines
It is known that Fumaria species can convert benzylisoquinolines into protoberberines, which in turn are the probable precursors of SBI. Bulgaramine, which has been identified in *F. officinalis*, occupies a central position in the biogenetic scheme linking SBIs with indenobentazepines. Fumarofine (Fumarostelline), and fumaritrine are also present in this species [3].

#### 3.1.2.7 Protopines
Protopines are found in most of the species of *Fumaria* and was found to be the main alkaloid (66–79% in total alkaloid) in 10 Fumaria species, including. *F. officinalis*, which contains protopine and cryptopine (Cryptocavine). The content of protopine was measured at 0.514 mg/g DW, being one of the highest among selected plants of the Ranunculaceae, Papveraceae and Fumarioides families [22]. Protopine protects oxidative stress-induced cell from death and has shown in vivo antiarrhythmic, antithrombotic, anti-inflammatory, and hepatoprotective activities, having also muscle relaxant, hydrocholeretic and antiviral effects.

#### 3.1.2.8 Benzophenanthridines
In this group, the quaternary benzophenanthridines (QBAs) are the most well known for their effects. Dihydrosanguinarine, sanguinarine [22] and chelerythrine [23] can be found in *F. officinalis*.

#### 3.1.2.9 Aporphines
Of this group, *F. officinalis* contains (+)-corytuberine [3].

#### 3.1.2.10 Other alkaloids
*N*-methylcorydaldine, also found in the plant, does not fall into any of the above groups [20].

### 3.2 Antioxidant activity
In a study using the β-Carotene-linoleic acid assay, the antioxidant activity of the *F. officinalis* extract was measured at 78.93%, comparing to 93.21% and 90.71% of standard BHA and BHT, respectively [15]. In a study using the TEAC assay, a value of 59.76 mg TE/g DW was found for F. officinalis, which was lower than that of *F. vaillantii*, *F. capreolata* and *F. rostellata*, higher than that of *F. jankae* and similar to that of *F. schleicheri* [4]. In a study with other four *Fumaria* species from Bulgaria, *F. officinalis* had the highest antioxidant activity in all the assays: 160.05 mM TE/g DW and 2.39 EC50 in DPPH, 131.14 mM TE/g DW in ABTS, 161.48 mM TE/g DW in FRAPS and 625.67 in CUPRAC [18]. In a study with several types of extracts, the methanolic extract exhibited the maximum percentage inhibition of DPPH (86.30%) [24]. Scavenging and anti-lipoperoxidation activity of some alkaloids (–)-fumarophycine, (−)-O-methylfumarophycine and protopine) and phenolic acids (caffeic and protocatechuic acids) in several *Fumaria* species including *F. officinalis* were tested. The alkaloid compounds showed higher antioxidant activity against tBH-induced lipoperoxidation than phenolic acids. On the other hand, the phenolic compounds were 2–3 fold as effective as scavengers of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical than the alkaloids. (–)-fumarophycine and caffeic acid were the most active constituents [3]. A study compared the antioxidant activity of the plant both as aqueous extract and as nanoparticles against DPPH free radicals. The $IC_{50}$ values were 253 and 145 mg/mL, respectively, which compare to the 107 mg/mL value of BHT [25]. Scavenging activity of the extract on radicals with: $IC_{50}$ of 0.02 mg/g on DPPH, 0.01 mg/g on FRAP, and 0.14 mg/g on H2O2, which compares with $IC_{50}$ values of vitamin C of 0.014, 0.01 and 0.02 respectively. The total antioxidant capacity of F. officinalis extract was 258.79 mg GAE/g dry weight [14].While some studies have found a correlation between the total amount of phenolics and the antioxidant activity, other studies have not found such correlation. This may be due to the presence of other antioxidant phytochemicals and synergistic effects among them, and to the fact that not all phenolic compounds have the same antioxidant activity. Besides, some methods do not measure accurately the total phenolic content [15].

### 3.3 Biological activities in vitro and in vivo
*F. officinalis* was officially recognized in 1986 by the French Health authorities as an herbal remedy traditionally used in renal and digestive elimination functions and to help digestion. It is included on the United Kingdom General Sales List (GSL) and is approved by the German Commission E Monograph. It has been used, in Europe and worldwide, as a traditional remedy for more than 30 years without safety problems [11]. The EMA assessment report on *F. officinalis* (2011) identified eight clinical studies in 617 patients as well as one double blind placebo trial (30 patients) and two open studies in 63 patients, conducted between 1968 and 1992, that fully support the traditional use with intended choleretic and digestive effects of the plant. It also recognized that the then existing data on amphocholeretic, mild antispasmodic on smooth muscle, mild diuretic and laxative antispasmodic activity, from studies between 1966 and 1992, supported the traditional use to increase bile flow for the relief of symptoms of indigestion (such as sensation of fullness, flatulence and slow digestion) [11]. In Germany, *Fumaria officinalis* is approved for the indication "colicky pain affecting the gallbladder and biliary system, together with the gastrointestinal tract" [26].

#### 3.3.1 Hepatoprotective
The hepatoprotective effects traditionally imputed to the Fumaria genus have been tested with positive results for some species. *F. indica* showed chemo preventive effect in in-vivo hepatocarcinogenesis models with rats, finding the butanol fraction, in which protopine was quantified as a major compound, to be the most effective. Protopine, which is a major alkaloid also in *F. officinalis*, showed to be equally effective as silymarin. In a study with an anesthetized dog, protopine produced a 2.5-fold rise in bile flow at 1 h after drug administration. *F. parviflora* also showed hepatoprotective effects, attributed mainly to its antioxidant efficacy, which derive not only from the alkaloids but also other components, like fumaric acid. However, further clinical studies are required to explore these properties. Both the methanol extracts of *F. densiflora* and *F. officinalis* showed a hepatoprotection against CCl4-induced toxicity in hepatocytes. The alkaloids showed a significant cytoprotective effect even at the lowest concentration of 1.0 mM, while the phenolic fraction exhibited no cytoprotective effect [3]. Three different studies by researchers in Tunisia have explored the protective effects of *F. officinalis* extract against mitochondrial bioenergetics disturbance and oxidative status perturbations induced by the insecticide permethrin (PER) both in the liver and in the kidneys. The protective

effects could be attributed to phenolics compounds such as polyphenols, condensed tannins, and flavonoids, as well as to some of the plant's alkaloids [27, 14, 28]. In the study focusing on the liver, the extract significantly restored food intake, body weight and liver weight towards normal in the PER-treated rats. It significantly reduced the impact of PER in hepatic AST and ALT activities, CRP level and ϒGT activity, as well as plasma transaminase activities and CRP level, an activity mainly imputed to its content of caffeic acid, which has shown to stabilize the membrane of hepatocytes. The extract reduced the increase of thiobarbituric acid reactive substances (TBARS), protein carbonyl (PCO), protein oxidation products (AOPP), and nitrite oxide (NO) levels in liver and mitochondria, which could be attributed to the inducing effect of quercetin on detoxifying pathways. It also restored significantly the liver and mitochondrial oxidative biomarkers, which could be due to the activity of naringenin both in scavenging free radicals and modulating GSH synthesis pathways. The improvements were visible under liver histopathology analysis, which saw a significant recovery from the PER-induced vacuolation and fragmentation of chromatin. *F. officinalis* also significantly decreased the DNA damage induced by PER, which could be due to quercetin, which is known to protect liver cells against mercury-induced DNA injury and redox status alterations [14]. The ethanolic extract at a dose of 200 and 500 mg/Kg body weight, showed significant ($p$<0.001) action against carbon tetrachloride induced liver damage, by reducing the serum marker enzymes like SGPT, SGOT and ALP. They also reduced the elevated levels of serum total and direct bilirubin, cholesterol, and triglycerides. The ascorbic acid quantity in the rats' urine and histopathological studies further confirmed the hepatoprotective activity of the extract [29].

### 3.3.2 Cytoprotective in kidney cells

In the study also using PER as toxic agent, but with a focus on nephrotoxicity, the flavonoid-rich fraction of the plant exerted a significant protective effect through several mechanisms. It effectively inhibited protein denaturation and heat-induced hemolysis, leading to the conclusion that membrane stabilization is a main mechanism of its anti-inflammatory activity. A strong correlation between the protein denaturation inhibition action and caffeic acid, ferulic acid and quercetin was found. It also improved the signs of metabolic syndrome including body and kidney weights, a modulatory action that could be attributed to ferulic acid, which had shown similar effects in cadmium-induced toxicity. The damage inflicted by the oxidizing species to the proximal tubules was decreased, which was attributed to the scavenging activity of the plants' phenolic compounds. The enhancement of antioxidant defenses also accounted for a decrease in the elevation of the levels of GSH and antioxidant mitochondrial enzymes. Kaempferol had demonstrated to scavenge oxygen free radicals, hinder lipid peroxidation and prevent the increase in membrane permeability resulting from renal oxidative injury, thus being probably involved in this activity. The fraction also attenuated the disturbance of mitochondrial activities, which could be attributed to naringenin that had shown some ability to rescue kidney mitochondrial function under oxidative stress conditions. Just like in the liver study, histopathological examination confirmed the protective effect on the renal tissue [28].

### 3.3.3 Cytoprotective in testicles cells

The protective effect of a *F. officinalis* extract from damaged caused by fluoxetine on the testicle tissue of rats was studied. Fluoxetine is used as an antidepressant, and it is known to induce sexual dysfunction and a decrease in sperm concentration, motility, and morphology. In the study, it caused several structural changes in the rat testis such as vacuolation within germinal epithelium of seminiferous tubules, decrease in the diameter of these tubules and decrease in the number of Sertoli and germinal cells. Treating the fluoxetine exposed rats with *F. officinalis* extract ameliorated these histological changes. The authors suggest that these results might be due to the antioxidant effect of the iso quinoline alkaloids such as protopine, fumaricine and sanguinarine and polyphenols such as rutin and apigenin that protect the cell membrane from damage. Previous studies had found related effects in other species of the genus, including increase of testosterone and more blood flow to the testis, with *F. officinalis* showing aphrodisiac activity in rats [30].

### 3.3.4 Antidiabetic, analgesic and anti-inflammatory

The extract of *F. officinalis* showed a significant and dose dependent reduction of blood glucose in diabetic mice, higher than glibenclamide. Either acutely (after 6 hours of administration) subchronically (after 8 days) and chronically (after 8 weeks), the effect was highest in the alkaloid-rich fraction, followed by the whole extract and finally by the two main alkaloids isolated in the fraction, stylopine and sanguinarine. Monitoring of the serum insulin levels suggest that the substances increase insulin secretion. The extracts showed dose-dependant alpha-amylase and alpha-glucosidase inhibitory effects. The pharmacokinetics of the tested substances was improved by its embedding in niosomes [19]. The effect on neuropathic complications of diabetes were also studied in this investigation. Improvements in tail-flick latency and hot-plate latency suggest that the extracts and alkaloids have anti-hyperalgesic potentials. The extracts also alleviated the mechanical allodynia provoked by hyperglycemia, observed as improvement in paw withdrawal thresholds. The observation of increased levels of serum CAT and GSH and reduction in TBARS levels suggest that antioxidant activity may be involved in the improvements observed [19]. In the same research, the extracts also showed anti-inflammatory potential, reducing the carrageenan-induced acute inflammatory-pain, and chronic hind-paw edema. Analysis of the serum identified reduced levels of cytokines, diminished levels of the pro-inflammatory cytokines TNF-alpha and IL-6, and elevated levels of anti-inflammatory factor IL-10 [19]. Another study performed in vitro and in vivo experiments to assess the antidiabetic potential of different extracts of the plant. The highest alpha-amylase inhibition activity was recorded with the methanolic extract at 16 mg/mL, and both the methanolic and aqueous extracts exhibited a significant hypoglycemic effect at all doses. At doses up to 750 mg/kg, administration in normo-glycaemic rats did not show any significant decrease in blood glucose level [24]. Hyperglycemia can induce non-enzymatic glycation of proteins. In a study involving several plants form Iran, the antiglycation activity of the methanolic extract of *F. officinalis* was measured using the TBA, DNPH and Congo assays. Inhibition of 94%, 60% and 93%, respectively, and $IC_{50}$ values of 1.04, 1.44 and 0.002 were found. This effect, along with the antioxidant properties demonstrate that the plant can be useful in diabetes [16].

### 3.3.5 Nervous system

*F. officinalis* effect on the nervous system is complex due to the different actions of its components. CNS depressing

effects have been reported for fumariline, anti-serotonine effects for fumarine, antispasmodic for protopine and coptisine. Protopine alone has a sedative effect at low doses and stimulating or even convulsant at high doses [6].

### 3.3.6 Nervous system: Anxiolytic

(+)-fumariline, the major alkaloid in *F. indica*, also found in *F. officinalis*, produced a moderate sedation effect in rats, although the effect of the whole extract of the plant (*F. indica*) was much higher. Further studies should be made to identify the compounds responsible for this effect [3].

### 3.3.7 Nervous system: CNS depressant and analgesic

In experiments with rats, the methanolic extract of *F. officinalis* showed significant skeletal muscle relaxant activity in the rotaroda and traction tests. At doses of 200 and 500 mg/Kg body weight it showed highly significant skeletal muscle relaxant activity at 30min of duration. Some flavonoids interact with the GABA/benzodiazepine receptor complex, suggesting a possible mechanism, however further elucidations are required. In the toxicity assessment, the LD50 was established as 2000 mg/Kg [31]. In another study, the ethanol extract was tested for analgesic activity. In the hot plate test, the pain reaction time significantly increased after the ingestion of the extract at 200 mg/Kg, and at 500 mg/Kg the analgesic effect was similar to that of diclofenac sodium. In the tail withdrawal response, the reaction time increased significantly at 100 mg/Kg, and at 500 mg/Kg, was similar to that of diclofenac sodium. Similar results were obtained in the acetic acid induced writhing test. In the toxicity assessment, the LD50 was also established as 2000 mg/Kg [32]. These results are in line with other studies where some *Fumaria* species, or some of their constituents, namely fumaric acid, had shown analgesic properties [3].

### 3.3.8 Nervous system: Alzheimer's disease

A study from 2016 evaluated the inhibitory activity of isolated alkaloids from the plant on four enzymes implicated in Alzheimer's disease, namely acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), prolyl oligopeptidase (POP), and glycogen synthasekinase-3β (GSK-3β). Four of the isolated compounds demonstrated better POP inhibitory activities than the standard baicalin, although inferior to the one of (Z)-pro-prolinal, the best being parfumidine and sinactine with $IC_{50}$ values of 99 µM and 53 µM, respectively. The ethyl-acetate alkaloid extract exhibited promising activity against AChE ($IC_{50}$ = 39.23 µg/ml) and BuChE ($IC_{50}$ = 40 µg/ml), but the effect obtained with any of the isolated alkaloids was much weaker ($IC_{50}$ > 200 µg/ml), suggesting that synergies among the compounds may happen in this effect. The activity on GSK-3β was practically null [20]. In a study with other *Fumaria* species form Bulgaria, the AChE inhibitory activity of the extract of *F. officinalis* had $IC_{50}$ = 0.26 mg/mL [33]. In an investigation on the AChE inhibitory activities of ten *Fumaria* species (not including *F. officinalis*) and their isolated alkaloids, protopine showed the highest activity (80.5%), as well as fumarophycine (37.9%) [3]. Further studies are required to identify the major compounds involved in this effect and their possibly synergistic mechanisms.

### 3.3.9 Diuretic

The diuretic effect has been studied with in vivo experiments. The extracts of *F. officinalis* produced a strong increase in urinary volumetric excretion of saline-loaded rats, 24 h after the oral administration of a single dose of 250 mg/Kg BW. It also significantly increased the urinary excretion of Na+ and K+ [4].

### 3.3.10 Cytotoxic and pro-apoptotic activity

The US National Cancer Institute (NCI) has classified the cytotoxicity of a compound as high cytotoxic activity if $IC_{50}$<20 µg/mL, moderate cytotoxic activity if $IC_{50}$ between 21-200 µg/mL, weak cytotoxic activity if $IC_{50}$ between 201-500 µg/mL, and no cytotoxic activity if $IC_{50}$ > 500 µg/mL [34]. In a study with fractional extracts of a different *Fumaria* species (*F. agraria*), a high antiproliferative effect against breast cancer cells ($IC_{50}$ of 17.6 µg/mL) was found in a fraction containing 60% protopine [3]. QBAs in cancer cells interact with DNA, induce apoptosis and cell cycle arrest, affect the cytoskeleton, and target important members of the signaling pathway. Sanguinarine and chelerythrine are the most well-known members of this group in the genus *Fumaria* and they have shown strong pro-apoptotic action against several cancer cell lines [3]. The extracts of *F. officinalis* slightly inhibited the viability of FaDu (human pharyngeal squamous carcinoma), SCC-25 (human tongue squamous carcinoma), and MDA-MB-231 (human triple-negative breast adenocarcinoma), with $IC_{50}$ = 86 µg/mL in the last one. The effect on MCF-7 (human breast adenocarcinoma) was weak ($IC_{50}$ > 200 µg/mL) [23]. Synthetized nanoparticles containing *F. officinalis* showed a weak inhibitory action on the viability of several human ovarian cancer cell lines: PA-1, Caov-3, SW-626, and SK-OV-3, with all the $IC_{50}$ above 200 µg/mL [25].

In a study with several leukemia and multiple myeloma cell lines and several extract fractions of the plant, a high cytotoxic effect was found in the chloroform fraction. It had an $IC_{50}$ of 12.52 and 14.48 µg/mL against CCRF-CEM (sensitive) and CEM/ADR5000 (multidrug resistant) leukemia cells and of 14.80 against NCI-H929 (multiple myeloma cells). Flow cytometric and morphological studies confirmed that the extracts induced apoptosis in NCI–H929 cells by loss of MMP, generation of ROS and morphological variations. The chloroform fraction stimulated iron-dependent cell death, while the ethyl acetate fraction, which also had remarkable cytotoxic effect, induced autophagic cell death [35]. In a recent study with several melanoma cell lines, the *F. officinalis* extract showed high cytotoxicity against G-361 cells, with an $IC_{50}$ value of 11.79 µg/mL and moderate activity against the other two tested cell lines, A375 and SK-MEL-3 [22]. Fumaria species are not used traditionally to treat cancers but the valuable alkaloids they contain may be a botanical source of chemotherapy agents in cancer. The mechanisms and compounds involved require further elucidation.

### 3.3.11 Antimicrobial

Both the methanol and aqueous extracts were tested for antimicrobial activity against 32 species. The aqueous extract had very limited activity, but the methanolic extract showed activity against 13 out of the 32 species. The highest inhibition zone (15 mm) was observed against *Staphylococcus aureus*. A good result was also obtained against *Cladosporium herbarum*, with an inhibition zone (14 mm) higher than that of amphotericin (10 mm) [15]. More than 20 isoquinoline alkaloids from *Fumaria* species in Turkey showed antibacterial activities against Gram-positive and Gram-negative bacteria at the concentration of 1 mg/mL. Among them, isoquinoline derivatives, PTIs and

tetrahydroprotoberberines were the most effective. The activity of protopines was similar to that of benzophenanthridines [3]. Several isoquinoline alkaloids from *Fumaria* species showed potent antiviral activities. (–)-fumarophycine and (−)-bicuculline, which can be found in *F. officinalis*, were 250- and 150-fold more potent, respectively, than tenofovir in reducing the level of Hepatitis B virus, and more than 10-fold less toxic than tenofovir, with (–)-fumarophycine showing a ten-fold increase in some inflammatory mediators. (–)-fumarophycine also showed antiviral activity against *Herpes simplex* and Parainfluenza [3].

### 3.3.12 Gastro-intestinal
The positive results, in studies dating to before 1979, with *F. officinalis* on abdominal pain, distension and stool irregularity, along with the fact that the irritable bowel syndrome (IBS) is frequently associated with low biliary secretion, led to the hypothesis of the plant's usefulness in IBS. However, in a randomized, double-blind, placebo-controlled trial with *F. officinalis* (standardized for protopine content) and *Curcuma xanthorriza* on 106 patients suffering from IBS, neither of the substances showed any therapeutic benefit over placebo [36]. A different species, *F. indica*, has shown antisecretory, gastroprotective, and in vitro antacid properties in rats. Further studies are required to identify the relevant compounds, some of which may also be present in *F. officinalis*. [3] *F. indica* and *F. parviflora* have shown antidiarrheal and antispasmodic effects, but the active substances involved are not yet well understood [3].

### 3.3.13 Cardiovascular
Fumarine has an anti-arryhthmic effect. Protopine has negative cardiac inotropic and chronotropic effects in rats. The plant is considered cardiac depressant [6].

### 3.3.14 Treatment of skin problems
Some plants of the genus have been studied for their usefulness in skin affections. The topical administration of a *F. vaillantii* gel formulation significantly accelerated wound healing process in rats [37]. In a randomized double-blind, placebo-controlled study with 44 patients with eczema, the application of a 4% cream of *F. parviflora* significantly reduced the eczema area and the severity index score [38]. In a randomized study with 40 patients with moderate eczema, the group treated with a herbal cream containing *F. officinalis* and *Silybum marianum* L. had improvements comparable to those in the group treated with mometasone [39].

## 3.4 Toxicological data
Most of the toxicological studies of Fumaria species, namely with *F. indica* [40], *F. capreolata*, *F. parviflora* and *F. officinalis* have shown that these herbal medicines exhibited few adverse effects. However, a significantly higher cytotoxic activity was observed in the n-hexane fraction, which deserves to be studied to assess the presence of specific toxic components [3]. In a study with rabbits, the plant's hydroalcoholic extract at 200 mg/ kg BW affected the kidney glomerular function, and at 400 mg/kg signals of renal and hepatic failure were observed [41]. In another study, also with rabbits, it decreased, in a non-dose-dependent way, the blood hemoglobin concentration, the packed cell volume percentage, the mean corpuscular volume, RBC and total WBC count and neutrophil percentage, as well as the lymphocyte, monocyte, neutrophil and eosinophil absolute counts. This suggests that, in the long run or at a high doses, the plant can be have an anemic effect and act as a suppressor of the body's immune system [42].So, caution should be exercised when using herbal medicines like *F. officinalis*, avoiding high doses or long periods of intake. The potential toxicity of its isolated compounds, especially alkaloids, deserve careful dosage studies. No studies have been found regarding its use in pediatric or gestational contexts, and so restrains in this field are advised.

### 3.4.1 Effects on cytochrome P450
The human cytochrome P450 (CYP enzymes play a major role in the metabolism of endobiotics and xenobiotics, including drugs. The induction of inhibition of these enzymes can alter pharmacokinetic or/and pharmacodynamic properties of administered drugs, which can pose a risk, especially with drugs that have a narrow therapeutic index such as warfarin, cyclosporine A and digoxin. Protopine and allocryptopine have been shown to increase CYP 1A mRNA levels starting from 25 to 10 μM but did not induce significantly CYP 1A activity in HepG2 cells and thus can be considered safe in this aspect [43]. Parfumine showed a moderate suppressing effect on CYP 1A2, 2B6, 2C9, 2C19, 2D6 ($IC_{50}$ between 10 and 100 μM), but it is a potent inhibitor of CYP 3A4 ($IC_{50}$ < 1 μM). Protopine inhibited CYP enzymes 1A1, 1A2, 2C8, 2D6, 3A4, and CYP 2B6 with $IC_{50}$ values ranging from 1 to 100 μM [44].

## 4. Conclusions
*F. officinalis* is rich in isoquinoline alkaloids, like protopine and sanguinarine. It contains organic acids like fumaric acid, and phenolic compounds like caffeic and ferulic acids and derivatives. Flavonoids like naringenin and quercetin are also abundant in this plant. These compounds are known for their important pharmacological effects, and their combination in the plant gives it a special range of activities. The investigations confirm the cytoprotective effect *in vivo* in the liver as well as in and other tissues, like the kidney and testicles. The antioxidant activity of its compounds and modulatory action on mitochondrial processes are involved in this effect. This plant is promising in the treatment of diseases associated with mitochondrial oxidative stress. The plant has also shown anti-inflammatory potential *in vivo*, by reducing levels of some pro-inflammatory cytokines and elevating the levels of anti-inflammatory factors.

Extracts of this plant have shown to be able to reduce blood glucose levels and protein glycation, increase insulin secretion and inhibit digestive enzymes. This, along with the antioxidant and anti-inflammatory effects, suggest it may be useful in diabetes. *F. officinalis* effect on the nervous system is complex due to the different actions of its compounds; however it tends to be CNS depressant with possible uses as anxiolytic. It has also shown analgesic activity *in vivo*, and inhibitory activity on enzymes involved in Alzheimer's disease. The traditional use as diuretic has been validated by *in vivo* experiments. Although not used traditionally in cancer, some extracts of the plant have shown strong activity against leukemia, multiple myeloma and melanoma cell lines. Moderate activity against oral squamous carcinoma was also observed. Some of the isolated alkaloids, like protopine, sanguinarine and chelerythrine have shown strong cytotoxic effects. The methanolic extracts and isolated alkaloids of the plant have exhibited antimicrobial activity against some bacteria, fungi and viruses, especially *Staphylococcus aureus*, *Cladosporium herbarum* and the Hepatitis B, *Herpes simplex* and Parainfluenza viruses. Studies about *F. officinalis* with

other plants, and studies of other *Fumaria* species point to potential in the dermatological area. The comprehension of the mechanisms of action of the plant requires further investigations about the workings of its individual compounds, namely alkaloids and phenolics, and their synergistic actions.

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