2. *Ferula gummosa* Boiss as a rich source of natural antioxidants with numerous therapeutic uses - A short review

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**Abstract.** The genus *Ferula* (Apiaceae family) comprises of more than 133 different species distributed throughout central Asia and the Mediterranean region. Among them, more than 30 species are found in Iranian flora of which 15 genuses, including *Ferula gummosa*, are endemic. In Iranian traditional medicine, an oleo-gum-resin obtain from *F. gummosa* is well known for treating various disorders including stomach ache, cholera and diarrhea, epilepsy, inflammation, and pain. Numerous chemical compound including terpenoids, sugars and aminoacids have also been reported from this species. Pharmacological studies on extracts obtained from the various parts of the plant have documented the
scientific evidence for some of the medicinal uses of the plant. In this systematic review, the antioxidant mechanisms that serve as the link between the reported medicinal uses and pharmacological actions of the plant is presented.

**Introduction**

Free radicals and various forms of reactive oxygen and nitrogen species are known to play crucial role in the pathogenesis of different disease conditions including cardiovascular, inflammation, neurodegenerative, cancer, hypertension and aging [1,2]. Antioxidants are compounds which are capable of reducing the formation of free radicals through direct interaction and inactivation of free radicals or restricting free radical-induced damages [3]. Our body is equipped with a variety of antioxidants ranging from proteins and enzymes to small molecular weight free radical scavengers. Under oxidative stress conditions, however, antioxidant defenses are overwhelmed either due to excessive production of free radicals or disease-induced diminished antioxidant defenses. One way of alleviating oxidative damage is by means of exogenous antioxidants [4,5] of which plants polyphenols are by far the most important [6]. The antioxidant properties and medicinal potential of culinary plants are also described in the various literatures [7].

The Apiaceae is a family of aromatic plant that constitutes about 3,700 species spread across 434 genera. Over 316 species of this family are known to occur in Iran of which around 25% are endemic [8,9]. Some of these species such as plants belonging to the genus *Ferula* are known to be used in Iranian traditional medicine for treating various disease conditions [10]. Among the reported traditional medicinal uses of *Ferula* species in Iran are for treating convulsion, neurological disorders, diabetes, rheumatism, pain and inflammation [13-22]. Numerous studies on chemical analysis of *Ferula* species also showed the rich phytochemical diversity of the genus: coumarin derivatives, sesquiterpenes, sulphur containing compounds and sugars are among the vast arrays of compounds isolated [12].

To date, over 170 species of the genus *Ferula* are known to be distributed in central Asia, Middle East and North Africa. There are over 30 *Ferula* species ("koma" in Persian language) of which about half are native and/or endemic to Iran [11]. The aim of the present review is to critically evaluate the traditional uses and pharmacological studies on one popular *Ferula species, F. gummosa*. The possible link between antioxidant effects of the plant and other reported pharmacological properties are also discussed.
**Botanical and socioeconomic considerations**

*Ferula gummosa* Boiss (Persian name, “baridje”) is one of the most well known species of the genus for its traditional uses [23]. The plant is a monocarpic perennial species with height between 0.8 and 3 m. The plant is known to grow in western and northern mountains of Iran between the altitude of 1800 and 3000 m [8,11]. The plant normally flowers in late stage of the plant development (normally 6-8 years old plants) [8]. *F. gummosa* stems contain elliptical ducts scattered in phloem tissue and its root has glandular tissues rich in oleo-gum-resin (commercially known as Galbanum) [24,25]. Hence, the plant is resinous with a strong odor that is of relevance to the perfumery industry. The viscous resins secreted out from the glandular root naturally or by physical means through vegetative period of this species. The correlation between resin property and ecological conditions has been reported and resin harvest is recommended from the beginning of June to beginning of November [25]. In addition to cosmetic and industrial applications, *F. gummosa* resins display various pharmacological properties such as antiepileptic, anti-catarrh, analgesic, carminative, digestive, expectorant, laxative, aphrodisiac and antimicrobial activities [24]. Traditionally, people collect the oleo-gum-resin through scraping the rhizomes/roots. *F. gummosa* resin and harvested roots are some of the most popular Iranian exports and have important source of income for the local populations [25].

**Chemistry**

To date, over 236 natural products belonging to various structural classes have been isolated and identified from plants belonging to the genus *Ferula* [12]. Numerous studies further reported that essential oil of *Ferula* contain nitrogenated and sulfurred compounds. For example *sec*-butyl-(*Z*)-propenyl disulfide and *sec*-butyl-(*E*)-propenyl disulfide were the most common sulfur-containing compounds in the *Ferula* essences [10]. Terpenoid compounds are by far the most prevalent components of this genus [10]. In this regard, chemical analysis of *F. gummosa* essential oil showed that α-pinene and β-pinene are major constituents [26]. Linalool, α-terpinolene, δ-3-carene, terpinolene, butyl isovalerate, α-campholenic aldehyde, hexyl isovalerate, butanoic acid-3-3-dimethyl, γ-terpinene, phellandral and limonene are also identified from *F. gummosa* oleo-gum-resin essential oil [26]. Other reports indicate that guaiole, pyrimidine and valencene are major constituents of the petroleum ether extract of *F. gummosa* oleo-gum-resin while the methanol extract of *F. gummosa* oleo-gum-resin predominantly contain benzene-1-3-
dimethyl, benzene-1-ethyl-2-methyl, benzene-1-2-dimethyl, diethylene glycol and benzene ethyl [26]. Recently, (+) norinone, (+) eremorphilene and β-amyrin have also been reported from oleo-gum-resin of *F. gummosa* [25]. Analysis of *F. gummosa* oleo-gum-resin’s for its mineral content also revealed significant level of calcium, potassium, magnesium, sodium, iron, manganese, aluminum, copper, zinc, strontium, boron, lithium and barium [25]. Among the polysaccharides, arabinan and galactan are the most abundant constituents *F. gummosa* oleo-gum-resin. The presence of amino acids such as glutamic acid, aspartic acid and leucine are also reported from *F. gummosa* oleo-gum-resin [25].

**Pharmacology**

Eftekhar et al. [27] demonstrated that the essential oil from *F. gummosa* seed displayed potent antibacterial effect against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis*. In comparison, a relatively weaker activity against *Pseudomonas aeruginosa* was recorded [27]. The antibacterial activity of essential oil of *F. gummosa* fruits has also been reported. According to Ghasemi et al. [24], the fruit essential oil was active against gram positive (*Staphylococcus aureus*, *S. epidermis*, and *Bacillus subtilis*) and gram-negative bacteria (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*) as well as fungi (*Candida albicans* and *Candida kefyr*). From these studies, the authors concluded that the essential oil of *F. gummosa* fruits can be used as an aromatic antimicrobial agent. In a further study by Jahansooz et al. [28], the effects of essential oils of *F. gummosa* aerial part on phylopathogenic fungi were investigated. The authors demonstrated that the growth of *Botrytis cinerea* could be inhibited by the essential oil in a dose-dependent manner while the growth of *F. verticillioides* was not affected. Interestingly, this potential therapeutic potential against plant pathogenic fungi was observed for essential oils obtained from plant samples collected from Semnan and Kashan regions of Iran. As antifungal effects against *Colletotrichum gloeosporides* and *Aspergillus niger* growth were also observed, essential oils of *F. gummosa* are likely to be used for management of wide range of fungal diseases.

The anti-proliferative and apoptosis inducing activity of ethanolic extract of leaves of *F. gummosa* was recently studied by Gharaei et al. [29]. They found that ethanolic extract of *F. gummosa* leaves prevented the AGS (gastric cancer) cell proliferation and caused apoptosis when assessed by DNA fragmentation and plasma membrane phosphatidylserine translocation studies.
Sayyah et al. [21,30] examined the anticonvulsant effects of acetone extract of *F. gummosa* root and methanolic, ethyl acetate and acetone extracts of seeds against pentylenetetrazole and electroconvulsive shock-induced seizures in mice. It was found that acetone extracts of roots and seeds possess anticonvulsant activity in dose-dependent manners. The ED$_{50}$ of extracts in these models established a better activity for the extracts when convulsion seizures was induced by pentylenetetrazole (ED$_{50}$= 154.4 mg/kg for root and 55 mg/kg for seed) than electroconvulsion (ED$_{50}$= 750 mg/kg for root and 198.3 mg/kg for seed). The study further established that *F. gummosa* extracts could generate motor deficits and sedation at the studied doses. Toxicity studies by the same authors also revealed that extracts of *F. gummosa* could be safely used for seizure therapies.

The possible alleviation of naloxone induced morphine withdrawal syndrome by *F. gummosa* aerial part was studied by Ramezani et al. [31]. They studied different fractions of methanol-chloroform (1-1) extract and suggested that non-polar fractions may be responsible for the observed activity. Significant suppression of morphine withdrawal syndrome by the extract and non-polar fractions possibly due to presence of lupidin was suggested, which has been reported from *Ferula* genus [31]. Further research is required to ascertain the mechanism of action of the extract but an effect through opioid or adenosine receptors are among the few suggested mechanisms of action.

Spasmodylic effect of hydro-alcoholic, ether, petrol and methanolic extracts and essential oil of *F. gummosa* oleo-gum resin on ileum contractions has been reported by Sadraei et al. [26]. It was found that the essential oil and hydro-alcoholic extract of *F. gummosa* oleo-gum resin have potent inhibitory action against potassium chloride- and acetylcholine-induced rat’s ileum contraction [26]. The observed smooth muscle relaxant effect of *F. gummosa* is in support of its traditional folk medicinal use for diarrhea and gastrointestinal spasm therapy.

*F. gummosa* was one of the Iranian medicinal plants who were recently screened for acetylcholinesterase inhibition by TLC bioautography and microplate colorimetric assay methods [32]. It was reported that the aerial parts of *F. gummosa* inhibits acetylcholinesterase [32] It has been also reported that antioxidants have acetylcholinesterase inhibition activity [61].

The antinociceptive and anti-inflammatory effects of aqueous, methanolic and acetone extracts of *F. gummosa* seeds and roots were studied using *in vivo* models [19]. The study included the tail-flick test in mice and models of formalin and cotton pellet granuloma tests in rats. The authors found that aqueous, methanolic and acetone extracts at non-sedative concentration failed to have any potent activity in pre- and post- latency time
in tail-flick test. The studied nociceptive models however indicated that mu-opioid receptors agonists’ type effect of antinociceptive action in acute pain was implicated. A further study on antinociceptive effect of this plant has been reported by Fazly Bazaz et al. [33] where the chloroform extracts of the aerial parts have an effect in hot plate model through opioid receptors.

Nabavi et al. studied the antioxidant and antihemolytic activity of stem, fruit and leaf of *F. gummosa* by employing different *in vitro* and *ex vivo* models [34]. They found that hydro-alcoholic extract of *F. gummosa* leaf showed better activity than other plant organs in iron chelating, 1,1-Diphenyl-2-picryl hydrazyl and nitric oxide radicals scavenging, reducing power, hydrogen peroxide scavenging and lipid peroxidation models. The study concluded that good antioxidant and antihemolytic activity of *F. gummosa* correlates with its phytochemicals content especially phenols and flavonoids. The antioxidant activity of *F. gummosa* roots was also reported very recently [35].

**Does antioxidant mechanism explain the observed medicinal uses and pharmacological effects?**

From ancient times, preparations of *F. gummosa* have been utilized in Iranian traditional medicine for treating various disease conditions. So far, scientific evidence for morphine withdrawal syndrome, antibacterial, antifungal, anticonvulsant, spasmyloytic, acetylcholinesterase inhibitory, analgesic, anti-inflammatory, antiproliferative, antioxidant and antihemolytic activities were reported on the plant. The reported potent antimicrobial activity could have been attributed to the presence of sesquiterpenoids [36] and sulfur-containing compounds [37] in the plant. It is also known that iron chelating compounds display bacteriostatic effect thereby suppressing the growth of various microorganisms [38]. The potent iron chelating activity reported for this plant [34] may thus in part explains the reported antibacterial effects. Other reports further indicate that antioxidant compounds with nitric oxide scavenging activity have a role in minimizing microbial induced pathogenesis [39]. For example, over production of viral infection is known to produce nitric oxide through expression of an inducible nitric oxide synthase. This further leads to the production of peroxynitrite and other radicals that contribute to microbial pathogenesis. In view of antioxidant activities demonstrated for the plant, its beneficial effect for treating microbial infections through direct and indirect mechanism is anticipated.

A further link between antioxidant activity and antimicrobial effects could be established with respect to the observed antifungal effect of the
Ferula gummosa as an antioxidant agent

plant [28]. Potent fungicidal effect may be explained by the presence of radical scavenging effect and sulfur-containing compounds in the plant [36]. A close correlation between transfusional siderosis and iron overload as an increased risk of mycosis has been reported by various authors [40,41]. Numerous studies further confirmed that iron chelators can prevent Aspergillus fumigates conidia through iron depletion mechanisms [42]. Some iron chelating compounds have also shown synergistic effect in inhibiting the growth of Aspergillus fumigatus with known antifungal drugs such as azole and polyene antibiotics [44]. Hence, iron chelation is now accepted as a therapeutic strategy for fungal infections [43]. The reported antifungal activity of F. gummosa is therefore in part explained by its iron chelating potential and/or antioxidant activity.

Apoptosis induction in cancer cell is another pharmacological activity established for F. gummosa [29]. It has been reported that hydrogen peroxide scavengers can cause the depletion of reduced glutathione level, mitochondrial dysfunction, activation of caspase-3, down-regulation of Bcl-2, and up-regulation of Bax in human leukemic HL-60 cells that eventually leads to tumor cell death [45,46]. Zheng et al. [47] further found that curcumin as nitric oxide scavenger inhibits cell growth at the G2/M phase and cause apoptosis in human melanoma cells through inhibition of NFκB activation and removal of endogenous nitric oxide. On the other hand, iron chelation therapy has been proposed as a novel strategy for inducing cytotoxicity in cancer cells [48]. Hence, hydrogen peroxide scavenging, nitric oxide depletion and iron chelation in cancer cells might account for the cytotoxic mechanism of F. gummosa.

Some reports suggest that seizure actions increase free radicals generation and reduce the antioxidant status in neural tissues [49]. There is also overwhelming evidence to suggest that antioxidant agents improve or mitigate seizure development and cognitive impairment by decreasing the level of brain peroxidation in traumatic damages and neurodegenerative disorders [50-52]. Oxidative stress is also known to play crucial role in the initiation and progression of epilepsy. Membrane polyunsaturated fatty acids peroxidation can generate toxic malone dialdehyde which compromises the dynamics of the membrane lipid matrix and cause seizures development [53]. In the pentylenetetrazole and maximal electroshock models, the level of malone dialdehyde has been shown to increase while reduced glutathione level decreases suggesting the development of an oxidative stress condition [54]. The observed anticonvulsant activity of F. gummosa is therefore in good agreement with its potent antioxidant potential. The presence of monoterpenes (e.g. linalool and pinene) and other constituents of F. gummosa
may also influence its anticonvulsant activity through other mechanisms [54,55].

Previous studies have confirmed that reactive oxygen species play crucial role during morphine withdrawal and treatment [56]. A close correlation between morphine treatment and a reduced level of endogenous antioxidant systems as well as increased lipid peroxidation level has been established [57]. Hence, antioxidants have been demonstrated to mitigate morphine withdrawal syndrome [58]. The reported beneficial effect of *F. gummosa* on morphine withdrawal syndrome is thus in part be accounted by its antioxidant effects.

The intestinal smooth muscle relaxant activity of *F. gummosa* explains the traditional uses of the plant for diarrhea and gastrointestinal spasm therapy [26]. It was further suggested that Ca\(^{2+}\) channels blockade is the possible mechanism of the observed antispasmodic activity [26]. It is well known that many calcium channels blockers have antioxidant and free radical scavenging activity [59]. Therefore antioxidant activity is possible mechanism of *F. gummosa* relaxant activity. Previous reports from our laboratories however suggest that *F. gummosa* extracts display direct metal chelating properties [34]. Such effect, like the known mechanism of EDTA, diminishes the availability of calcium ions for muscle contraction resulting in smooth muscle relaxation. The link between antioxidant activity and metal chelation with antihaemolytic effect has also been established in recent years. Red blood cells are by far the most sensitive cells to oxidative stress [63] as free radical attacks to membrane lipids and hem protein results in haemolysis process [64]. Since numerous literatures report that iron chelators and free radical scavengers [65] can protect erythrocytes from haemolysis, the reported antihaemolytic activity of *F. gummosa* could be attributed to antioxidant and/or iron chelation mechanisms.

Acetylcholinesterase is an enzyme responsible for acetylcholine hydrolysis in neuronal tissues in the brain [60] and its inhibition is one of the most important strategy for improving cholinergic functions in neurodegenerative diseases (e.g. Alzheimer’s disease). This approach would increase the life span of acetylcholine in cholinergic synapses thereby compensating the loss of neurons in disease state. Many known clinically useful enzyme inhibitors such as physostigmine and tacrines have short half life, many side effects and toxicity including hepatotoxicity. As a result, there is currently an increasing level of interest to find novel inhibitors of acetylcholinesterase enzyme. Recently, Adhami et al. [32] reported that *F. gummosa* has acetylcholinesterase inhibition potential. Since nitric oxide has also suggested having direct role in the pathogenesis of Alzheimer disease [62], the reported effect of *F. gummosa* could have potential
therapeutic effect for Alzheimer’s disease through multiple targets. It is worth noting that anticholinesterase mechanisms, inhibition of amyloid toxicity through oxidative stress and neuroprotection are some of the key validated targets for Alzheimer’s therapy that are all likely to be modulated by extracts of \textit{F. gummosa}.

\textbf{Conclusion}

\textit{F. gummosa} is an endemic Iranian plant with interesting arrays of biological activities. The plant is currently exploited for local medicinal uses and commercially for its oleo-gum resin. The antioxidant activity and metal chelation effect of the plant extracts are prevalent. Our critical review has highlighted that such effects are linked with the medicinal uses and pharmacological activities reported on the plant so far. In view of the therapeutic and economic potential of the plant, further studies including \textit{in vivo} models are well merited. The chemistry of the plant is also barely studied and further bioassay-guided isolation of the pharmacologically active principles is required.

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\textbf{References}