

# A Review on Ethnomedicinal, Pharmacological, **Phytochemical and Pharmaceutical Profile of** Lady's Finger (Abelmoschus esculentus L.) Plant

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Abstract

Abelmoschus is a genus of about fifteen species belongs to the family Malvaceae. The herb, popularly known as Lady's Finger or Okra (in English) is a nutritional source used for both medicinal and culinary purposes. The plant is widely distributed from Africa to Asia, Southern Europe, and America. This comprehensive account provides a botanical description of the plant, its phytochemical constituents and pharmacological activities focusing anti-diabetic, anti-oxidant, anti-adhesive, gastro-protective, hepatoprotective, and immunomodulating actions. Most of the pharmacological effects can be explained by the constituents like tannins, terpenoids, flavonoids and glycosides present in all plant parts. However, future efforts should concentrate more on in vitro and in vivo studies and also on clinical trials in order to confirm traditional wisdom in the light of a rational phytotherapy. The present review is an overview of phytochemistry and ethnopharmacological studies that support many of the traditional ethnomedicinal uses of the plant.

# **Keywords**

Lady's Finger, Abelmoschus esculentus, Medicinal Plants, Phytochemicals, Pharmacological Activities

# 1. Introduction

Abelmoschus esculentus L. (Family: Malvaceae), also known as Hibiscus esculentus, is an important vegetable, widely distributed from Africa to Asia, Southern Europe, and America that is more commonly known as Ladies finger, okra, or gumbo [1]. Nutritionally, the richest part of the plant is the dried seeds. The seed oil is edible and the residual meal after oil extraction is rich in protein [2]. Lady's finger ensures recovery from psychological and mental conditions like depression and weakness. It has an effective remedy for ulcer and joint healthiness. It is applied for pulmonary inflammations, bowel irritations and sore throat. The fibers of Ladies finger help to stabilize blood sugar by regulating the rate at which sugar is absorbed from the intestinal tract. Previous studies reported that Ladies finger polysaccharide possesses hepatoprotective activities [3].

## 2. Origin and Geographic Distribution

The lady's finger was previously included in the genus *Hibiscus*. Later, it was designated to *Abelmoschus*, which is distinguished from the genus *Hibiscus* by the characteristics of the calyx, spatulate, with five short teeth, connate to the corolla and caduceus after flowering [4]. Okra is grown in many parts of the world, especially in tropical and sub-tropical countries [5] [6]. The plants are grown commercially in many countries such as India, Japan, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Myanmar, Malaysia, Thailand, India, Brazil, Ethiopia, Cyprus and in the Southern United States [7] [8] [9].

#### **Physical Characteristics**

It is an annual herb and often grows to around 2 meters tall. The leaves are long-petioles, orbicular around 10 - 20 cm long, broad and rough, palmately-lobed with 5 - 7 lobes (**Figure 1**). Flowers of this plant are axillary and solitary, 4 - 8 cm in diameter having five white to yellow petals, often with a red or purple spot at the base of each petal (**Figure 2**). Fruits are elongated to 10 - 25 cm long, 1.5 - 3 cm in diameter and contain numerous seeds (**Figure 3**). Depending on the cultivar, its fruits are matured after 60 - 180 days of sowing. The plant requires moist and well-drained soil. It cannot grow in the shade [3] [10].

#### **3. Chemical Composition**

The estimated average chemical composition of Lady's finger or okra bast fiber



Figure 1. Leaves of Abelmoschus esculentus.



Figure 2. Flowers of Abelmoschus esculentus.



Figure 3. Fruits of Abelmoschus esculentus.

(OBF) are 67.5% a-cellulose, 15.4% hemicelluloses, 7.1% lignin, 3.4% pectic matter, 3.9% fatty and waxy matter and 2.7% aqueous extract and the rest are very minor in proportion [11]. Okra seeds are a significant source of proteins and minerals, such as Ca, K, Fe and Zn. Seeds of all the genotypes contained significant amounts of gamma-tocopherols, liposoluble pigments, and linoleic and palmitic acid [12].

## 4. Nutritional Value

Lady's finger plays an important role in the human diet by supplying carbohydrate, minerals and vitamins. Na, Mg, K and Ca are found to be the principle elements with Fe, Zn, Mn and Ni. Its seeds could serve as alternate rich sources of protein, fat, fiber and sugar [13] [14]. Raw nutritional values of lady's finger are shown in **Table 1**.

# 5. Ethnomedicinal Uses

Lady's finger is widely used in ethnomedicine in diverse cultures (**Table 2**). In Ayruveda, it is used as an edible infusion and in different preparation for diuretic effect. The roots are very rich in mucilage, having a strongly demulcent action.

Compounds	Quantity	Compounds	Quantity
Energy	33 kcal	Niacin (B <sub>3</sub> )	1 mg (7%)
Carbohydrates	7.45 gm (140 kj)	Vitamin C	23 mg (28%)
Sugars	1.48 gm	Vitamin E	0.27 mg (2%)
Dietary Fibers	3.2 gm	Vitamin K	31.3 µg (30%)
Fat	0.19 gm	Calcium	82 mg (8%)
Protein	2 gm	Iron	0.62 mg (5%)
Water	90.19 gm	Magnesium	57 mg (16%)
Vitamin A	36 µg (7%)	Potassium	299 mg (6%)
Thiamine (B <sub>1</sub> )	0.2 mg (17%)	Zinc	0.58 mg (6%)
Riboflavin (B <sub>2</sub> )	0.06 mg (5%)		

Table 1. Raw nutritional value of lady's finger per 100 gm [15].

Percentages are related to US recommendations for adults (Source: USFDA database).

### Table 2. Lady's finger in ethnomedicine.

Parts	Form	Name of the Medicinal system where it is used	Used for	References
	Infusion of the roots	Ethnomedicine of Nicaragua's Atlantic Coast and Turkey	Used as stomachic to treat diabetics, ulcer; used as laxative to treat jaundice.	[16] [21] [22]
Root	The juice of the roots	Ethnomedicine of Nepal	To treat cuts, wounds and boils.	
	Infusion of the roots	Malaya's Indian ethnomedicine	Treatment of syphilis.	
	Extract of roots	Indian ethnomedicine	Used for demulcent and emollient action.	
	Leaves	Latin America	Remedies for tumour.	
Leave	Extract of leaves	Indian ethnomedicine Extract of leaves mixed with egg albumin and app on hair, which makes black and silky hair.		d [16] [21]
Flower	The decoction of the flowers	Indian ethnomedicine	Used for the treatment of bronchitis and pneumonia.	[17] [28]
Fruit	For treating dys inflammation a Infusion of the fruit mucilage Indian ethnomedicine bowelsand kidne urinae, dysuria replacer		For treating dysentery and diarrhoea in acute inflammation and irritation of the stomach, bowelsand kidneys catarrhal infections, ardour urinae, dysuria, diuretic, antipyretic, plasma replacement and gonorrhoea.	[16] [18] [19] [20]
	A decoction of immature fruit	Indian ethnomedicine	Used for demulcent and emollient poultice.	
	Seeds	Indian ethnomedicine	Use as antispasmodic, cordial and stimulant; To treat spermatorrhoea.	
Seed	Infusion of the roasted seeds	Indian ethnomedicine	an ethnomedicine Has a sudorific property.	
	Infusion of the roasted seeds	Turkish folk medicine	To treat diabetes mellitus by increased blood glucose level.	[26] [27]
	Seeds	Latin America	Remedies for tumor.	

The leaves are used as an emollient poultice. A decoction of the immature capsules has demulcent, diuretic and emollient action. The seeds are used as antispasmodic, cordial and stimulant [10].

## **6. Phytochemical Properties**

The word phytochemical came from Greek word Phyto-plant and chemicals. The term phytochemical is usually used to those chemicals that may have biological significance but are not established as important nutrients. But in narrower sense the term phytochemical describe the number of secondary metabolic compounds found in plants. The scientists estimate that metabolic compounds like tannins, terpenoids and glycosides are found from seeds of lady's finger [29] [30]. The chemical constituents are shown in **Table 3**.

# 7. Various Pharmacological Properties

#### 7.1. Hepatoprotective Activity

Plant extract having antioxidant activities also leads to the inhibition of oxidative

Table 3. Isolated chemica	l constituents from	Abelmoschus a	esculentus [31].
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Serial No.	Compound	Name	Plant sources	Structure
1	Hyperoside/Hyperin	dihydroxyphenyl-3-[(3R,4S,5R,6R) -3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy-4H-chromene-4,5,7-triol	Abelmoschus esculentus	
2	Flavonoid glycoside	5,7,3',4'-tetrahydroxy-4''-O-methyl flavonol-3-O- <i>β</i> -D-glucopyranoside	Abelmoschus esculentus	
3	Flavonoid glycoside	5,7,3',4'-tetrahydroxy flavonol-3-O- [β-D-glucopyranosyl-(1→6)] -β-D-glucopyranoside	Abelmoschus esculentus	
4	Coumarin scopoletin	7-hydroxy-6-methoxychromen-2-one	Abelmoschus esculentus	OH OCH3
5	Uridine	1-[(3R,4S,5R)-3,4-dihydroxy-5- (hydroxymethyl)oxolan-2-yl] pyrimidine-2,4-dione	Abelmoschus esculentus	

damage to a targeted tissue. Considering such antioxidant potential, the *in vivo* hepatoprotective effect of *A. esculentus* was evaluated using CCl<sub>4</sub> intoxicated HepG<sub>2</sub> cell line and Wistar rats by estimating the levels of hepatic and antioxidant markers. The root extract of *A. esculentus* showed IC<sub>50</sub> values of 270.99 and 532.86  $\mu$ g/mL for DPPH and hydroxy radical scavenging assays, respectively. The incubation of HepG<sub>2</sub> cells with CCl<sub>4</sub> drastically decreased the cell viability and increased the leakage of transaminases. Pre-treatment with the extract significantly restored the cell death by 31.25% and 39.04% at 200 and 400  $\mu$ g/mL concentrations, respectively. The reduction of ALT leakage by the treatment was 18.62%, 38.59% and 52.15% compared to the CCl<sub>4</sub> treated cells at 100, 200 and 400  $\mu$ g/mL, respectively. In *in-vivo* experiments, the treatment reduced the levels of transaminases, ALP, MDA, total bilirubin and hepatic TNF $\alpha$  levels as well as increased the anti-oxidant levels in a dose dependent manner. Histological observations of liver sections showed reduction in steatosis, necrosis and inflammation [32].

## 7.2. Hypolipidemic and Anti-Diabetic Effects

Diabetes is a serious complication attributed to several metabolic disorders. A literature review revealed that *A. esculentus* fruit extract and its isolated constituents possessed mild to moderate antidiabetic property, which was experimented through *in vitro* antidiabetic assays. The ethanolic extract of Lady's finger reduced blood glucose and serum insulin levels and improved glucose tolerance in obese mice. The serum triglyceride levels and liver morphology in the mice was significantly ameliorated by the treatment of isoquercitrin. Total cholesterol levels in isoquercitrin and quercetin 3-O-gentiobioside treated mice were also reduced. So Lady's finger may serve as a dietary therapy for hyperglycemia and hypertriglyceridemia [33].

### 7.3. Anti-Fatigue and Anti-Oxidant Effects

Literature review revealed that okra pods contain high contents of polysaccharides, polyphenols and flavonoids [34]. It has also been demonstrated that polyphenols and flavonoids possess strong antioxidant and anti-fatigue effects [35] [36]. Antioxidant assays, including 1-diphenyl-2-picrylhydrazyl scavenging, ferric reducing antioxidant power, reducing power test and weight-loaded swimming test showed its seeds possessed significant antioxidant and antifatigue effects. Moreover, biochemical determination revealed that anti-fatigue activity of okra seeds is caused by reducing the levels of blood lactic acid and urea nitrogen, enhancing hepatic glycogen storage, promoting anti-oxidant ability by lowering malondialdehyde level and increasing superoxide dismutase and glutathione peroxides levels [37].

#### 7.4. Gastro Protective Effect

*Abelmoschus esculentus* is a medicinal plant widely used in traditional medicine for the treatment of various diseases such as ulcers and gastritis. The gastropro-

tective effect of okra against ethanol-induced acute gastric mucosal injury in animal models was evaluated. The *in vivo* data indicated that okra has a gastroprotective effect against ethanol and could reduce the gastric ulcer as seen from biochemical and histopathological results [38]. Another study of *Abelmoschus esculentus* lectin (AEL) gives gastro protective effect on gastropathy induced by ethanol. 0.2 ml/animal; p.o. given to fasted mice of ethanol 99.9% received previously AEL (0.01, 0.1, 1.0, 10 or 50 mg/kg; i.v.), saline (5 ml/kg; i.v.) or ranitidine (80 mg/kg; p.o.). The mice were euthanized 30 min after ethanol challenge to verify the stomach damages. Gastric oxidative stress, tissue hemoglobin content and microscopic features were taken in order to characterize the AEL gastro protective effect. AEL (1 mg/kg) was capable of protect mucosa against ethanol damages in presence of two evaluation of microscopic features, oxidative stress, and Hb levels pointed the protective effects of AEL. AEL simultaneously showed antioxidant effect that is probably implicated in its intricate defensive mechanism of action [39].

## 7.5. Neurological Disorders

It has been reported that okra treatment reverses cognitive deficits and protect against morphological changes in the CA<sub>3</sub> region of dexamethasone-treated mice [40]. Okra extract and its bioactive constituents, quercetin and rutin, also protect neuronal function and improve learning and memory deficits in dexamethasone-treated mice [41]. Recently, a study demonstrated that okra treatment decreases ROS and  $H_2O_2$  production, tau phosphorylation, and cellular events associated with AD in a stably expressed HFE neuroblastoma SH-SY5Y cell line [42]. Literature review revealed that okra attenuates the production of the proinflammatory mediators, NO and ROS, as well as production of TNF- $\alpha$  and IL-1 $\beta$ , in LPS-stimulated BV2 microglial cells by suppressing Akt-mediated NFxB pathway. The findings provide evidence that okra possesses potential anti-oxidative and antiinflammatory activities in neuronal disorders induced by activated microglia [43]. The lady's finger provides important in vitro data on the effects of various AlD-associated cellular processes in H63D variant HFE cells. These results suggest it may be beneficial in people expressing the H63D variant to reduce the risk of Alzheimer's disease and other neurodegenerative diseases related to oxidative stress [44] [45].

#### 7.6. Cardiovascular Disease

Lady's finger is also used to treatment of cardiovascular disease. This reduces serum cholesterol and therefore decreases the chance of heart disease. It is additionally loaded with pectin that can help in reducing high blood cholesterol simply by modifying the creation of bile within the intestines [46]. This study investigated the effect of mucilage of okra (crude water extract and water fraction) fruit on lipid parameters in a high-fat diet fed rats. Okra crude water extract (500 and 1000 mg/kg body weight) or water fraction (50 and 100 mg/kg body weight) was provided with a high fat diet to hypercholesterolemic rats for one week. The effect of treatment of okra fruit on lipid parameters of hypercholesterolemic rats were evaluated and compared with the negative control and positive control rats. Crude water extract (1000 mg) and the water fraction of okra (50 mg and 100 mg) had the potential to reduce (p < 0.01) different lipid fractions (total cholesterol, triglycerides, LDL and VLDL) and atherogenic index in the test group. The mucilage had the potential to increase the HDL fraction (p < 0.01) of the test group. These results suggest that crude water extract and the water fraction of the okra fruit modulate the blood lipid levels favorably and have the potential to be used as a "heart friendly" vegetable [47].

### 7.7. Immunomodulating Activity

A water soluble polysaccharide (OFPS11) was obtained from lady's finger (A. esculentus) flowers using aqueous extraction and purification with DEAE-52 cellulose and Sephacryl<sup>TM</sup> S-500 column. Its preliminary characterization and immunomodulating activity were investigated. Results showed that OFPS11 is mainly composed of galactose and rhamnose in a molar ratio of 2.23:1 with molecular mass of 1700 kDa. RAW264.7 cells pretreated with OFPS11 significantly inhibited the proliferation of HepG-2 cells. Additionally, OFPS11 enhanced the phagocytic ability and induced the elevation of NO production, TNF-a and IL-1 $\beta$  secretion of RAW264.7 cells. OFPS11 can strongly increase NF- $\kappa$ B levels in nucleuses, which is an important transcription factor that can modulate expressions of iNOS, NO and TNF-a. These outcomes support that OFPS11 exerts its antitumor activity by probably stimulating macrophage activities through nuclear NF- $\kappa$ B pathway [48]. Their immunomodulatory activity was evaluated with an in vitro cell model (RAW264.7 cells). In vivo immunomodulatory activity of RPS-2 was evaluated in normal and cyclophosphamide-induced immunosuppressed mice. The results showed that the molecular weights of RPS-1, RPS-2, and RPS-3 were 600, 990, and 1300 kDa, respectively. RPS-1 and RPS-2 were mainly composed of galactose, rhamnose, galacturonic acid, and glucuronic acid, while RPS-3 was mainly composed of galactose, rhamnose, galacturonic acid, glucuronic acid, and glucose. FT-IR and NMR spectrum data indicated a rhamnogalacturonan I characteristic of polysaccharide. Both RPS and its purified fractions RPS-1, RPS-2, and RPS-3 significantly increased RAW264.7 cell proliferation, nitric oxide (NO) production, inducible nitric oxide synthase (iNOS) expression, and tumor necrosis factor (TNF)-a, interferon (IFN)-y, and interleukin (IL)-10 secretion (p < 0.05). The purified fraction RPS-2 also increased the spleen index, splenocyte proliferation, and cytokine secretion in vivo. These results indicate that okra polysaccharides may potentially serve as novel immunomodulators [49].

### 7.8. Anticancer Activity

Cancer is an abnormal type of tissue growth in which the cells exhibit an uncon-

trolled division, relatively in an autonomous fashion, leading to a progressive increase in the number of dividing cell. Cancer is one of the ailments, which cannot be completely subdued by chemotherapy. Literature review revealed that okra is used as a chemo-preventive agent by inhibiting the growth of cancer cells by proper signaling mechanisms. Metabolic product such as indole-3 carbinols from the seeds and flowers of *A. esculentus* are known to be examined in the treatment of several cancer. The flowers of *A. esculentus* were tested for its anti-cancer activity against human liver cancer HePG<sub>2</sub> cell line by MTT assay. The MTT assay of the compound isolated from the ethyl acetate fraction of flowers of *A. esculentus*. The CTC<sub>50</sub> value of the sample was 444.22 µg/ml against liver cancer HePG<sub>2</sub> cell lines for MTT assay. Results showed that phytochemical compounds of this plant from flowers have great potential to act as a source of useful anticancer drug [50].

#### 7.9. Anti-Adhesive Effects against H. pylori

Polysaccharide containing extracts from immature fruits of okra (*A. esculentus*) are known to exhibit anti-adhesive effects against bacterial adhesion of *Helicobacter pylori* to stomach tissue. The study reported that ammonium sulfate precipitation of an aqueous extract yielded two fractions at 60% and 90% saturation with anti-adhesive effects against *H. pylori* after pre-incubation of the bacteria at 1 mg/mL [51]. A standardized aqueous fresh extract (Okra FE) from immature okra fruits was used for a quantitative *in vitro* adhesion assay with FITC-labled *H. pylori*. Okra FE dose-dependently (0.2 to 2 mg/mL) inhibited *H. pylori* binding to AGS cells. FE inhibited the adhesive binding of membrane proteins BabA, SabA, and HpA to its specific ligands. Non-specific interactions between high molecular compounds from okra fruits and the *H. pylori* surface lead to strong antiadhesive effects [52].

#### 7.10. Nootropic Potential

The seed extracts of *Abelmoschus esculentus* L. possess anti-oxidant, anti-stress and nootropic activities, which promisingly support the medicinal values of this plant. So the pretreatment of mice with aqueous and methanolic seed extracts of *A. esculentus* (200 mg/kg; p.o.) for seven days significantly (p < 0.01) attenuated scopolamine-induced cognitive impairment in the passive avoidance test. These extracts reduced the blood glucose, corticosterone, cholesterol and triglyceride levels elevated by acute restraint stress [2].

#### 7.11. Vital Substance for Optimum Pregnancy

Lady's finger is full of both foliate and vitamin C that are responsible for maintaining and creating fresh cells. Foliate is a vital substance for optimum pregnancy that increases the growth and development of the fetus brain. It is preventing birth defects. Vitamin C also as well required for baby development. The high quantity of folic acid within okra performs a huge role within the neural tube formation of the fetus through the fourth to the  $12^{th}$  week of pregnancy [53].

#### 7.12. Eye-Sight Improvement and Skin Nourishment

For eyesight improvement lady's finger pods are used. These pods are implausible options for vitamin A and  $\beta$  carotene that are both important nourishment for sustaining an excellent eye-sight along with healthy skin [52].

#### 8. Pharmaceutical Applications

Okra mucilage or okra gum can be used as pharmaceutical excipients includegelling, thickening, suspending and emulsifying agents to formulate solid oral dosage form. Literature review revealed that okra gum has acceptable  $p^{H}$  and organoleptic properties. It is extracted from the fruits of *Abelmoschus esculentus* using organic solvent such as acetone. It has diverse pharmaceutical applications [54].

#### 8.1. Emulsifier in Emulsion

Extracts of lady's finger showed an emulsifying activity in acidic environments, producing fine emulsions with good stability against coarsening in case of oil in water emulsions with n-hexadecane as dispersed phase [55]. The emulsifying properties of okra mucilage were determined by evaluating its emulsifying capacity (EC), emulsifying stability (ES), water holding capacity (WHC), oil holding capacity (OHC) at different temperature ( $10^{\circ}$ C,  $30^{\circ}$ C,  $50^{\circ}$ C and  $70^{\circ}$ C). The study showed for EC, an oil-in-water emulsion system was prepared at room temperature ( $\sim 26^{\circ}$ C) by adding 6 mL of corn oil into 60 mL of 1% (w/w) of mucilage solution. Next, the emulsion was mixed at 2000 rpm for 10 minutes. After that, the emulsion was homogenized at 9600 rpm for 1 minute. Finally, the suspension was centrifuged at 4000 rpm for 10 minutes. However, for ES, the emulsion was heated first in water before the mixture was centrifuged at 4000 rpm for 10 minutes. The EC and ES of okra mucilage were calculated using following formula:

EC or ES (%) = 
$$\frac{ev}{tv} \times 100$$

where, ev is the volume of emulsion after being centrifuged and tv is total volume of mixture.

Results revealed that okra mucilage at 1.0% percentage in coconut milk obtained the highest value in emulsion capacity (EC) and emulsion stability (ES). It is found that okra plant has potential to be used as emulsifying agent and stabilizer in food emulsion system [56].

#### 8.2. Retarding Agent

Gum of lady's finger is an effective retarding polymer to develop sustained release tablets. It is able to formulate propranolol hydrochloride tablets, showing a release up to 24 hours as compared to HPMC and sodium alginate as retarding agents [53].

### 8.3. Sustained Release Candidate

Ibuprofen and calcium acetate tablets contain lady's finger gum and it showed sufficient hardness, desirable disintegration time and low friability. The percent of drug released after 45 minutes was 15%, 44% and 96% for Acetaminophen, Ibuprofen and Calcium acetate tablets, respectively. The gum produces some tablet formulations with good hardness and friability. However, this binder prolongs the dissolution rate of some slightly soluble drugs and hence may be a good candidate for sustained release formulations [57].

#### 8.4. Controlled Drug Releaser

Polymer swelling studies were carried out with both poorly soluble drug (Flurbiprofen) and soluble drug (Theophylline). The study revealed that the release rate of flurbiprofen was slower than theophylline [55].

#### 8.5. Release Modifier

Lady's finger gum was successfully employed for formulating the sustained release matrix tablets of diclofenac sodium and 10% - 15% concentration of gum was capable of prolonging the release of drug for 10 hours. Drug release was found to follow near zero order kinetics and mechanism of drug release was observed to be following the korsmeyer-peppas model [58].

#### 9. Conclusion

Medicinal plants are the nature's gift to human being to have disease free healthy life. *Abelmoschus esculentus* is very popular phytomedicine for local healers. This review demonstrated the diverse ethnomedicinal practices, phytochemical properties, pharmacological activities, nutritional importance and pharmaceutical applications of *A. esculentus* species. It is a rich source of vitamins, minerals and phytoconstituents which are used in the treatment of various types of ailments. The scientific community continues to unravel the mechanisms involved in disease prevention and determine how food bio-actives from such foods as lady's finger can influence human health. Due to the importance of the plant as a source of new medicinal agents, a review is worthwhile. The data may support future multidisciplinary studies and promote rational use of the plant as a therapeutic resource.

## **Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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