

# *Solanum nigrum*: Current Perspectives on Therapeutic Properties

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## Introduction

*Solanum nigrum* (black nightshade) is a medicinal plant member of the Solanaceae family of plants. This family comprises many genera, well known for their therapeutic properties. In addition to *S. nigrum*, this family includes fruits and vegetables such as potato (*Solanum tuberosum*), tomato, and peppers, ornamental plants such as petunia, and other medicinal plants such as *Atropa belladonna* L. (deadly nightshade), *Datura stramonium* L. (Jimson weed), and *Hyoscyamus niger* L. (black henbane).

*S. nigrum* commonly known as Makoi or black nightshade, usually grows as a weed in moist habitats in different kinds of soils, including dry, stony, shallow, or deep soils, and can be cultivated in tropical and subtropical agro climatic regions by sowing the seeds during April-May in well-fertilized nursery beds; it can be used for reclaiming the degraded land as well.<sup>1</sup>

*S. nigrum* has been extensively used traditionally to treat various ailments such as pain, inflammation and fever.<sup>2,3</sup> The plant is also used in the Oriental systems of medicine for various purposes – as an antitumorigenic, antioxidant,<sup>4</sup> anti-inflammatory,<sup>3</sup> hepatoprotective,<sup>5</sup> diuretic,<sup>3</sup> and antipyretic agent.<sup>3</sup> Various compounds have been identified which are responsible for diverse activities.

*S. nigrum* is widely used in many traditional systems of medicine worldwide for disparate ailments (Table 1), but has not garnered attention for modern therapeutic use.

## Active Constituents

*S. nigrum* possesses various compounds that are responsible for diverse activities. The major active components are glycoalkaloids, glycoproteins, and polysaccharides. It also contains polyphenolic compounds such as gallic acid, catechin, protocatechuic acid (PCA), caffeic acid, epicatechin, rutin, and naringenin.<sup>17</sup>



The glycoalkaloids include solamargine, solasoline, and solanine that belong to the tropane group of compounds. Solanine's function and activity has been extensively studied. It comprises 95 percent of the total alkaloid concentration present in the plant and is found naturally in any part. It is one of the plant's major natural defenses as it is toxic even in small quantities. With a molecular weight of 868.04 and formula  $C_{45}H_{73}NO_{15}$  it consists of an aglycone, solanidine (alkaloidal portion), and three sugar moieties

Table 1. Traditional Uses of *S. nigrum*<sup>6-16</sup>

State, Country	Part Used	Preparation	Conditions	References
Tanzania, Africa	Leaf	Leaves are pounded and applied topically	Treatment of ringworm	Moshi et al (2009)
	Leaf	Leaves are pounded and baked	Used for dressing of warts	
	Fruit	Ripe fruits in edible form	Given to kids to stop bed-wetting	
Tunisia, Africa	Sap		Erysipelas (acute Streptococcus bacterial infection)	Leporatti and Ghedira (2009)
United Republic of Congo, Africa	Whole plant	Maceration	Snake bite/sting by a venomous animal	Chifundera (1998)
Algeria, Africa	Fruit	Diluted infusion of berries	Blindness; conjunctivitis; glaucoma; trachoma; cataract	Boulos (1983)
	Whole plant	Decoction	Burns and dermal affections	
Tamil Nadu, India	Leaf	Fresh leaves cooked with onion bulbs and cumin seeds or leaf juice can also be taken orally	Stomachache; stomach ulcer	Sivaperumal et al (2010); Ramya and Jayakumarara (2009); Ignacimuthu et al (2006); Muthu et al (2006)
	Leaf paste	Applied directly	Rabies; wound healing	
	Whole plant	Taken as food	Cough	
Himalayan region, India	Leaf		Liver tonic; indigestion	Kala (2005)
Thar Desert, India	Roots	Roots are boiled with a little sugar	Increase fertility in women	Parveen et al (2007)
Assam, India	Roots	Juice of roots is extracted	Asthma and whooping cough	Sikdar and Dutta (2008)

**Key words:** solanum, *s. nigrum*, hepatic, liver, hepatoprotectant, nightshade, night shade, solanaceae, cancer, seizure, epilepsy, antiproliferative, antiseizure, anti-inflammatory

(glucose, galactose, and rhamnose, collectively known as solatriose), which are attached to the third position of the aglycone.<sup>18,19</sup> It is generally present in the form of  $\alpha$ -solanine, but can be hydrolyzed to  $\beta$ - and  $\gamma$ -solanine with one or two carbohydrate molecules each.<sup>20</sup> These glycoalkaloids demonstrate marked antitumor effects on various tumor cell lines.<sup>21</sup>

The glycoproteins are obtained from *S. nigrum* by precipitation with 80-percent ammonium sulphate. SNL glycoproteins I of 150 kDa and 100 kDa and SNL glycoprotein II of 210 kDa are obtained from fruits, stems, and leaves, respectively.<sup>4</sup> The

glycoproteins consist of carbohydrate (69.74%) and protein (30.26%, mainly hydrophobic amino acids containing glycine and proline).<sup>22</sup> A 150 kDa phytyglycoprotein isolated from seeds by affinity chromatography and ammonium sulphate precipitation<sup>23</sup> has been shown to exhibit antitumor effects on HCT-116 cells, as well as diuretic and antipyretic effects.<sup>22</sup> Polysaccharides isolated from aqueous extracts of *S. nigrum* have been shown to possess antiproliferative activity that can be attributed to their immunomodulatory ability.<sup>24,25</sup>

## Mechanisms of Action/Clinical Indications

*Solanum nigrum* demonstrates a diversity of therapeutic properties (Figure 1).

### Antiproliferative Activity/Cancer Preventive

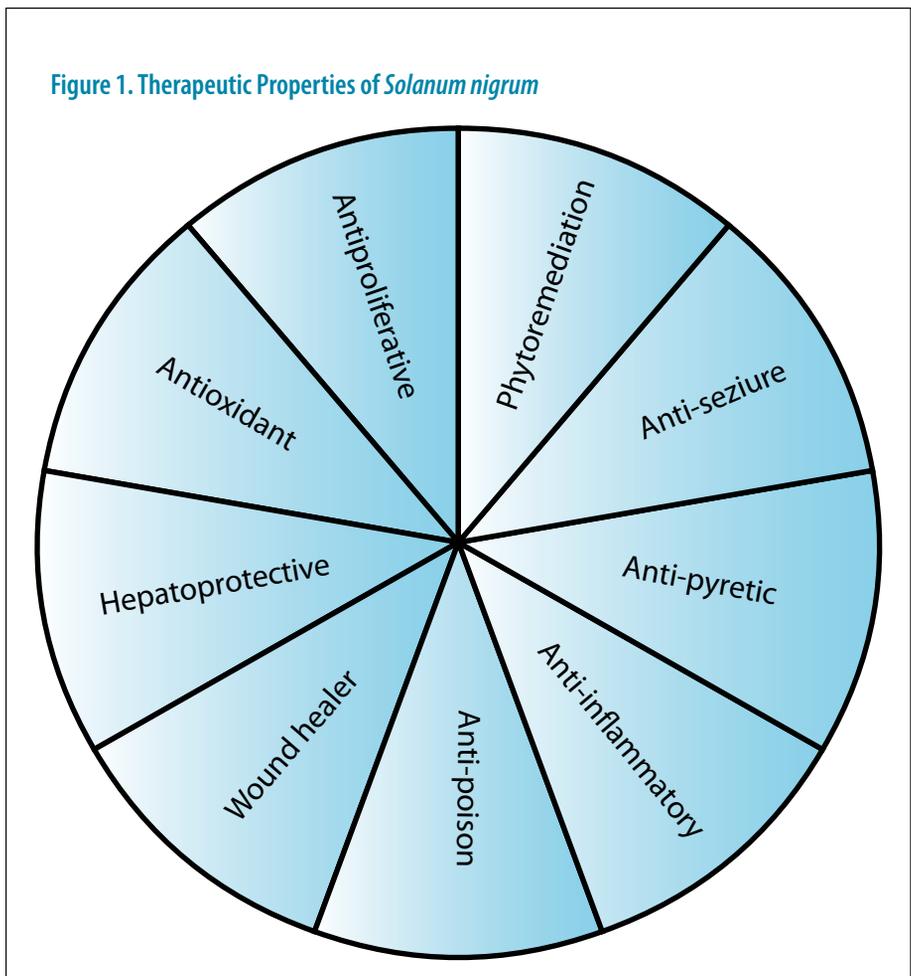
Both the crude extracts and isolated components of *S. nigrum* possess antiproliferative activity on various cancer cell lines. Crude extract is usually prepared with dried berries, but can also be prepared from the whole plant. The antiproliferative activities of the crude organic extract and isolated compounds were studied on tumor cell lines of liver (HepG2),<sup>21,22</sup> colon (HT29 and HCT-116),<sup>21,22</sup> breast (MCF-7),<sup>26</sup> and cervical (U14<sup>24,25</sup> and HeLa<sup>27</sup>). The antiproliferative activity of these extracts was examined by studying the cytotoxicity of the extract on cells. DNA fragmentation, a hallmark of apoptosis, was used to analyze the extent of apoptosis in treated cells. Apoptosis or programmed cell death is mediated by two pathways. The extrinsic pathway is activated by death receptors; whereas, the intrinsic pathway is mitochondria dependent. Apoptosis at mitochondria is controlled by antiapoptotic (Bcl-X<sub>L</sub>, Bcl-2, and Mcl-1) and proapoptotic (Bax, Bak, Bid, Bim, and Bad) proteins.

It has been observed that crude extracts induce different responses in cells *in vitro* at high and low concentrations. This is evidenced by the fact that when liver cancer cell lines (HepG2) are treated with high concentrations of the crude extract, c-Jun N-terminal kinase (JNK) is activated, which results in activation of proapoptotic factors like Bax. It further results in release of cytochrome c from mitochondria that activates caspases and triggers apoptosis.<sup>20,27</sup>

When the same cell lines are treated with low concentrations of extracts it leads to the induction of autophagy, in contrast to apoptosis.<sup>17</sup> Autophagy is a lysosomal degradation pathway in which the cell's damaged organelles or defective pathway prepares the cell to adapt to stressful conditions. That might be why the low concentration leads to autophagy.<sup>28</sup>

Aqueous plant extracts possess antiproliferative activity as demonstrated by growth inhibition of cervical carcinoma.<sup>24</sup> The mechanisms of actions of aqueous

extracts and isolated polysaccharides (SNL-P) were found to be identical,<sup>24,25</sup> indicating that the components responsible for the antiproliferative activity in aqueous extracts are probably the polysaccharides. SNL-P did not have any direct cytotoxic effect on U14 cell lines isolated from cervical cancer. Its antitumor property can be attributed to its immunomodulatory ability, which alters the host's immune response.<sup>25</sup> This is a crucial characteristic since, during the evolution of cancer, the immune system becomes weakened and is further undermined by chemotherapy.<sup>29</sup> SNL-P treatment arrests these cells in G2/M phase and results in an increase in the percentage of CD-4<sup>+</sup> T cells.<sup>25</sup> SNL-P activated CD-4<sup>+</sup> cells are mainly T-helper 1 (Th-1) cells that are activated by interferon-gamma (IFN- $\gamma$ ). These Th-1 cells help in the fight against intracellular pathogens and killing self-altered or tumor cells.<sup>30-32</sup> Similar immunomodulatory and anticancer properties of SNL-P have been confirmed in U14 cervical cancer-bearing mice.<sup>25</sup>



The mechanism of action of isolated glycoalkaloids on various cancer cell lines such as HepG2 has also been studied. The antiproliferative activity of solanine, a glycoalkaloid, on transformed cell lines is mainly due to its ability to facilitate the opening of the permeability transition (PT) channels of mitochondria by lowering the membrane potential. This results in an increase of the intrinsic calcium ion level that culminates in apoptosis. Solanine also inhibits Bcl-2, an antiapoptotic protein leading to an increase in cytochrome c, which activates caspases and triggers apoptosis.<sup>20</sup> Structure and antiproliferative activity of other glycoalkaloids from *S. nigrum* have not yet been studied.

A 150 kDa phytyglycoprotein isolated from the plant has been shown to possess antiproliferative activity on HCT-116 cells,<sup>22</sup> HeLa cells,<sup>27</sup> HT29 cells,<sup>33</sup> Hep3B cells,<sup>34</sup> and MCF-7 cells.<sup>35</sup> It activates caspase-3 and induces apoptosis. In addition, it inhibits transcription factor nuclear factor-kappaB (NF- $\kappa$ B), protein kinase C alpha (PKC $\alpha$ ), and inducible nitric oxide (iNO).<sup>22</sup> PKC $\alpha$  is a serine threonine kinase that plays an important role in tumor progression.<sup>36</sup> NF- $\kappa$ B is a known eukaryotic transcription factor involved in downstream signal transduction paths of phosphorylated PKC $\alpha$ . NF- $\kappa$ B protein is present in all cell types.<sup>37</sup> It is responsible for activation of transcription in mature B cells and plasma cells by binding to 10 base pair regions of nuclear kappa enhancer.<sup>38</sup> Its activity in other cell types is inhibited due to the presence of a substance such as inhibitor-kappaB (I $\kappa$ B) in the cytoplasm.<sup>39</sup> In the presence of the appropriate signal, I $\kappa$ B is phosphorylated, releasing NF- $\kappa$ B. Phosphorylated I $\kappa$ B undergoes proteasomal degradation and NF- $\kappa$ B is translocated to the nucleus where it activates the transcription of genes involved in tumor progression. The activated NF- $\kappa$ B results in the expression of the iNO synthase promoter-dependent gene and leads to the production of inducible nitric oxide, which also culminates in apoptosis.<sup>36</sup>

### Antiseizure Activity/Epilepsy

Seizures are defined as alterations of behavior due to disordered, synchronous, and rhythmic firing of populations of brain neurons.<sup>40</sup> Modern drugs have not exhibited sufficient effectiveness in their ability to cure seizures; in addition there are many side effects such as impairment of the central nervous system (CNS), aplastic anemia, hepatic failure, or even death. Medicinal plants such as *Ficus sycomorus*,<sup>41</sup> *Sclerocarya birrea*,<sup>42</sup> *Annona diversifolia*,<sup>43</sup> *S. nigrum* and many more used in

traditional medicine have proven anticonvulsant properties in animal models and may be a source of new antiepileptic drugs.

Experimentally, seizures were induced by picrotoxin, pentylenetetrazole, or electric shock in the adult albino rats. The aqueous extract of *S. nigrum* leaves provided protection against induced seizures in rats and a significant dose-dependent protection in chicken.<sup>44</sup> The mechanism of action of the extract still needs to be elucidated.

### Antioxidant Activity/Degenerative Disease; Anti-aging

The uncontrolled production of free radicals results in the onset of many neurodegenerative diseases, can accelerate aging, and can be controlled to some extent by exogenous antioxidants.<sup>45</sup> Methanol extracts of *S. nigrum* have shown significant antioxidant activity in various assays, including 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, estimation of the total phenolic compounds in the plant extracts, and determination of the 5-lipoxygenase activity.<sup>46</sup> Methanol extracts of *S. nigrum* inhibited the DPPH by 92 percent; whereas, the aqueous extracts showed considerably less effective radical scavenger activities. A quantitative correlation between the antioxidant activity and the content of polyphenols was seen, signifying that the phenolic compounds present in the plant contribute to the radical scavenging activity.

Other than methanolic extracts, purified *S. nigrum* glycoproteins also possess antioxidant activity. Their activities are distinctively specific, when demonstrated on MCF-7 cell lines using assays like DPPH radical scavenging assay, 2-deoxyribose oxidation assay, and superoxide anion scavenging assay.<sup>4</sup> *S. nigrum* glycoproteins effectively inhibited hydroxyl radicals in a dose-dependent manner. But the mechanism of scavenging action by stimulating cytokines (interleukin [IL] -2, IL-4, IL-12, IFN- $\gamma$ , and tumor necrosis factor-alpha [TNF- $\alpha$ ]) remains to be elucidated.<sup>4</sup>

### Anti-inflammatory Activity/Inflammatory Conditions

Inflammation is a disorder caused by the release of leukocytes and various other complex mediator molecules such as prostaglandins, leukotrienes, histamines, bradykinin, platelet activating factor, and IL-1 from tissues and migrating cells.<sup>47,48</sup> Various drugs and extracts derived from grapes, turmeric, mint, clove, eucalyptus, lavender, and many more have been used to alleviate inflammation.<sup>49</sup>

*S. nigrum* has been used in the traditional Indian medicinal system to treat inflammation, edema, and mastitis. The most widely used method to study anti-inflammatory effects in animal models is by inducing local edema in a rat paw by injecting an irritant agent such as carrageenan.<sup>47</sup> The methanolic extract of the plant showed good dose-dependent anti-inflammatory effect on induced edema in the rat model.

Cai et al<sup>50</sup> isolated the compounds responsible for the anti-inflammatory activity from the ethanolic extract of the *S. nigrum*. Leukotrienes such as LTC<sub>4</sub> are the lipid mediators that are found in increased concentration in the inflammatory reactions. Anti-leukotrienes are currently prescribed to treat various inflammatory diseases such as asthma and atopic rhinitis. (E)-ethyl caffeate, one component isolated from *S. nigrum*, possesses maximum inhibition for leukotrienes and thus could be considered as a potential anti-inflammatory therapeutic compound.

### Hepatoprotective Activity/Liver Disease

The protective effects of the aqueous extract of *S. nigrum* whole plant were evaluated in carbon tetrachloride (CCl<sub>4</sub>)-induced chronic hepatotoxicity in rats. CCl<sub>4</sub>-induced experimental hepatic damage leads to substantial increase in the serum activities of glutamate-oxaloacetate aminotransferase (GOT), glutamate-pyruvate aminotransferase (GPT), alkaline phosphatase (ALP), and total bilirubin that are indicators of cellular leakage and loss of functional integrity of cell membrane in liver.<sup>51</sup> CCl<sub>4</sub> also decreases the levels of antioxidant enzymes such as glutathione (GSH) and superoxide dismutase (SOD). The utilization of *S. nigrum* aqueous extract at the dose of 0.2 g kg<sup>-1</sup> body weight for six weeks restored the CCl<sub>4</sub>-caused increased levels of the hepatic enzymes (GOT, GPT, ALP); the higher dosage of *S. nigrum* aqueous extract (0.5 and 1.0 g kg<sup>-1</sup>) restored the diminished levels of SOD and GSH, indicating repair of the hepatic tissue damage caused by CCl<sub>4</sub>.<sup>52</sup>

Glutathione-S-transferases (GSTs) are a family of enzymes involved in detoxification of xenobiotics. CCl<sub>4</sub> treatment decreases the expression of hepatic GST isoforms, GST Mu and GST Al; whereas, the GST Pi expression is up-regulated. *S. nigrum* extract restored expression levels of the GST subunits to control levels. Whether the mechanism of action of *S. nigrum* is attributed to its direct action on the GST subunits or its neutralization of CCl<sub>4</sub> remains to be elucidated. Liver histopathological analysis also confirms that *S. nigrum* reduced the incidence

of liver lesions.<sup>52</sup> In another study, the ethanol extract of the fruits of *S. nigrum* (at a dose of 0.25 g/kg) was found to be hepatoprotective in a rat model of CCl<sub>4</sub>-induced hepatic damage.<sup>5</sup>

The effect of *S. nigrum* extract was also evaluated on thioacetamide (TAA)-induced liver fibrosis in mice. Liver fibrosis generally involves accumulation of the extracellular matrix protein, mainly collagen. Hydroxyproline levels are indicative of collagen and hence are used to determine the extent of fibrosis. Histological examination confirmed that *S. nigrum* whole plant extract reduced the degree of fibrosis caused by TAA treatment by reducing the amount of hydroxyproline and hence collagen. Also, the treatment with the *S. nigrum* extract reduced the elevated levels of plasma alanine aminotransferase (ALT) and total bilirubin to normal levels.<sup>53</sup> Therefore, these results suggest that *S. nigrum* could protect liver against CCl<sub>4</sub>- and TAA-induced oxidative damage in rats.

So far no clinical trials have been conducted on *S. nigrum* and its purified components. However, there are reports on clinical trials conducted using polyherbal formulations, such as Liv 600<sup>54</sup> and Liv 52,<sup>55</sup> that contain *S. nigrum* as one of the components and have been used as hepatoprotective agents.

### Side Effects and Toxicity

Most species in the Solanaceae family are poisonous to humans as well as to livestock. The toxic effects of the plants are mainly reported in the older literature. For instance, deadly nightshade contains tropane alkaloids. The toxin, when ingested by humans in large quantities, causes anticholinergic effects.<sup>56</sup> Although *S. nigrum* is considered to be an edible plant, its toxicity is mainly due to the presence of solanine, a glycoalkaloid causing varying degrees of toxicity in a dose-dependent manner.<sup>20</sup> The symptoms of poisoning in humans due to solanine are reported to include nausea, vomiting, diarrhea, headache, dizziness, loss of speech, fever, sweating, tachycardia, pupil dilation, blindness, mental confusion, convulsions, coma, and death.<sup>57,58</sup> The amount of toxic compound in a plant depends on the climate, soil type, season, and maturity.<sup>58</sup> The green unripe berries are generally considered more toxic than the ripe berries.<sup>59</sup> It is probable that by boiling the plant, the toxic components are destroyed as the plant is reported to be edible after cooking.

Traditionally, consumption of nightshade vegetables like tomato, potato, and eggplant has been considered to be problematic for arthritic

patients, leading to aggravation of joint pain. It has been reported that solanine present in the green parts of these vegetables is probably responsible for joint pain. However, scientific documentation on a correlation between *S. nigrum* consumption and inflammation of joints is lacking.

### Therapeutic Dosage

*S. nigrum* crude extracts as well as its purified compounds have been used to study antitumor, antiseizure, anti-inflammatory, and hepatoprotective activities in various animal models such as rat, mice, and chick. In most of the studies the extract has been either orally fed (p.o.) or administered intra-peritoneal (i.p.).

*S. nigrum* aqueous extract of the whole plant was prepared by hot extraction at 100 °C for 40 minutes and fed to mice at a dose of 2 mg/kg daily for 15 days to study its effect on melanoma cells metastasized to the lung. This dosage resulted in more than 50-percent reduction in tumor weight and lung metastatic nodules. The metastasis was probably suppressed due to decreased expression of signaling molecules PKC $\alpha$ , RAS, NF- $\kappa$ B and AKT phosphorylation.<sup>60</sup>

In another study, polysaccharides isolated from dried whole plant powder were further purified on DEAE-cellulose and Sephadex G-100 columns into three subfractions (SNL-P-1a, 1b and 1c). These purified subfractions were given i.p. to mice at a dose of 25 or 50 mg/kg daily for 12 days and SNL-P1a was found to be more effective in inhibiting cervical cancer than SNL-P1b and SNL-P1c. SNL-Ps protected the T-cells from tumor-induced apoptosis, resulting in host immune counter surge to fight the tumors.<sup>61</sup>

Antiseizure activity of *S. nigrum* aqueous extract from leaves was evaluated in rats, mice, and chicks. The extract was prepared using the Soxhlet apparatus for 72 hours at 60 °C. Seizures were induced by picrotoxin, pentylenetetrazole, or electric shock. The therapeutic dose for anticonvulsant activity was found to be 30-60 mg/kg in rats and mice, while the dose of 10-40 mg/kg was effective in electroshock-induced seizures in chicks. The study concluded that, although *S. nigrum* exhibited anticonvulsant activity, it did not have antiepileptic activity.<sup>44</sup>

Dose-dependent anti-inflammatory, antipyretic and antinociceptive activities were observed using the chloroform extract of *S. nigrum* leaves. The extract was administered subcutaneously (s.c.) in rat and mice models at a dose of 20-200 mg/kg.<sup>3</sup>

Kaushik and co-workers also documented anti-inflammatory activity using higher doses of an ethanolic extract of *S. nigrum* fruits (500 mg/kg p.o.).<sup>62</sup>

The above studies show that the aqueous and organic extracts were prepared from different parts of *S. nigrum* using different protocols to study its therapeutic effects. Though the scattered studies have indicated *S. nigrum* as a promising therapeutic agent, further studies need to be carried out using standardized preparation methods from specific plant parts.

### Conclusion

*S. nigrum*, a widely used plant in oriental medicine, has been shown to possess various activities such as antitumorogenic,<sup>17,20-27</sup> antioxidant,<sup>4</sup> anti-inflammatory,<sup>48</sup> hepatoprotective,<sup>51-55,63</sup> diuretic, and antipyretic.<sup>44</sup> Major compounds have been isolated and characterized. The *in vivo* activity of these compounds has also been studied. Although it is mentioned as a component in several popular polyherbal formulations in the form of alcoholic or hydroalcoholic extracts, it is an attractive candidate plant for formulating targeted drugs. A combined approach of parallel preclinical studies involving *in vitro* and *in vivo* models could provide necessary data to assess its suitability in this regard. While the exact mechanism of action remains to be elucidated in many cases, this plant with wide-ranging therapeutic properties needs to be investigated in well-designed clinical studies.

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