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Review Article

A REVIEW ON MEDICINAL PROPERTIES OF *CENTELLA ASIATICA*

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ABSTRACT

Plant-based drug discovery has drawn the attention of researchers, especially the one used as traditional medicines. *Centella asiatica* is traditional Ayurveda medicine widely used in India and across Asia for treating a variety of diseases. The aerial parts and roots are used for medicinal purpose, and its chemical constituents have wide therapeutic applications in areas of antimicrobial, anti-inflammatory, anticancer, neuroprotective, antioxidant, and wound healing activities. Many of its uses have been proven scientifically, and bioactive ingredients have been validated. In this review, we have done a critical evaluation of available literature looking for the pharmacological importance of *C. asiatica*. Further studies will be helpful to discover more bioactive compounds their exact mode of actions.

Keywords: *Centella asiatica*, Pharmacology, Chemical constituents, Therapeutic usage.

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INTRODUCTION

The plant is commonly known as Gotu Kola, Asiatic pennywort, Indian pennywort or Spadeleaf and belongs to Umbelliferae/Apiaceae family. In China, Southeast Asia, India, Sri Lanka, Oceania, and Africa, the plant has long been used as vegetable. In Southeast Asia, it is traditionally used for the treatment of a wide variety of disorders such as skin diseases, rheumatism, inflammation, syphilis, mental illness, epilepsy, hysteria, dehydration, and diarrhea [1,2]. *Centella asiatica* (Gotu Kola) is used in Indian systems of medicine for enhancing memory and for the treatment of skin diseases and nervine disorders [3]. The plant medicinal properties have long been utilized by the people of Java and Indonesia. In China, it is indigenously called as Gotu kola, and over 2000 years ago, it was one of the documented "miracle elixirs of life" [4]. Herbal medicines can be used as adaptogens, these plant derived drugs either reduce stress reactions in the alarm phase and provide a certain degree of safety against long-term stress [5]. *C. asiatica* (Umbelliferae) syn. Hydrocotyle asiatica is used to treat various ailments across India which includes body aches, headaches, insanity, asthma, leprosy, ulcers, eczemas, and wound healing [6]. To find out new potential compounds for therapeutic use, screening of medicinal plant is vital [7].

The plant has significantly drawn the attention of scientific groups in the recent years as it has multiple usages in the treatment of ailments. In Fig. 1, major pharmacological usage in the treatment of ailments has been outlined followed by its detailed description.

CHEMICAL CONSTITUENTS

The plant has long been used as folklore medicine for the treatment of a variety of diseases. Chemically been identified leading to therapeutic properties. Asiatic acid, asiaticoside, and madecassoside form the major constituents responsible for pharmacological value apart from being rich in flavonoids and terpenoids [8]. Centelloid was term given for different constituents of secondary metabolites produced by plant which mainly comprised of pentacyclic triterpenoid saponins [9]. P-cymene-(44%) along with other volatile compounds was found to be in a prominent amount in the essential oil of *C. asiatica* on analysis with gas chromatography-mass spectrometry (GC-MS) [10]. Centellin, asiatic, and centellicin were isolated from the aerial part of the plant, and further, their structures had been determined using 2D nuclear magnetic resonance technique [11]. From plant extract using high-performance liquid chromatography to identify bioactive compounds, madecassoside, asiaticoside, madecassic acid, and asiatic acid were found in the significant amount [12]. A quantitative estimation of triterpenoids showed highest asiaticoside content (6.42%) in leaf samples collected in Mangoro region [13]. New triterpene and a saponin, 2α,3β,23-trihydroxyurs-20-en-28-oic acid and 2α,3β,23-trihydroxyurs-20-en-28-oic acid O-α-l-rhamnopyranosy-(1→4)-O-β-d-glucopyranosyl(1→6)-O-β-d glucopyranosyl ester, have been isolated from the aerial part of *C. asiatica*, and their structures were determined using spectral methods [14].

ANTICANCER ACTIVITY

On A549 and PC9/G lung cancer, cell line inhibitory concentration 50 (IC_{50}) values of A-3 were 26.03±2.47 and 25.57±0.51, respectively, due to the presence of asiatic acid as major component [15]. Against the cell lines of human breast cancer (MDA MB-231), mouse melanoma (B16F1), and rat glioma (C6), the aqueous extract of *C. asiatica* had shown inhibitory activity with IC50 values of 698.0, 648.0, and 1000.0 μg/mL, respectively [16]. The methanolic extract of C. asiatica (Linn) showed inhibitory effect on MCF-7 cell lines, and induced apoptosis in MCF-7 cells as indicated by nuclear condensation, increased annexin staining, loss of mitochondrial membrane potential, and induction of DNA breaks was identified by TUNEL reactivity [17]. The effect of *C. asiatica* juice was checked on human HepG2 cell line using MTT assay, and it showed cytotoxic effects on tumor cells in a dose-dependent manner. At a concentration above 0.1% of juice, a higher amount of DNA damage and apoptotic cell death was observed on human HepG2 cell line [18].

Asiatic acid was evaluated for antiproliferative effect in lung cancer cells using MTT assay. Oral administration of AA inhibited weight and tumor volume significantly in lung cancer xenograft model [19]. In another study, asiatic acid showed induced apoptosis and decreased viability in human melanoma SK-MEL-2 cells in a dose-dependent manner [20]. Asiatic acid derived from *C. asiatica* showed antiproliferative effects on RPMI 8226 cells. It decreased the expression levels of focal adhesion kinase (FAK), and the probable mechanism of AA may be related to the inhibition of signal transduction mediated by FAK [21]. Asiatic acid, asiaticoside, and madecassic acid was the major composition of titrated extract of *C. asiatica*, and asiaticoside reduces melanogenesis in B16F10 mouse melanoma by checking tyrosinase mRNA expression [22]. In long-

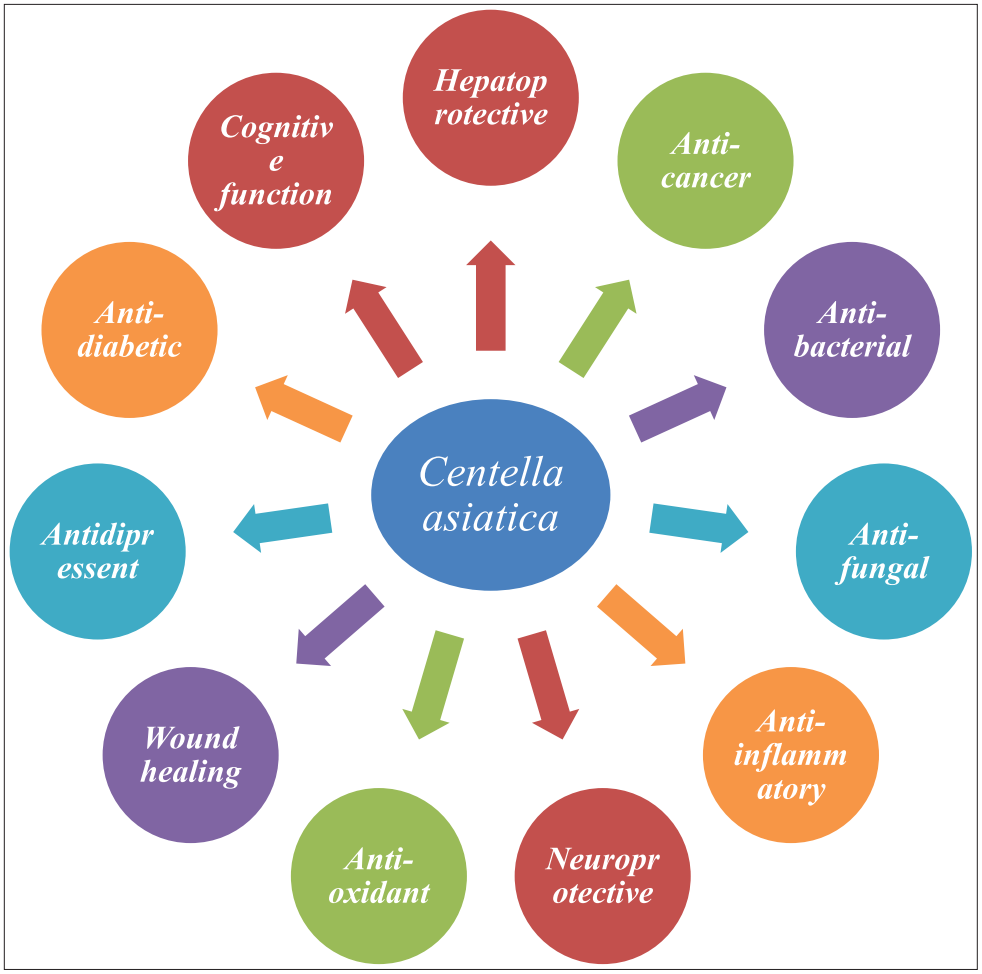

Fig. 1: Pharmacological activities of *Centella asiatica*

term culture at a concentration of 8 ug/ml, partially purified fractions inhibited the growth of mouse lung fibroblast (L-929) cells [23]. Reduction up to 50% in viability was observed in ovarian cancer cells treated with 40 ug/ml concentration of asiatic acid, and it also showed cell cycle arrest at the G0/G1 phase and increased apoptosis by 7-10 folds [24]. Induction of apoptosis was observed in A-549 cell line due to presence of asiatic acid which helped in regulation of miR-1290, BCL2 protein level, and cell cycle regulation [25].

ANTIBACTERIAL ACTIVITY

Methanol hot extract from *C. asiatica* leaves was taken to check the antibacterial activity which was assessed by zone of inhibition and minimum inhibitory concentration (MIC) value (2 µg/disc) by disc diffusion method. *In vitro* antibacterial activity of the plant extract against *Staphylococcus aureus* ATCC 25923 and methicillin resistance *S. aureus* (wild type) showed a zone of inhibition of 5 mm and 7 mm respectively [26]. In a study, it was observed that essential oil extract showed antibacterial properties against Gram-positive (*Bacillus subtilis* and *S. aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Shigella sonnei*) with MIC values ranging from 1.25 to 0.039 mg/ml [27]. *Bacillus cereus* and *Listeria monocytogenes* 10403S were selected to study the antibacterial activity in *C. asiatica* under both normal and osmatic stress condition. At 95% ethanolic extract, antibacterial activity was enhanced twice under osmotic stress condition. The MIC of *C. asiatica* was observed to be 16 µl/ml against *B. cereus* while 8 µl/ml for *L. monocytogenes*10403S [28]. MS media was used to culture leaf explants, and its antibacterial activity against *B. cereus, E. coli, S. aureus,* and *P. aeruginosa* was evaluated; methanol extracts of leaf and callus displayed maximum inhibitory effect against the tested organisms [29].

ANTIFUNGAL ACTIVITY

The petroleum ether, ethanol, chloroform, n-hexane, and aqueous extract of *C. asiatica* showed activity against *Aspergillus niger* and *C. albicans* with a zone of inhibition of 14, 16, 13, 13, and11 mm and 13, 15, 15, 11, and 9 mm, respectively. The control Ketoconazole (10 µg) showed the inhibition of 12 mm [30]. Ethanolic extract of *C. asiatica* was checked for antifungal activity against *Aspergillus flavus,* and *Penicillium citrinum* exhibited the strongest antimold activity (percentage mycelial inhibition = 26.3 mm) [31]. 100% ethanolic extract of *C. asiatica* showed a zone of inhibition of 15.4 mm against *A. niger* [32]. Following agar well diffusion method, antimicrobial activity was checked for ethanolic extract of plant against *A. niger* and *Candida albicans*, an inhibition of 16 and 15 mm was observed, respectively, while control ketoconazole (10 µg) gave a inhibition zone of 12 and 10 mm [30]. Against *Candida albicans*, on an average 5 mm, zone of inhibition was observed while the standard miconazole nitrate showed an inhibition of 20 mm [33].

ANTI-INFLAMMATORY ACTIVITY

Anti-inflammation is widely used methodology in experimental oncology which helps to examine the inflammation defensive potential of natural products (betulinic acid, α-amyrin acetate, lupeol acetate, oleanolic acid, and ursolic acid) and synthetic entities [34]. Terpenoid is major constituents among of secondary metabolites secreted by plants. It helps to cope up stress condition and supports defense activities. Plants with medicinal properties are rich in these compounds such as ceramide and different forms of terpenoids [35]. Pentacyclic triterpenoid and saponins are collectively known as centelloids. Among different constituents, triterpenoid saponins were mainly

expected to be responsible for therapeutic actions [9]. Hypotonicity-induced human red blood cell membrane breakdown was inhibited by *C. asiatica*. At different concentrations, membrane stabilization was observed for diclofenac sodium and methanolic extracts, and at a dose of 2000 µg/ml, maximum membrane stabilization of *C. asiatica* extracts was noticed to be 94.97% [36]. At concentration of 2 mg/kg, the CA extract showed moderate anti-inflammatory property on prostaglandin E2-induced inflammation in a dose-dependent manner [37]. In another study, the aqueous and alcoholic extract of *C. asiatica* showed 46.31% and 71.18%, respectively, inhibition of edema after 3 hrs, while the standard ibuprofen showed an inhibition of 66.66% [38]. At the 4th and 5th hrs, after λ-carrageenan (Carr) supplementation, the asiatic acid reduced paw edema by regulation of catalase, superoxide dismutase (SOD), and glutathione in the liver tissue [39]. In another study, measurement of paw size was taken before carrageenan injection and then 1, 2, 3, and 4 hrs after carrageenan injection. It was observed that the methanolic extract showed significant inhibition was the highest at 3 hrs at 200 mg/kg dose which was slightly lower than indomethacin effect [40].

NEUROPROTECTIVE ACTIVITY

Neuroprotection aspect of *C. asiatica* mainly involves enzyme inhibition, prevention of amyloid plaque formation in Alzheimer's disease, dopamine neurotoxicity in Parkinson's disease, and reducing oxidative stress [41]. Water extract of *C. asiatica* was evaluated on the activity of subtypes of phospholipase A2 (PLA2) in primary cultures of rat cortical neurons, asiaticoside present in extract inhibited cPLA2 and sPLA2 activities [42]. In male Sprague-Dawley rats, improved learning and memory were observed on acute administration of asiatic acid [43]. Neuroprotective potential of modern medicine constituents of the plant includes asiatic acid, madecassic acid, and brahmaside as well as flavonoids madecassoside and madesiatic acid [44]. *C. asiatica* was explored for neuroprotective effect on cell death and cognitive irregulation in aluminum-treated rat. Significant improvement in memory performance, oxidative defense was observed on chronic administration of CA [45]. The plant is known to utilize neuroprotective effects by attenuating the changes in an animal model such as pathological neurobehavioral and neurochemical properties. Phosphoinositides-assisted cytodynamics and synaptic function show the neuroprotective effects of asiaticoside in the rat which includes mode of ROT-infused hemiparkinsonism [46].

ANTIOXIDANT PROPERTY

Free radical and reactive oxygen species (ROS) are the main reason behind aging. All organisms have a mechanism to deal with such reactive groups, free radical scavengers provide protection to the organism. The plant extract obtained from Turkey and India was compared with a standardized extract of plant obtained from China. The three extract at concentration of 250, 500, 1000, and 2000 µg/mL showed radical scavenging activity for 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay: CA-STD>CA-TR>CA-IND [47]. *C. asiatica* extract and powder was evaluated for reduction in oxidative stress in Sprague-Dawley rats. Results showed a decrease in the generation of ROS and oxidative stress in the rats. It was also noted that there was a significant decrease in SOD level [48]. Essential oil of *C. asiatica* extracted through steam distillation showed to be excellent antioxidant for food containing lipids. Its activity was quite comparable with the synthetic antioxidant butylhydroxyanisole (BHA) [49]. Polyphenol, flavonoid, β-carotene, tannin, Vitamin C, and DPPH compounds are readily found in *C. asiatica* contributing to significantly higher antioxidant activity in the herb [50]. Crude methanolic extract on continuous supplementation for 14 days resulted in increase in level of antioxidant enzymes and ascorbic acid level reduced in lymphoma-bearing mice [51]. Extracts of *C. asiatica* in different solvents such as chloroform, hexane, acetone, ethyl acetate, methanol, and water were assessed for antioxidant potential. The DPPH and hydroxyl radical scavenging activity were tested for methanolic extract which showed the IC_{50} value of 0.07 mg/ml and 500 µg/ml, respectively [52].

WOUND HEALING

An increase in DNA, protein, and collagen content of granulation tissues was observed on supplementation of extract of *C. asiatica* resulting in collagen synthesis and cellular proliferation at wound site [53]. The extract of *C. asiatica* considerably enhanced the wound breach power in incision model when compared to controls ($p<0.001$), wound contraction rate was noticeably enhanced as compared to control wounds ($p<0.001$), and wounds epithelized faster when treated with an extract of CA [54]. Rats treated with extract showed a better tensile strength of wound after 7 days of wound infliction when compared with control [55]. A study on ethanol-induced gastric lesion oral administration of CE (0.05, 0.25, and 0.50 g/kg) before ethanol administration considerably lowered mucosal myeloperoxidase activity checked gastric lesions formation (58% to 82% reduction) and in a dose-dependent manner [56]. Fibroblast division and collagen synthesis were enhanced in wound on treatment with extract of *C. asiatica* [57].

ANTIDEPRESSANT

Compared to diazepam *C. asiatica* possesses antianxiety effect but has no effect on behavioral despair [58]. Total triterpenes and imipramine from *C. asiatica* were evaluated for antidepressant activity using forced swimming test, the result showed a reduction in stillness duration and regulated amino acid levels [59]. In another study, decrease in corticosterone level in serum and enhanced 5-HT, NE, DA and their metabolites 5-HIAA and MHPG in rat brain were observed [60]. Standardized extract showed a reversal of physiological and behavioral changes following OBX-induced depression in rats [61]. Forced swim test was performed in male Sprague-Dawley rat treated with asiatic acid and midazolam+asiatic acid, significant result was observed in the ration of open-arm time, maximum speed, and time spent in the AA group and the midazolam+AA group ($p<0.05$) [62].

ANTIDIABETIC ACTIVITY

Antidiabetic properties of leaves extract of *C. asiatica* was evaluated in alloxan-induced rat by administering extract at a concentration of 250, 500, and 1000 mg/kg after 3 hrs of ingestion reduction in blood glucose level was noticed by 32.6%, 38.8%, and 29.9%, respectively [63]. Effect of ethanol extract was tested in streptozotocin (50 mg/kg)-induced Wistar rats. Studying the serum glucose, urea cholesterol, lipid, liver glycogen level, and body weight, the antidiabetic activity of extract at concentration of 200 mg/kg was noticed [64]. In a study, lower inhibitory activities of α-amylase of C. asiatica extract and rutin were observed when compared to acarbose and an anti-diabetic drug [65]. Extract of C. asiatica led to reducing blood glucose level in dose-dependent manner by 29.4%, 32.8%, 33.6%, and 35.7%, respectively, at doses of 50, 100, 200, and 400 mg per kg body weight [66]. In alloxan-induced rats, reduction in blood glucose level was observed at a dose level of 50 mg/kg bwt of *C. asiatica* juice [67]. The effect of intestinal disaccharides and alpha amylase was inhibited, and lowered glucose absorption was observed when supplemented with plant extract [68]. Long-term administration of plant extract reversed the blood glucose level to normal in obese diabetic rat [69]. Asiatic acid was found to reduce blood glucose level in Goto-Kakizaki (GK) rat by enhancing fibrosis of islets in diabetes which plays a vital role in the prevention of islets dysfunction [70]. In diabetic Wistar rat model, asiatic acid showed to preserve and restore beta cell mass [71].

COGNITIVE FUNCTION

Asiatic acid was found to prevent spatial working memory and reduction of neurogenesis defects in the hippocampal region caused by 5-FU chemotherapy [72]. Water extract of *C. asiatica* was observed to enhance synaptic differentiation and dendritic arborization with reference to Aβ which causes cognitive improvement [73]. In a study, gotu-kola extract was supplemented for weeks in defined concentration results showed to be effective in the treatment of cognitive function impairment after stroke [74]. Asiatic acid has potential to restore the

impairment of cell proliferation, spatial working memory caused by treatment with valproic acid [75]. Water extract helped to improve cognitive function by activation of antioxidant response gene and mitochondrial biogenesis [76], normalized calcium homeostasis [77]. In another study, asiatic acid was found to enhance hippocampal neurogenesis which can serve as potent cognitive enhancer [78]. Asiaticoside isolated from Hydrocotyle sibthorpioides helps in scavenging free radical enhancing activity of antioxidant enzymes, improving synaptic plasticity, reducing the level of Aβ, and reversing abrupt changes in AchE activity [79]. In cognitive-related disorders, mitochondrial dysfunction and oxidative stress have major role, in a study carried out aluminum-induced cognitive dysfunction and mito-oxidative damage *C. asiatica* have proved to carry neuroprotective potential [45]. The study was done in primary culture of rat cortical neurons to check the activity of subtypes of PLA2, asiaticoside present in extract inhibited cPLA2 and sPLA2 activities [42]. On experiment conducted on mice model oral supplementation of asiatic acid (100 mg/kg), significant improvement in cognitive function was observed in the Morris maze test and retained glutathione and lipid peroxidation and SOD activity in cortex and hippocampus to control levels [80]. In another study, *C. asiatica* proved to have a protective role against D-glucose-induced biochemical, behavioral, and mitochondrial dysfunction in mice [81]. Acute administration of asiatic acid was studied on male Sprague-Dawley rats for memory and learning, treatment at 30 mg/kg of asiatic acid significantly improved memory [43]. The healing effect of *C. asiatica* was observed against colchicine-induced cognitive impairment [82] and lead acetate-induced changes in oxidative biomarkers in the central nervous system [83]. Cyclic AMP response element binding protein phosphorylation level was enhanced in primary culture of rat embryonic cortical cells on treatment with plant extract, thus improving memory function [84]. In another finding, it was found that high dose of plant extract-enhanced memory and increased N100 constituent amplitude of event-related potential [85]. In an intracerebroventricular streptozotocin model of Alzheimer's disease in rat plant, aqueous extract was found effective in combating the cognitive deficit and oxidative stress [86].

HEPATOPROTECTIVE

Effect of methanolic extract of CA was evaluated in Type 2 diabetes mellitus, hepatic concentrations of interleukin-1β, MCP-1, and tumor necrosis factor alpha in diabetic control rats orally treated with deionized water, group were reduced to 68%, 75%, and 63% of normal control rats orally treated with deionized water values, respectively [87]. In dimethylnitrosamine-induced liver injury *C. asiatica* noticeably enhanced fibrosis of liver tissues by mass periportal±bridging necrosis, intralobular degeneration, and focal necrosis [88]. On INH-treated albino rats, CA (100 mg/kg bw) dose was found effective to improve liver histology [89]. In cyclophosphamide (CYP)-induced hepatotoxicity in rats, *Centella asiatica* triterpene saponins saponins regulated hepatic function by restoring cytokine production [90]. Hepatoprotective activity of plant extract was checked against Ccl4-induced liver injury, and the extract showed hepatoprotective activity most probably due to the presence of asiaticoside (14.5%) in the extract [91]. The functional group of asiatic acid was modified at C2, C3, C23, and C28. Compound 9 showed hepatoprotective effects against GalN-induced hepatotoxicity (66.4% protection at 50 μM) and moderate hepatoprotective activities against CCl4-induced hepatotoxicity (20.7% protection at 50 μM) [92]. Asiatic acid protects liver injury by onset of Smad7-dependent inhibition of TGF-beta/Smad-assisted fibrogenesis [93]. Conventionally, used plants to get rid of liver dysfunction might, therefore, could be potential source for new hepatoprotective compounds for development as pharmaceutical entities [94].

CONCLUSION

C. asiatica is potential herb with an array of health-care applications. It is widely accepted that plant has got neuroprotective activities and helpful in brain improvement. Plants have proved to bear low toxicity and higher efficacy in clinical treatment with prominent activities such as anticancer, antibacterial, antifungal, anti-inflammation, neuroprotection, antioxidant, wound healing, and antidepressant as mentioned in above manuscript. As *C. asiatica* is an endangered species using plant tissue culture mass propagation major can be helpful, and callus and suspension culture techniques can be harnessed for secondary metabolite extraction. Germplasm conservation could be a possible way to preserve this precious plant. More studies are required to characterize and establish the chemical compounds responsible for a wide range of therapeutic activity. Due to the presence of wide bioactive compound, the plant has vast application. The plant can be a safer alternative for the formulation of new drugs. Further research is needed to confirm their activities mentioned in ancient scripts followed by clinical studies for their safe application for humans.

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