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Review

An Overview of *Justicia adhatoda*: A Medicinal Plant but Native Invader in India

Isha †, **Pardeep Kumar** †,‡ **and Anand Narain Singh** *

Soil Ecosystem and Restoration Ecology Laboratory, Department of Botany, Panjab University, Chandigarh 160014, India; sandhuisha70@gmail.com (I.); pardeepmor989@gmail.com (P.K.)
* Correspondence: ansingh@pu.ac.in or dranand1212@gmail.com
† These authors contributed equally to this work.
‡ Current address: Department of Botany, Mata Gujri College, Fatehgarh Sahib 140406, India.

Abstract: *Justicia adhatoda*, also known as Adulsa or Vasaka, is a notable member of the family Acanthaceae, with a broad geographic distribution across varied climatic conditions, and is known for its extensive medicinal properties for treating respiratory disorders, tuberculosis, malaria, and dysentery. It possesses several pharmacological activities, including anti-bacterial, anti-fungal, and anti-cancerous ones. In addition to discussing its morphology, phytochemistry, and pharmacological aspects, the present review also focuses on its several unexplored facets, such as pollination mechanism, cytology, molecular and genetic aspects, conservation, and ecological attributes. The literature survey indicates that, despite its medicinal value, *J. adhatoda* is also a strong invader in various ecosystems, suppressing associated species and cause vegetation homogenization. It also provides several ecosystem services, including soil stabilization, ecological restoration, and phytoremediation by removing contaminants such as chromium and mercury from wastewater. Additionally, its leaves increase the nitrogen content in compost, promote the proliferation of earthworms, and help suppress plant diseases. This highlights its potential for sustainable land management and integrated disease control. The urgency of this research is underscored by the significant gap in the literature regarding the ecological interactions of *J. adhatoda*, particularly its allelopathic effects on other plant species. The findings underscore the need for sustainable utilization and conservation strategies, emphasizing the dual importance of *J. adhatoda* as a medicinal resource and an ecological disruptor.

Keywords: medicinal plants; ethnomedicine; native invader; phytochemistry; conservation; ecological attributes

Academic Editor: Antoni Margalida

Received: 9 November 2024
Revised: 20 December 2024
Accepted: 29 December 2024
Published: 3 January 2025

Citation: Isha; Kumar, P.; Singh, A.N. An Overview of *Justicia adhatoda*: A Medicinal Plant but Native Invader in India. *Conservation* **2025**, *5*, 2. https://doi.org/10.3390/conservation5010002

1. Introduction

India's rich and diverse medicinal plant resources have played a vital role in developing traditional medicinal systems such as Ayurveda and Siddha [1]. These systems utilize approximately 1500 plant species in Ayurveda and about 1200 species in the Siddha medicinal system for drug preparation [1]. The Acanthaceae family, consisting of over 4300 species worldwide, is particularly significant for its many medicinally important plants [2]. *Justicia*, the largest genera in the Acanthaceae family, is home to more than 600 species in tropical and pantropical regions. In India, *Justicia* contributes significantly to the country's medicinal plant diversity, with approximately 50 species located primarily in temperate areas [3]. One of the notable members of the Acanthaceae family is *Justicia adhatoda*, also known as Adulsa or Vasaka [4]. This medicinal shrub, native to tropical regions of Southeast Asia, including India, Malaysia, Singapore, Sri Lanka, Burma, and southern

China, is known for its rich phytochemical composition [4,5]. Traditional medicines extensively utilize it to address various health issues, including respiratory, circulatory, hepatic, and skin problems [5].

J. adhatoda is rich in phytochemicals, including alkaloids, phytosterols, polyphenolics, and glycosides [1,4,5]. Notably, alkaloids such as anisotine, vasicinone, vasicinol, adhatodine, adhatodinine, and adhavasinone are the main phytoconstituents. Additionally, vascicine, vascinone, and adhatonine are the key phytoconstituents reported from different plant parts of Vasaka [5]. The phytochemicals discussed have demonstrated significant potential for the development of novel pharmaceuticals. Building on this potential, the plant showcases an impressive array of pharmacological properties, including anti-bacterial, anti-fungal, anti-cancer, immunomodulatory, antitussive, antihelminthic, hepatoprotective, antioxidant, antimalarial, anti-Alzheimer's, and insecticidal activities [5]. These compounds have shown promise in developing drugs that target specific molecular pathways. For example, anisotine and vasicinone inhibit viral proteases, making them potential candidates for antiviral therapies. Other phytochemicals such as phytol and amrinone, exhibit antioxidant and cardiotonic effects, highlighting the plant's significance in modern pharmacology [6].

In addition to its pharmacological benefits, *J. adhatoda* leaf extract is recognized as a valuable source of chiral bioactive compounds, with significant potential for drug development [7]. Furthermore, the plant exhibits allelopathic properties, which means it can exert phytotoxic effects on other plants [8]. This highlights its multifaceted importance in both traditional medicine and modern pharmacology [7]. The plant extracts of *J. adhatoda* are used as an ingredient in famous marketed drugs such as Bisolvon, which is commonly used to relieve airway obstruction by reducing mucus production and promoting airway clearance [9]. Several herbal formulations, including Kada, Femiforte, and Spirote, also feature *J. adhatoda* and are used to manage various respiratory conditions [9].

Moreover, the unprecedented collection of Vasaka for medicinal and other socioeconomic purposes has caused a decline in its natural populations in some parts of the world. Therefore, implementing guidelines for sustainable use can help prevent further population decline and ensure the plant's availability for future generations [10]. Due to the plant's broad applicability and significant medicinal value, various studies have reviewed its ethnomedicinal, phytochemical, and pharmacological characteristics [5,11,12].

Despite its significant medicinal value, *J. adhatoda* is recognized as a formidable invader of natural habitats [13]. However, it is a matter of thorough investigation whether it is detrimental to local vegetation or propagates aggressively [13]. This review aims to consolidate the available information on the least explored aspects such as molecular phylogeny, ecological distribution, diversity attributes (species and genetic diversity), ecosystem services, pollination mechanisms, biotechnology, and cytology. Furthermore, this study aims to elucidate the factors influencing the invading behavior of *J. adhatoda* in natural habitats. To our knowledge, no previous study has integrated these aspects in a single study. This study serves as a baseline reference for future research on this plant, particularly in understanding its cohabitation effects within the invaded ecosystem.

2. Material and Methods

Literature Search

This study used search engines and databases such as Google Scholar (http://scholar.google.com) (accessed on 14 September 2023), Web of Science (https://www.webofscience.com/wos/woscc/basic-search) (accessed on 5 October 2023), Scopus (https://www.scopus.com) (accessed on 21 October 2023), PubMed (https://pmc.ncbi.nlm.nih.gov) (accessed on 24 January 2024), and NCBI (https://www.ncbi.nlm.nih.gov) (accessed on 10 March 2024) to find published literature. To search for relevant information, we entered keywords like "*Adhatoda vasica*", "*Justicia adhatoda*", "Malabar Nut", "Vasaka", and "Basuti". Regional floras, monographs, research papers, and online resources like eFlora of India [14] were also consulted to gather additional information. Furthermore, the GBIF database [15] was utilized to retrieve the plant's geographical records and prepare its distribution map using QGIS software version 3.2.0. Additionally, ChemDraw Professional 16.0 software was employed to draw the chemical structures.

3. Botany

3.1. Classification

The taxonomic identity (Table 1) of *J. adhatoda* is quite confusing and debatable. Separate names have been used for the plant based on its geographical records, such as *Adhatoda vasica* in northern India and *Adhatoda zeylanica* in southern India. Berry et al. [16] recognized two separate morphotypes of *Justicia adhatoda*, named 'S' and 'B', based on distributional records, anatomical, biochemical, and genetic variations. The 'S' morphotype is mainly confined to dry regions of northwestern India, whereas the 'B' morphotype is in the wet tropics of southern India. They designated the S and B morphotypes as *Justicia adhatoda* var. vasica and *Justicia adhatoda* var. zeylanica, respectively. Furthermore, based on phylogenetic analysis, Chrungoo et al. [17] considered *Justicia adhatoda* as a sister group of *Justicia beddomei*, both sharing a common ancestor. However, to resolve the taxonomic identity of *J. adhatoda*, further studies that include sampling populations from across India must be considered for both morphological and phylogenetic analysis.

Table 1. Taxonomic position of *Justicia adhatoda*. Adapted from [5,12].

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Scrophulariales
Family	Acanthaceae
Genus	*Justicia*
Species	*adhatoda*

3.2. Vernacular Names

J. adhatoda is recognized by various vernacular names across different dialects and languages in India, highlighting its extensive use and cultural importance in traditional medicine. As shown in Table 2, the regionally diverse names emphasize the plant's local recognition within different communities.

3.3. Botanical Description

J. adhatoda is a small, evergreen, branched shrub that grows to a height of 2.5 m with moderate to high primary and secondary branching. The leaves are large, lance-shaped, and grow to be around 4 cm broad and 6 to 18 cm long with an opposite phyllotaxy. The stomata have an extended, oval form, and the epidermis contains tiny glandular hairs with quadricellular secreting glands and simple warty hairs with one to three cells. The flowers are small, irregular, and zygomorphic, arranged in a spike or panicle inflorescence [18]. The fruit is a four-seeded, non-fleshy, dehiscent capsule with longitudinal channels, while the corolla is large and white with a lower lip stained purple or pink. The seeds are globular, non-endospermic, and are borne on the minute, hook-like outgrowths called "reticular" [19].

Figure 1 illustrates the morphological attributes of *J. adhatoda*. Panel (a) highlights the leaves and unopened floral bracts, while panel (b) showcases the inflorescence with fully opened flowers, displaying distinct pink coloration on the petals.

Table 2. A list of vernacular names used for *Justicia adhatoda* in different dialects/languages in India. Adapted from [2,5,12].

Language/Dialect	Vernacular Name
English	Arusa, adusa, rusa, baansa, adulsa
Hindi	Adosa, adalsa, vasaka
Bengali	Basak
Gujarati	Aradusi, adulso
Kannada	Adusoge, kurchigida, pavate, bansa
Marathi	Vasuka
Malayalam	Ata-lotakam
Punjabi	Bhekkar, bansa, basuti
Tamil	Adatodai
Telegu	Adasaram
Sanskrit	Amalaka, bashika

Figure 1. Morphological attributes of *Justicia adhatoda* are (**a**) leaves and unopened floral bracts and (**b**) inflorescence with opened flowers depicting pinkish colorings on petals.

4. Ecology

4.1. Ecology and Distribution

The plant is native to subtropical and tropical parts of Southeast Asia, including China [20,21], India [11,22], Pakistan [11], Burma [22], and Sri Lanka [11,22]. In addition, it is frequently found in Bangladesh [5,22,23], Malaysia [22], and subtropical parts of Bhutan [21], as well as Burma [22]. Studies have also recorded its presence in China [20,21], Hong Kong [21], Thailand [11], and Nepal [11]. This tiny evergreen shrub mainly grows along roadside ditches, waste areas, and stony, dry, low-humid soils [24]. In natural forests, it mostly prefers open canopy and sunny habitats [25]. However, it grows in whole light in meadows and flood plains. Owing to its subtropical to tropical nature, its habitat is restricted up to 1450 m altitude [25]. In India, the plant is commonly found in the lower

Himalayan region or Siwalik hills, experiencing a subtropical environment, ranging from Hamirpur in Himachal Pradesh [26] to Chandigarh [27], Morni hills [26], and the Kalesar National Park of Haryana state [28]. It also grows luxuriantly in the semi-arid climate of southern Haryana (Narnaul, Rewari, Mahendragarh) [28] and southeastern parts of Rajasthan state (Sariska Tiger Reserve) [13]. It also experiences a favorable environment in the Gangetic plains, and therefore, heavy infestations have been reported in almost all parts of Uttar Pradesh [11] and some parts of Bihar [11]. It also has scattered populations across the northeast Indian states of Manipur and Nagaland [11]. The geographical distribution of *J. adhatoda*, as depicted in Figure 2, provides essential insights into its adaptability and prevalence across diverse regions. Geographical records indicate that *J. adhatoda* is predominant across several parts of southern India, including coastal areas of Odisha, Andhra Pradesh, and Tamil Nadu [11]. Furthermore, a few studies have documented its frequent occurrences in the tropical wet and dry forests of Kerala and Tamil Nadu [11]. A few studies have shown its occurrence under *Pinus roxburghii* and *Prosopis juliflora* plantations in the Siwalik region [29]. Numerous studies have documented variations in the plant density of *J. adhatoda*, underscoring the significant influence of diverse environmental conditions on its growth and distribution. A summary of these findings is presented in Table 3.

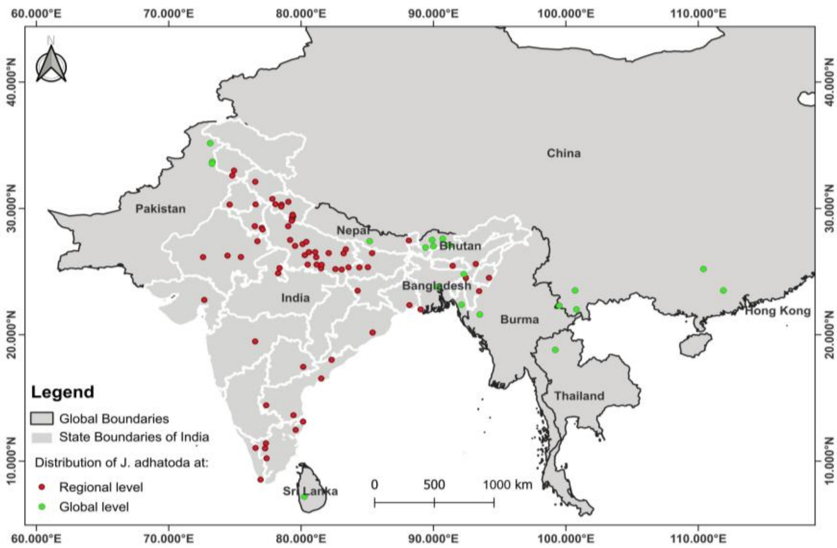

Figure 2. Geographical distribution of *Justicia adhatoda*.

Table 3. Variations in plant density of *Justicia adhatoda* across different studies.

Locality	Density (Individuals ha^{-1})	Reference
Ridge Forest, Delhi	1193.3	[30]
Garhwal Himalaya, Rajasthan	364	[31]
Savannas of STR, Rajasthan	29,058.8	[13]
Gurugram, Haryana	600	[28]
Gurugram, Haryana	466.7	[28]
Gurugram, Haryana	766.8	[28]
Mahendragarh, Haryana	436	[28]

Data concerning the physicochemical status of *J. adhatoda*-invaded habitats is very obscure. A recent study by Naeem et al. [32] investigated the adaptability potential of *J. adhatoda* to different xeric conditions. They observed that under dry conditions, the plant showed substantial modifications in morphological (increased root length, root biomass), physiological (low concentration of Ca^+, K^+ ions, and high concentration of Na^+ ions), and anatomical traits (thick epidermis, enlarged and sclerified vascular bundles). They further inferred that morpho-plasticity and broad adaptability to environmental conditions are mainly responsible for its wide geographic distribution.

4.2. Invading Properties

Given its gregarious and monoculture habitat formation, *J. adhatoda* colonizes quickly wherever it grows. For example, a study by Kumar and Bhatt [33] found that *J. adhatoda* displayed the highest shrub density (190 individuals/ha) among the sampled plant communities. Furthermore, a study conducted by Bhatt et al. [13] in Sariska Tiger Reserve, Rajasthan, observed that tree species, including *Acacia leucophloea* and *Acacia catechu*, displayed interrupted regeneration in *J. adhatoda* occupied patches. Furthermore, they also inferred that the manual removal of Vasaka plants enhanced sapling density, shrub density, and grass cover. Several features that make *J. adhatoda* an aggressive suppressor of associated vegetation can be hypothesized as the (1) prevention of sunlight reaching the ground surface, which might hinder the sapling's growth and the(2) release of certain allelochemicals preventing neighboring plant species from growing and developing [34]. The allelopathic potential of a plant species is partly determined by its phytotoxicity [35]. Knowledge of a plant species' phytotoxicity makes creating natural plant growth regulators and biological herbicides easier. In a study, researchers tested the phytotoxic effects of crude methanolic extracts from various parts of thirteen medicinal plants on radish seeds. The phytotoxicity of *J. adhatoda* was demonstrated against the dry weight, fresh weight, germination velocity, germination, and moisture of the radish seeds [35]. In another study, Mitra and Prasad [36] examined the effects of aqueous extracts from the leaves and flowers of *J. adhatoda* on the root weight of turnips (*Brassica rapa*). Their findings revealed a gradual delay in flowering time with increasing doses of the plant extract. In a separate study, Devkota and Sharma [8] assessed the allelopathic potential of the leaf extract of *J. adhatoda* on wheat (*Triticum aestivum*) and pea plants (*Pisum sativum*). They found that different plant extract concentrations significantly slowed seed germination and stopped the growth of hypocotyls and roots. Additionally, Kundu et al. [37] reported a considerable decrease in sunflower and grass pea seed viability and germination rate upon treatment with the *J. adhatoda* plant extract. Their analysis suggested that the plant extract reduces overall dehydrogenase activity and nucleic acid content, decelerating seed vigor. This implies that the plant contains some allelochemicals that inhibit the growth of other vegetation, which follows the novel weapon hypothesis. However, further studies are required on a broader scale to ascertain the role of *J. adhatoda* in potential allelopathic interactions. (3) Vegetative propagation by stem cuttings is crucial in its rapid multiplication, enabling the plant to colonize quickly to new habitats. In addition, the plant's adaptability to varied environmental conditions, such as semi-arid, lower Himalayan subtropical, and tropical moist, indicates its morpho-plastic nature, aligning with the phenotypic plasticity and ideal weed hypotheses. However, no study has tested the above hypothesis concerning this plant's invasive nature.

4.3. Ecosystem Services

J. adhatoda has a solid regenerative and competitive ability. It typically outcompetes native vegetation and is considered a weed in many areas [13]. Ungulates, such as Sambhar and Chital, use their patches for shelter and hiding [13]. The plant also serves as a tool for phytoremediation [35,38]. According to Sekhar et al. [39], *J. adhatoda* leaf powder has a strong affinity for chromium (VI) ions and can help eliminate chromium from wastewater. Another study by Aslam et al. [40] showed that the plant can effectively absorb Hg (II) ions from water, removing mercury from contaminated waters. Duwadi et al. [41] found that

Vasaka leaves are ideal for earthworm proliferation as they enhance the nitrogen content of compost materials. However, further research must confirm its role as a suitable material for vermicompost.

The plant supports various ecosystem services, including soil binding, regulation, and succession support [42]. For example, Hayyat et al. [38] investigated the role of *J. adhatoda* in the ecological restoration of degraded limestone mining lands. They observed substantial plant growth and accumulation of minerals such as Ca, Mg, and P without any soil improvement, highlighting its role as a bioengineer of degraded lands. In addition, the green manure of Vasaka leaves at the dose of 30 g kg^{-1} soil substantially suppresses the bacterial wilt disease of tomatoes, signifying the role of the plant in nature-friendly integrated disease management [43].

5. Pollination Mechanisms

The field of pollination biology investigates how plants achieve pollination and the significant impact of these events on fertilization, seed production, and the resulting progeny [44]. The flower of *J. adhatoda* shows geitonogamy (transfer of pollen grains from the anther to the stigma of a flower on the different branches but on the same plant); however, the geitonogamous flowers fail to set seeds, and cross-pollination is the only option for seed formation [45]. The flowers of *J. adhatoda* are reported to attract many visitor insects, either for pollen or nectar reward. However, the pollinating species vary geographically. For example, Shivanna [45] reported that the plant is mainly pollinated in southern India by two carpenter bees, *Xylocopa verticalis* and *Xylocopa* sp. However, Berry et al. [16] found that the honeybee *Apis dorsata* is the chief pollinating agent for *J. adhatoda* growing in the semi-arid environment of Rajasthan. The discrepancies in pollinating agents could be linked to variations in flower shape and size caused by different environmental conditions. To ensure cross-pollination, the flowers exhibit self-incompatibility and protandry (maturation of anthers when stigma becomes receptive). For successful germination and population establishment, the viability and vigor of pollen grains is essential. Viability refers to the ability of the pollens to transfer viable sperm cells to the embryo sac after suitable pollination. In this context, Wagh and Bathe [46] assessed the pollen viability of *J. adhatoda*. They observed that the plant showed a good fertilization potential, with pollen viability ranging between 73.26% with acetocarmine and 80.11% with the TTC (2,3,5 triphenyl tetrazolium chloride) test, respectively.

6. Phytochemistry

Chief Constituents

Given its high medicinal importance, various researchers have extensively investigated the plant for its chemical profile. All plant parts, including leaves, roots, stems, flowers, fruits, and seeds, yield several bioactive compounds [5,11,20]. However, a majority of bioactive phytochemicals are restricted to the leaves and roots. More than 233 phytochemical components have been identified, including 33 alkaloids, 12 flavonoids, 47 essential oils, 14 phenolic compounds, 23 terpenes and steroids, 47 organic acids, and 59 other substances [5]. Furthermore, the plant contains various macro- and micro-minerals such as calcium (Ca), copper (Cu), iron (Fe), potassium (K), zinc (Zn), manganese (Mn), vanadium (V), and chromium (Cr), with calcium showing the highest concentration [5]. Additionally, the plant comprises fatty alcohol, alkylamine alkenes, acetylene, naphthalene, and naphthoquinone hydrocarbons [5]. The plant is a rich source of quinazoline alkaloids such as vasicine, vasicinone, adhatonine, and anisotine, which are responsible for their medicinal properties [5,11,20,47]. In addition, several compounds like dibutyl phthalate, octadecadienoic acid, butylated hydroxytoluene, tannic acid, and hydroquinone have been exclusively isolated from its flowers. Some of the chief constituents of the plant are depicted in Figure 3. These compounds have shown promise in drug development by targeting specific molecular pathways. For instance, anisotine and vasicinone act as inhibitors of viral proteases, making them strong candidates for antiviral therapies. Other phytochemicals, such as phytol and amrinone, possess antioxidant and cardiotonic properties. This highlights the significance of these plants in modern pharmacology [6].

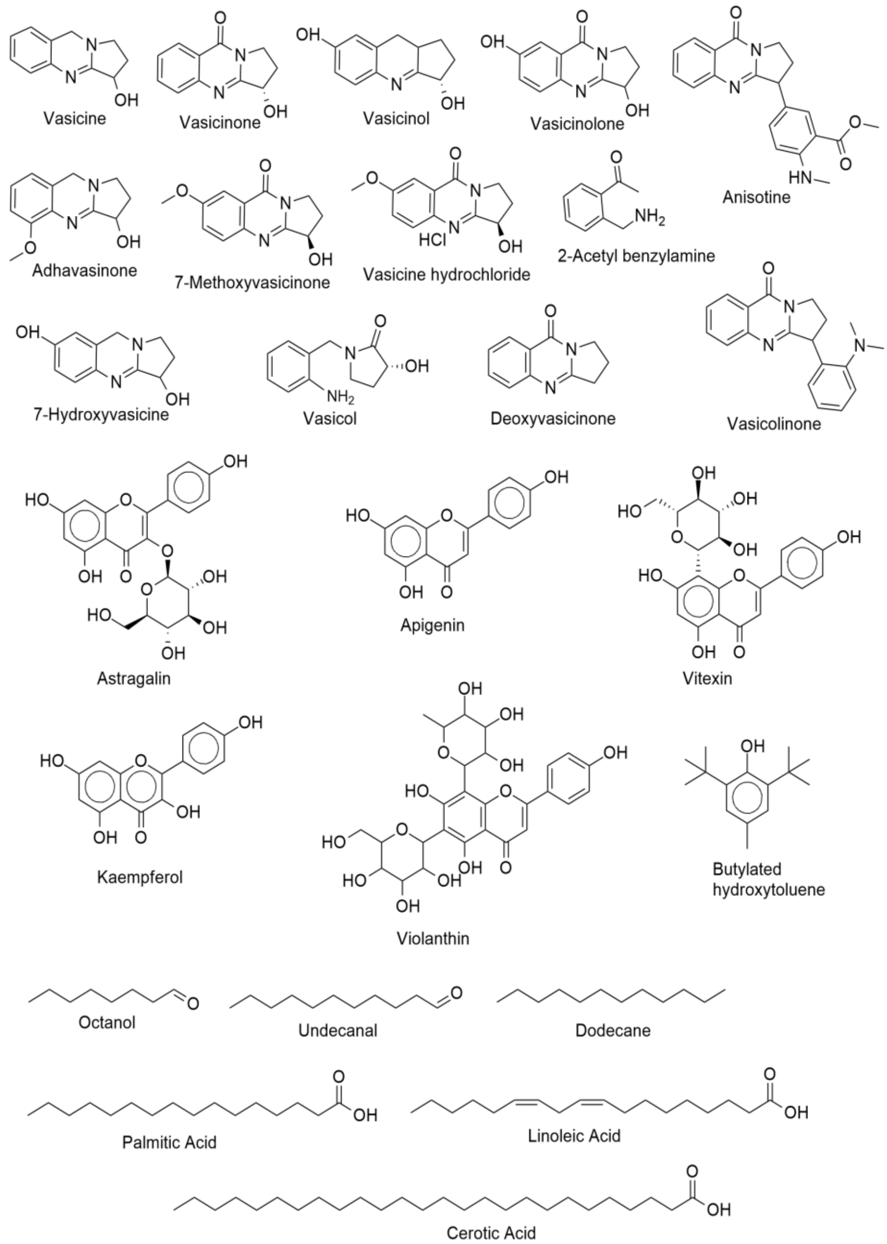

Figure 3. Some of the chief phytoconstituents of *Justicia adhatoda* (Produced by Authors) [5,20,48].

Notably, the roots of *J. adhatoda* are rich in lipids, protein, vitamin C, and vasicine. In contrast, the leaves exhibit minimal levels of these chemicals, barring sugar, vasicinone, fibre, Zn, and Fe [48]. The seeds of Vasaka are a rich source of several fatty acid compounds such as Behenic acid, Arachidic acid, and Linoleic acid [20,47]. A recent study by Maddineni et al. [49], using cold methanolic extracts of *J. adhatoda* leaves, revealed the presence of novel chemicals such as amrinone, arsenous acid, silicic acid, methyl tris (trimethyl siloxy) silane, and tetra siloxane decamethyl. Each of these novel drugs has demonstrated therapeutic efficacy. A comprehensive list of reported phytoconstituents in *J. adhatoda* is given in

Supplementary Table S1 [5,20,47,50–70]. Several factors, including geographic and climatic variations and the plant's developmental stage, significantly impact medicinal plants' proportion of bioactive constituents [71]. For example, a study conducted by Pandey et al. [72] observed that the plant, upon exposure to elevated UV-B radiations, synthesized some new compounds such as oridonin oxide (diterpene) and α-Bisabolol oxide-B (sesquiterpene). In contrast, a decrease in saturated fatty acids and other sesquiterpenes was observed.

7. Pharmacological Activities

The plant contains various chemical components contributing to its diverse pharmacological effects [5,7,11]. One of its primary alkaloids, quinazoline, is extensively extracted from the leaves and other plant parts [5,11,20,47,48]. Another significant alkaloid in Vasaka is vasicine, which has various physiological effects on the human body and potential medical applications [73]. Chirality, the property of molecules that makes them non-superimposable mirror images, plays an essential role in novel drug discovery [7]. The effectiveness of pharmaceuticals is determined by their interaction with biological targets such as proteins and nucleic acids [7]. Research has shown that the active form of a chiral drug is used to treat specific illnesses, while the inactive and potentially harmful form remains in the body. As a result, chirality significantly influences a drug's effectiveness. Phytochemical analysis has identified 27 chiral components in the leaves of *J. adhatoda*. Also, GC-MS analysis of the crude methanolic extract of *J. adhatoda* leaf has found several chemical compounds with different biological effects [7].

Various researchers have ascertained the multifaceted properties of *J. adhatoda*, including anti-bacterial, anti-fungal, antiasthmatic, antihistaminic, anti-inflammatory, antiulcer, antioxidative, antitubercular, antitussive, larvicidal, anti-Alzheimer, anthelmintic, anticancer, wound healing, and hepatoprotective effects [5,20,47,48,66,71,72].

7.1. Anti-Bacterial Activity

One of the most pressing issues facing the world today is antibiotic resistance. The discovery of novel antimicrobials in plant extracts offers promise in addressing this problem. For example, *Streptomyces aureus*, a bacterium resistant to antibiotics such as tetracyclines, gentamicin, macrolides, and lincosamides, was once susceptible to these treatments [74]. Disc diffusion studies have shown that leaf petroleum ether and ethanolic leaf extracts of *J. adhatoda* exhibit potent anti-bacterial properties against various microorganisms, including *Vibrio cholera* and *Bacillus subtilis* [75]. Additionally, research indicates that leaf extracts in various solvents demonstrate vigorous anti-bacterial activity against bacteria such as *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas eugeniae*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* [76].

Furthermore, the biological characteristics of zinc oxide nanoparticles can be improved by bi-metal doping (silver and gold), highlighting the plant's potential as an effective anti-bacterial agent [77]. Alcoholic extracts of the plant have been found to significantly impact antibiotic-resistant bacteria, such as methicillin-resistant *S. aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Klebsiella pneumoniae* [78]. Moreover, the *J. adhatoda* leaf extract, which contains alkaloids, exhibits cytotoxic and antimicrobial properties. For example, vasicine acetate, a component obtained by acetylation of vasicine, inhibits *Enterobacter aerogenes*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*, with a minimum inhibitory concentration range of 125 µg/mL against each bacterial species [67]. Similarly, Shahwar et al. [79] observed the anti-bacterial efficacy of vasicine against *Salmonella typhimurium* with 8 mm zone of inhibition. A recent study conducted by Nithya et al. [80] using disc diffusion assay, showed that cerium oxide-loaded silver and gold nanoparticles synthesized from *J. adhatoda* leaf extract displayed the highest zone of inhibition (29 mm and 32 mm at 100 mg/mL) against strains of *S. aureus* and *E. coli.* Similarly, Bose and Chatterjee [81] found that silver nanoparticles significantly inhibit the growth of *Pseudomonas aeruginosa* (zone of inhibition at 7–9 mm). In contrast, no inhibitory effect of raw leaf extract was found on bacterial growth. Additionally, the molecular docking approach can utilize natural alkaloids from *J. adhatoda* as inhibitory agents against the beta-ketoacyl synthase III enzyme (mtFabH) of *Mycobacterium tuberculosis*. The alkaloids in *J. adhatoda* demonstrate inhibitory activity towards mtFabH, indicating their potential as suitable tuberculosis inhibitors [68]. However, further studies are required to support the current findings.

7.2. Anti-Cancerous Activity

The anti-cancer potential of *J. adhatoda* is linked to compounds like l-vasicinone, deoxyvasicine, maiontone, vasicinolone, and vasicinol, all of which have been widely studied for their broad pharmacological activities [44,71]. For example, Duraipandiyan et al. [67] synthesized vasicine acetate from vasicine (a novel alkaloid of Vasaka) that showed a substantial cytotoxic effect against A549 lung adenocarcinoma cancer cell lines under in vitro conditions. In another study, Balachandran et al. [82] isolated a novel alkaloid 2-acetyl-benzylamine from the leaves of *J. adhatoda*. The compound showed suitable cytotoxic activities against MOLM-14 and NB-4 cells by arresting the cell cycle at the G2/M phase in the former and the G0/G1 phase in the latter. Nikhita et al. [83] found the antimetastatic activity of ethanol leaf extract against ovarian PA1 cell lines in mice. They observed that the plant extract reactivates the expression of tumor-suppressing p53 and p21 genes. However, further studies are required under in vivo conditions to ascertain the efficacy in humans.

Similarly, Kumar et al. [71] found a cytotoxic effect of methanolic leaf extract on human breast cancer cell lines. The extract-treated cells showed increased production of NO and ROS, which damage cancer cells. In addition, extract-treated cells stop migration, colony formation, and arrests at the sub-G0 phase of the cell cycle. Thus, the plant *J. adhatoda* possesses excellent potential for developing non-toxic and cost-effective natural therapies to fight against the deadly cancer disease.

7.3. Anti-Diabetic Activity

Diabetes, also referred to as diabetes mellitus, is a metabolic disorder that has been becoming more prevalent over the past few years [5]. The potential role of *J. adhatoda* as an anti-diabetic agent has been explored by researchers globally. For example, Gulfraz et al. [84] observed that after administering the dose of 50 mg/kg and 100 mg/kg leaf and root extract of *J. adhatoda*, a significant reduction in blood glucose level was observed in alloxan-induced diabetic mice. Gao et al. [69] observed the inhibitory effect of the methanolic extract of *J. adhatoda* leaves on α-glucosidase activity. The inhibitory effect was due to two quinazoline alkaloids, such as vasicine and vasicinol, showing high sucrase inhibitory activity, with IC50 values of 125 lM and 250 lM, respectively. However, the observed results were not comparable to the commercial α-glucosidase inhibitors. A recent study by Ameer et al. [85] investigated the anti-diabetic potential of different leaf extracts (methanol, ethanol, and ethyl alcohol) in mice. Their results showed that the administration of 400 mg/kg methanol extract for 28 days showed the best results by reducing blood glucose levels. Various alkaloids, phenols, flavonoids, and other bioactive compounds could be attributed to its hypoglycemic activities.

7.4. Anti-Fungal Activity

According to Pa and Mathew [86], methanolic leaf extracts of *J. adhatoda* showed significant anti-fungal activity against *Aspergillus flavus*, *Candida albicans*, and *Cryptococcus neoformans*, with zones of inhibition varying from 9.6 mm, 14.8 mm, and 10.9 mm, respectively. Furthermore, they also inferred that *C. albicans* was highly sensitive to methanolic leaf extract compared to the standard anti-fungal drug Nystatin. In a recent study, Vazhacharickal et al. [87] synthesized copper and zinc nanoparticles using a leaf extract of *J. adhatoda*. The nanoparticles showed more significant anti-fungal activity against the fungal pathogen *Fusarum oxysporum*.

7.5. Anthelmintic Activity

The efficacy of *J. adhatoda* as an anthelmintic agent against gastrointestinal nematodes in sheep was investigated by Al-Shaibani et al. [88]. The plant's aqueous and ethanolic extracts were assessed using assays for egg hatching and larval development [88]. Compared to the other, the ethanolic extract performed better. At 800 mg/kg, the plant extract exhibits anthelmintic or anticestodal activity. The extract shows a significant decline in the recovery rate for the juvenile worms. The root powder has the most remarkable effects in vitro; however, the aqueous and methanolic extracts exhibit significant inhibitory activity. Each model's anthelmintic activity is directed against nematodes [89].

7.6. Antioxidant Activity

Products of the various chain reaction mechanisms that result from free radicals are known as antioxidants [79]. Previous studies have reported a more significant antioxidant potential of *J. adhatoda* leaves. For example, Rao et al. [90] determined the high antioxidant potential of the methanolic leaf extract of *J. adhatoda* using several assays such as 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), reducing power potential, and iron chelating activity. The presence of high levels of polyphenolic compounds, primarily flavonoids and phenolic acids, is probably responsible for the antioxidant potential of *J. adhatoda* [90,91]. Another method of estimating the antioxidant activity is using oxidative parameters such as reduced glutathione, liver lipid peroxidase enzyme, and DPPH, as well as the 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). A study conducted by Padmaja et al. [92] found that the plant's aqueous leaf extract possesses the ability to scavenge hydroxyl and DPPH radicals in the Fe^{3+}/ascorbate/EDTA/H_2O_2 system, lipid peroxidation inhibition brought on by $FeSO_4$ in egg yolk, and the activity of metal chelation by in vitro assessment. Their results showed that the total antioxidant and reducing power activity in *J. adhatoda* leaves extract was 83.7 $\pm$ 1.76 AAE/g and 40.3 $\pm$ 0.288 Vit E/g, respectively. Additionally, the shoot tissue cultures of *J. adhatoda* when grown on a supplemented medium (Murashige and Skoog), accumulated compounds such as vasicine and vasicinone that exhibited the highest DPPH radical scavenging activity in the water extracts [70]. In another study conducted by Shahwar et al. [79], it was found that vasicine exhibited a concentration-dependent increase in reduced ferric ion in the ferric reducing ability of plasma assay and considerable free radical scavenging activity in the DPPH assay, contributing to its antioxidant properties.

7.7. Antiviral Activity

The plant's aqueous and methanolic extracts exhibit antiviral solid action against the influenza virus by preventing the virus from attaching to the host surface and replicating. As a result, we may state that it has a prophylactic application in managing viral infection [93]. Gheware et al. [94] observed that the whole plant aqueous extract of *J. adhatoda* modulates hypoxic conditions in mice lungs by reducing the expression of HIF-1α, TGF-β, and collagen proteins, thus reducing the disease severity. In another study, Ghosh et al. [95], using molecular approaches, identified an alkaloid anisotine that strongly inhibits the proteolytic activity of SARS-CoV-2 main protease MPro. However, the efficacy of this compound needs to be tested under in vivo conditions. Verma et al. [96] recently elucidated the influential role of *J. adhatoda* against the SARS-CoV-2 (COVID-19) virus. They observed that when patients with mild COVID-19 symptoms were administered 500 mg of *J. adhatoda* and *Tinospora cordifolia* herbal drugs, the viral symptoms cleared away in 13.92 days and 13.44 days, respectively. However, when *J. adhatoda* and *Tinospora cordifolia* drug combinations were administered (250 mg each), the mean viral clearance time was 11.86 days without any side effects. This highlights the role of Vasaka as a potent substrate for novel drug development against global pandemics like COVID-19.

7.8. Phytotoxic and Insecticidal Properties

A study by Khan et al. [97] investigated the phytotoxic effect of *J. adhatoda* leaves on several plant species. They found that the methanolic leaf extract significantly inhibits the growth of roots and shoots, as well as seed germination of monocots such as foxtail millet and barnyard grass when compared to dicots like cauliflower, broccoli, and tomato. Matharu and Mehta [98] studied the insecticidal activity of a 5% hexane leaf extract from *J. adhatoda* on tomato fruit borer (*Helicoverpa armigera*). They found that the extract caused 36.41% mortality of the tomato fruit borer. The extracted plants from *J. adhatoda* showed 70–89% inhibition of shoot growth and over 90% inhibition of root growth [99]. In another study, Budhathoki et al. [100] reported that Vasaka extracts had antifeedant, larvicidal, suicidal, and ovicidal effects against the field pest *Spodoptera litura*. The study also revealed that at a 5% extract concentration, *J. adhatoda* ethanol extracts had the highest antifeedant action, effectively reducing the feeding rate of *S. litura* larvae.

Thanigaivel et al. [101] reported that *J. adhatoda* leaf extract substantially suppresses the growth of the Dengue mosquito (Aedes aegypti). The methanolic leaf extract damages the insect midgut cells by inhibiting antioxidant enzymes such as glutathione-Stransferase, superoxide dismutase, cytochrome P450, and α- and β-esterases, which protect the insect cells from metabolic damage.

8. Ethnomedicinal Importance

In Ayurvedic and Unani medicine systems, the plant has been used for thousands of years [102]. According to the WHO manual "The Use of Traditional Medicine in Primary Health Care", the plant is frequently used to treat diseases related to the bronchial system and bleeding piles [103]. A more significant number of studies in the literature revealed its effectiveness in treating respiratory conditions such as asthma, bronchitis, cough, and cold symptoms [11]. The leaf extract contains oxytocin and stimulates uterine contraction during childbirth in India. According to a survey, 70% of expectant women in Gora village, Lucknow, had previously used the leaves of *J. adhatoda* orally to induce abortions [104]. However, the abortifacient property of its leaves is subjected to scientific validation. The leaf extract is used in various formulations to treat skin diseases, peptic ulcers, diarrhea, dysentery, malarial fever, jaundice, and headache [5]. Apart from the leaves, the flowers and roots of Vasaka are also essential components of various herbal remedies and dietary supplements aimed at enhancing the immune system, aiding in weight management, and providing respiratory relief.

In several parts of India, the bark and wood decoction are used to cure asthma. The leaves and flowers of Vasaka are also consumed as vegetables by locals in India and Nepal [11,105]. Leaf decoction of Vasaka, along with mustard oil, is used to relieve joint pain in the Hamirpur district of Himachal Pradesh, India [106]. Leaves and roots are used by the local people of Himachal Pradesh, India, to cure rheumatism [2]. The study of Claeson et al. [11] provides detailed information about the ethnomedicinal uses of *J. adhatoda*.

9. Cytology

Various researchers investigate the karyomorphological details of *J. adhatoda*. However, in most studies, only the meiotic chromosomal details are evaluated. The reported meiotic chromosome number in the plant ranges from n = 17 to 2n = 34 [11,107–109]. However, Datta and Maiti [110] observed some variations in chromosome number (2n = 40, 46, 50) among specimens collected from different locations in West Bengal.

10. Genetics and Molecular Insights

Knowledge about genetically diverse populations of a species is essential not only to understand its evolutionary history but also for its efficient conservation and management. The greater the geographical distribution of a species, the higher the chances of variability in its gene flow and adaptation to a wide range of environmental conditions. This is probably attributed to its substantial genetic diversity, given that *J. adhatoda* has adapted to various climatic conditions. Only some studies have attempted to evaluate the genetic variations in its wild populations. For example, a study by Kumar et al. [111] studied genetic divergence in *J. adhatoda* using RAPD and ISSR markers on populations from different localities and climates across eastern and northern India. Their results showed a high level of genetic diversity, with polymorphism percentages ranging between 94.2 and 92 for RAPD and ISSR markers, respectively.

Furthermore, they observed that the specimens collected from hilly regions showed substantial genetic variations compared to the ones from plains, emphasizing the role of environmental variables between populations [111]. In another study conducted in Pakistan, Gilani et al. [10] used P-450-based analog (PBA) markers and found 80% polymorphism in different populations of *J. adhatoda*. Garg et al. [112] evaluated genetic diversity using RAPD markers among different populations of *J. adhatoda*. They observed that out of the 20 primers used for genome amplification, five primers (OPA-06, OPA-07, OPA-11, OPA-12, OPA-18) were solely responsible for 94% of DNA polymorphism, showing high genetic diversity among accessions. They attributed the role of altitude and geographic locations to higher genetic diversity [112].

11. Conservation Status

Due to its higher significance in traditional medicine, *J. adhatoda* has gained enormous attention from pharmaceutical industries to prepare drugs. In addition, the plant is widely collected by herbalists/local practitioners to treat various diseases [15,18]. This has put immense pressure on the plant's natural populations. Several other factors may also be responsible for declining populations, such as the growing demand for land for industries, urbanization, agriculture [19], habitat fragmentation, and competition from other species.

Biotechnological Approaches for Conservation

Mass multiplication of the plant is required to maintain a viable supply of plant germplasm to local people and industries. Given that *J. adhatoda* has a low seed set formation and germination rate and artificial propagation through stem cuttings is time-consuming, biotechnological applications (in vitro propagation) provide an efficient tool to multiply its germplasm rapidly. Several studies have investigated the in vitro regeneration potential of *J. adhatoda* using various plant parts such as young leaves, nodal segments, and shoot tips [113–115] as potential explants. In addition, a few studies have induced in vitro direct organogenesis and callus formation [116–118]. Recently, a study by Rathour et al. [119] developed synthetic seeds from the shoot tips culture of *J. adhatoda*. They employed a slow-growth approach (storing encapsulated shoots under reduced nutrient levels for 60 days). They observed that after storage, 50% of the synthetic seeds could grow into plantlets, thus providing a vital approach for the short-term storage of plant germplasm and its transportation to distant places. Apart from germ conservation, biotechnological approaches can enhance secondary metabolite production in micro-propagated plants. For example, a study by Bhambhani et al. [120] investigated the role of an elicitation technique on secondary metabolite (vasicine) production in *J. adhatoda* cell cultures. They observed a 3.7- and 3.2-fold higher vasicine production in leaf cell cultures of *J. adhatoda* when it was provided with elicitor molecules such as 20 lM Methyl Jasmonate and 50 mg L^{-1} yeast extract, respectively. Similarly, in another study, Singh et al. [121] found that by feeding root cell cultures of Vasaka with elicitors such as tryptophan and sorbitol, the pyrroloquinazoline alkaloids, i.e., vasicinone and vasicine, showed a 12- and 8-fold higher production.

12. Conclusions and Knowledge Gaps

In traditional medicines, *J. adhatoda* has been used to cure various ailments since time immemorial. The efficacy of traditional claims has been validated scientifically as the different plant parts exhibit pharmacological properties such as anti-bacterial, anti-fungal, anti-inflammatory, anti-cancerous, insecticidal, and phytotoxicity. The recent advancement in nanotechnology offers new approaches to unravel its therapeutic potential. Overall, this plant has a novel potential for pharmacological research and drug development in future scenarios. At the same time, given its medicinal value and industrial demand for research and drug development, its natural populations have declined at specific locations. However, recent advancements in vitro propagation and synthetic seed formation are vital for germplasm conservation.

A growing body of literature on *J. adhatoda* is restricted to its phytochemistry, ethnomedicinal uses, and pharmacological importance. However, the ecological status of the plant remains unexplored. Despite its significant medicinal value, there are still key knowledge gaps regarding *J. adhatoda*:

1. The ecological impact of *J. adhatoda* is not well-studied, particularly its role in vegetation homogenization and allelopathic interactions with other species;
2. There is limited information on how this plant affects soil health, including its influence on nutrient composition and physicochemical properties;
3. Its classification as a native invader, which leads to the formation of monospecific thickets, necessitates further ecological studies to better understand its impact on surrounding ecosystems.

Future research should address these gaps to optimize the plant's pharmacological potential and ecological sustainability.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/conservation5010002/s1, Table S1: A list of key constituents isolated from various parts of *Justicia adhatoda* and their pharmacological activities.

Author Contributions: Conceptualization, P.K. and A.N.S.; methodology, I. and P.K.; software, I.; validation, P.K. and A.N.S.; formal analysis, I., P.K. and A.N.S.; investigation, I. and P.K.; resources, I. and P.K.; data curation, I. and P.K.; writing—original draft preparation, I. and P.K.; writing—review and editing, I., P.K. and A.N.S.; supervision, A.N.S. All authors have read and agreed to the published version of the manuscript.

Funding: The first author acknowledges the funding from the University Grants Commission (UGC), Govt. of India, New Delhi, as a Junior Research Fellowship (NTA Ref. No.: 221610214193/CSIR-UGC NET June 2022).

Acknowledgments: The authors would like to thank the Chairperson of the Department of Botany, Panjab University Chandigarh, for providing the necessary research facilities.

Conflicts of Interest: The authors declare no conflicts of interest.

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