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A review on critically endangered species of Acanthacea: *Justicia beddomei* (Clarke) bennet: An immune booster

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Abstract
Justicia beddomei (Clarke) Bennet, a member of Acanthaceae, is endemic to the Southern Western Ghats. This plant shows remarkable similarities with *J. adhatoda* L., and the only morphological difference is the smaller size of both leaves and inflorescence. *J. beddomei* has an abundance of phytochemicals and widely used in traditional medicinal systems such as *Ayurveda, Siddha* and *Unani*. The phytochemicals present in the spewcies possess immense anti-bacterial, cytotoxic, anthelmintic, analgesic, antioxidant activities. Vasicoline, a major phytochemical proved for the treatment for Covid 19. At present, the plant is listed under IUCN Red List as Critically Endangered category. Protecting species from extinction, enhancing ecosystem services and protecting biological diversity are important for maintaining a healthy ecosystem. The review reveals the importance of conservation of this plant for the fitness of the ecosystem and the development of traditional medicine for the future.

Keywords: *Adathodai*, critically endangered, conservation, substitute, *Vasaka*

Introduction
India's traditional medicinal system flourished because of the diversity and abundance of medicinal plants. These plants and their derived parts play a key role in the treatment of several ailments of human beings. Nearly 1500 and 1200 species of plants are used in drug preparation for *Ayurveda* and *Siddha* respectively [1]. Acanthaceae is a large family of dicotyledonous plants comprising of more than 4300 species and distributed worldwide having a lot of medicinally important plants [2]. The largest member of this family is *Justicia* which comprises about more than 600 species distributed in the tropical and pantropical regions, nearly 50 species occur in India and are distributed in the temperate regions [3, 5].
J. beddomei is endemic to the Southern Western Ghats in locations like Kerala, Valparai (South Arcot), Akkamalai (Coimbatore) and Mahendragiri (Kanniyakumari) [6]. It is included in the IUCN Red List category as Critically Endangered Species [7]. The plants are commonly known as Malabar Nut because of the resemblance with *J. adhatoda*. It is morphologically similar to *J. adhatoda* and the only visible difference is the smaller size of leaves and terminal spike inflorescence with more tailed anthers [8]. *J. beddomei*, very commonly used in the traditional medicinal systems like *Ayurveda*, *Siddha* and *Unani* as diuretic, antispasmodic, expectorant, anti-asthmatic, febrifuge, styptic and tonic [9]. The leaves are very effective for the treatment of irritable cough, diarrhoea and haemoptysis [10]. The medicinal properties of plants are broadly used for the treatment of leprosy, blood disorders, heart troubles, thirst, fever, vomiting, cough, asthma [11], diseases of eyes, bleeding diarrhoea, dysentery, bronchitis, inflammation, jaundice, tumours, mouth-troubles, sore-eye, gonorrhoea, tuberculosis, haemorrhage and haemorrhoids [12].
The prevailing status of this plant reveals that the unconstrained use of natural products inversely affects the balance of the ecosystem. Crucial conservation of the ecosystem is important for the healthy existence of each member.

Scientific classification
Kingdom: Plantae
Division: Angiosperms

Class: Asterids
Order: Lamiales
Family: Acanthaceae
Genus: *Justicia*
Species: *J. beddomei*

Common names
English: Malbar Nut
Hindi: Adusa, arusa
Kannada: Sann-adusoge, aadusoge, addalasa, addasara, byaaladamara, vaasa
Malayalam: Cheriyaatalotakam, cittatalotakam
Sanskrit:Vasa, vrsah
Tamil: Adutota, cittadalodakam
Telugu: Addasaramu

Habit
These are large diffusely branched shrubs wit cylindrical striated stem with swollen nodes. The simple leaves arranged opposite and nearly 5 to 10 cm long and 3 to 4 cm wide and glabrous; apex ovate to acuminate with entire margin; base acute and petiole short; 8 paired main nerves arranged laterally.
The flowers are small terminal spikes; calyx with 5 sepals of 5 mm in length; petals 1.25 cm long with 2 lips; pubescent outside and dull white in colour; upper lip emarginated and deflexed lower lip with 3 lobes; 4 stamens with hairy filaments at the base; 2 celled ovary and each cell with 2 ovules; style pubescent. Fruit a clavate capsule with a long solid base and the seeds 1 or 2, suborbicular, compressed and rugose [13, 14].

Pharmacognosy
Pharmacognostical studies are the easiest way to sort out morphologically similar adulterant/substitute plants used in the medicinal system. *J. beddomei* and *J. adhatoda* are morphologically similar and the one who is unaware of plants will misidentify these two. The characteristic features of the family Acanthaceae are the presence of cystoliths in leaf lamina and petiole. But in *J. beddomei* these characteristic cystoliths are absent[15]. There is no further anatomical or pharmacognostical studies about this plant reported yet.

Phytochemicals
Preliminary phytochemical analysis of the plant *J. beddomei* reported various types of phytochemicals. The phytochemical investigation of different parts revealed the presence of the bioactive compounds like alkaloids, tannins, disaccharides, flavonoids, phenolics and glycosides [16, 17]. The important biologically active chemicals and their properties are tabulated in Table 1. The chemicals in which detailed pharmacological studies are not carried out are 7-octadecyne; 2-methyl, 9,12,15-octadecatrienal; *β*-sophoroside; anisotine; hexadecanoic acid, ethyl ester; phosphoric acid, diethyl pentyl ester; flavonoids like luteolin; alkane like tritriacontane; quinazoline alkaloid like adhavasinone, deoxyvasicinone, vasakin, vasicinine, vasicinol, vasicinolone, vasicol, vasicolinone, vasicolone and Vasicine[18-21].

Table 1: Chemicals and properties of *Justicia beddomei* (Clarke) Bennet

SN	Chemicals	Chemical formula	Property
1.	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl ester) [21]	$C_{16}H_{22}O_4$	Cytotoxic [22]
2.	Adhatodic acid [18]	-	Tuberculosis, sore throat [23], expectorant, bronchodilator, antibacterial [24], antiasthmatic [25]
3.	Adhatodine [18]	$C_{20}H_{21}N_3O_2$	Anti-malarial[26]
5.	Aminophylline [18]	$C_{16}H_{24}N_{10}O_4$	Asthma or other chronic lung diseases like chronic bronchitis and emphysema, prevent apnea in preterm infants [27].
6.	Campesterol [21]	$C_{28}H_{48}O$	Lowering LDLs and cholesterol [28]
7.	Carotene [18]	$C_{40}H_x$	Precursor of vitamin A [29], anti-cancer [30], antioxidant [31]
9.	Kaempferol [18]	$C_{15}H_{10}O_6$	Antioxidant by reducing oxidative stress, antibacterial agent, human xenobiotic metabolite, blood serum metabolite, urinary metabolite and currently under consideration as a possible cancer treatment [32]
10.	Lupeol [18]	$C_{30}H_{50}O$	Anti-cancerous [33]
11.	O-Ethyl S-2-dimethylaminoethyl methylphosphonothiolate [21]	$C_7H_{18}NO_2PS$	Used as a quick-acting military chemical nerve agent [34].
12.	Phytol [21]	$C_{20}H_{40}O$	Antinociceptive, antioxidant[35], anti-inflammatory, anti-allergic [36], immunostimulant, activation of both innate and acquired immunity [37].
13.	Squalene [21]	$C_{30}H_{50}$	Advantages for the skin as an emollient and antioxidant, and for hydration and its antitumor activities [38]
14.	Stigmasterol [21]	$C_{29}H_{48}O$	Maintain the structure and physiology of cell membrane [39], lowering the levels of LDLs [40]
15.	Vasicine (peganine) [19]	$C_{11}H_{12}N_2O$	Bronchodilator activity *in-vitro* and *in vivo* [41], uterine stimulant, respiratory stimulant, cardiac depressant (combined with vasicinone) [42]
16.	Vasicinone [20]	$C_{11}H_{10}N_2O_2$	Bronchodilatory (*in vitro)* bronchoconstictory (*in vivo*) [43], antianaphyactic [44]
17.	Vasicoline [18]	$C_{19}H_{21}N_3$	Effect for the treatment for COVID 19, diseases related to respiratory problems [45]
18.	Vitamin E [21]	$C_{29}H_{50}O_2$	Antioxidant [46]
19.	Vitamin C [18]	$C_6H_8O_6$	Antioxidant [47]
20.	*β-Sitosterol* [19]	$C_{29}H_{50}O$	Antiinflammatory[48], chemoprotective [49], hypocholesterolemic [50], immunomodulatory [51]

Pharmacological activities
J. beddomei has a predominant role in the traditional medical systems in India. The pharmacological activities are studied for the further development of drug research. The following activities of the plant are studied.

Anthelmintic activity
The antihelminthic activity of ethanolic as well as chloroform extract of *J. beddomei* leaves was tested against Indian earthworms. Different doses of 10 mg/ml, 20 mg/ml and 50 mg/ml of each extract were tested and compared with the

standard drug Piperazine citrate. The result observed that the death/paralysis of the worms increased with increasing concentration. Effectiveness of the extracts was inversely proportional to the time taken for paralysis of the worms. The 50 mg/ml ethanolic extract was more efficient than the chloroform extract for the killing of worms. The current study suggested that the ethanolic extract may be effective against the worms in human [52].

Analgesic activity

The analgesic activity of different concentration of ethanolic extract of *J. beddomei* leaves was evaluated in albino rats using Eddy's hot plate method [54]. The activity of the 100 mg/kg and 50 mg/kg was compared with the standard 15 mg/kg morphine sulphate. The result showed that the 90 minutes administration of test extract of 100 mg/kg possesses significant analgesic activity. Due to the presence of alkaloids, carbohydrates and tannins, the extract engenders noticeable analgesic effect. The exact modes of action of the biologically active compound responsible for the activity are not studied, which minimise the efficacy of the results[53].

Antioxidant activity

The powdered aerial parts of *J.beddomei* were extracted using petroleum ether, chloroform, ethyl acetate and methanol. Standard protocols followed for screening the preliminary phytochemicals. The tests like DPPH, hydroxyl radical, superoxide anion radical scavenging abilities, β-carotene-linoleic acid model, reducing power ability, nitric oxide scavenging assay of all the extracts were evaluated for analyzing their potential antioxidant activities. The results were compared with ascorbic acid, Butylated hydroxytoluene and catechin standards and the concentration and efficacy were directly proportionate. Because of the presence of phenolic and flavonoid, all the extracts showed strong antioxidant activity [16, 21].

Anti-cancer and XOI activities

In vitro anticancer and xanthine oxidase inhibitory (XOI) activities were investigated with the methanolic extract of dried aerial parts of *J. beddomei* which was compared with the standard 2.4-40 µg/ml Allopurinol. The extract was exposed to MTT colourimetric assay in HeLa and MCF-7 cell lines for XOI activity and cytotoxic activity. Increased dosage of the methanolic extract showed increased anticancer and XOI activities (200 µg/ml and 40 µg/ml respectively). The presence of flavonoids and phenolic compounds of the extract contributed towards the inhibitory activities against cancer and Xanthine Oxides [54].

Anti-diabetic activities

Whole plant ethanolic extract induced to the alloxan-induced diabetic rats showed a reduction of diabetes in rats. Further studies needed for the identification of specific phytochemicals involved in this [55].

Cytotoxic activity

Endophytes are known for their cytotoxic activities. The endophytes found in *J. beddomei* were tested for its cytotoxicity. Ethyl extracts of the plant *J. beddomei* and its endophytic fungi showed cytotoxic activity. The preliminary phytochemicals were screened out and the MTT assay of the extract was carried out on lung adenocarcinoma cells. The results revealed that the bioactivity was three times than that of the host plant. Endophytes are known for the production of novel secondary metabolites with a broad spectrum of activity according to their host. *Aspergillus fumigates* found in *J. beddomei* increased the cytotoxicity [56].

Other activities

The pharmacologically active phytochemicals present in this plant revealed important activities like antipyretic, anti-inflammatory, anthelmintic, antiseptic, antidiabetic, blood coagulant, a bronchodilator, disinfectant, antioxidant, hepatoprotective, anti-jaundice, expectorant and has many other medicinal applications [57, 60]. The unavailability and the increasing demand of the plant may have reduced further studies.

Molecular studies

The morphological characters of *J. adhatoda* and *J. beddomei* are almost similar. It is very difficult to distinguish them based on the taxonomic or phenotypic characters. Analyzing the molecular aspects is the correct identification strategies in such cases. PCR-RFLP of selected nuclear ribosomal ITS amplicon along with sequence variability was used for the molecular studies. The already sequenced *J. adhatoda* was reported in NCBI as 687 bp and the direct sequencing of the gel-purified ITS amplicon yielded a 624 bp sequence for *J. beddomei*. It is clear that through the phylogenetic tree *J. beddomei* and *J. adhatoda* were sister groups and distinct species with common ancestors. The ITS sequences of *J. beddomei* and *J. adhatoda* contained unique recognition sites for specific restriction enzymes for which all the species were distinct in their PCR-RFLP patterns. When the ITS amplicons of the four selected *Justicia* species were subjected to restricted digestion with EcoRI or SfoI, it yielded the expected restricted products. The ITS sequences and PCR-RFLP were successful in resolving the ambiguity that existed among the species of *Justicia* [61].

Propagation

Seed germination and stem cutting are the main propagules for *J. beddomei.* Studies showed that seed germination and propagation through stem cutting are very low [62]. Micro-propagation ways like tissue culture are the easiest way to propagate these type of plants. Rapid propagation through nodal explants in MS medium supplemented with BAP achieved shoot multiplication. Increasing concentration of BAP resulted in an increase in shoot development and IAA and NAA concentration affected the root development. It was suggested that hardening of the plant in an organic supplemented soil environment will get the better result[63]. Clonal propagation of explants was achieved through callus free axillary meristem proliferation in SH medium from the stem node explants. Shoot multiplication increased by the result of cytokinin along with the synergetic effect of auxin. Five to 10 shoots was obtained in 5 to 6 weeks with the effect of 3.0 $mg.l^{-1}$ BAP, 0.5 $mg.l^{-1}$ 2-ip and 1.0 $mg.l^{-1}$ IAA. Rooting was obtained in the medium containing 0.2 $mg.l^{-1}$ IBA or IAA. Hardening the plants in humidity chamber showed 95% of survival rate. They flowered in 15 months with no cytological defects shows a good result of micropropagation [64].

Discussion

J. beddomei, the plants are medicinally very important and its presence is inevitable. The detailed pharmacological activities about the phytochemicals found in *J. beddomei* are absent or insufficient. Primary phytochemical studies revealed the

presence of several chemicals. Most of these chemicals are similar to *J. adhatoda*, which are efficient for the treatment of various disorders. Molecular studies revealed that these two plants emerged from a common ancestor, so shows the remarkable similarities. Recently in traditional medicine, *J. adhatoda* acquired its position. The unavailability of *J. beddomei* makes limited studies and restricted use in medicinal fields. The distribution of the plant is restricted at the elevation of 1000 m, overexploitation for the medicinal and research purpose leads to the rapid depletion of this important plant from its natural habitat[65]. From 1998 the plant is listed in IUCN Red List as endangered and again the count is reduced to become critically endangered[6,7]. The conservation of the plants is very important for the development of the traditional medicinal system. Recent studies show that the phytochemicals found in *J. beddomei* are used against viral infected diseases such as COVID 19. The propagation of the plants is very difficult, so we have to develop new propagation strategies like tissue culture. The ecosystem is balanced because of the equal distribution of flora and fauna. The involvement of human being makes a disturbed ecosystem which will adversely affect the whole. The plant *J. beddomei*, not used as an adulterant or substitute, shows its effect on the medicinal field confronts adverse riddles from the ecosystem. The extinction of these types of medicinally important plants will lead to developing new strategies to conserve them.

Conclusion

From the current review, we concluded that the conservation of *J. beddomei* could be useful for the development of commercial as well as traditional drugs on a detailed exploration of phytochemicals and pharmacological actions after needful mass cultivation is practised.

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