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A comprehensive review on a less explored medicinally important plant *Justicia betonica* L.

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ABSTRACT

Justicia betonica L., family Acanthaceae is a widely used traditional folk medicinal herb. It is a unique source of jusbetonin and the leaves yield bluish purple dye. Traditionally, the plant is used to cure constipation, diarrhea, malaria, orchitis, pain, snake bite, stomach ache, vomiting etc. The whole plant possess significant biologically active principles like steroids, triterpenoids, alkaloids, saponins, glycosides, carbohydrates, gum and mucilage, proteins, fixed oils and fat, phenolics and tannins. It is reported to exhibit antiviral, antioxidant, anti-inflammatory, analgesic and antimalarial activities. The intention of the study was to endow an overview of the ethnomedicinal properties, phytochemistry and related pharmacological applications of *J. betonica*, and to make an authenticated evidence base for further research on this important medicinal plant.

Keywords: *Justicia betonica*, White shrimp, Folk medicine, Chemical constituents, Biological activities

1. INTRODUCTION

Plants signified in enormous drugs production due to the synthesis of wide variety of secondary metabolites to protect themselves from various biological and non-biological factors (Wink, 2015). The Rig Veda (oldest manuscript on plants in India), written between 4000 and 6000 B.C. reveals that plants were known for medicinal practices to combat a number of dreadful diseases since time immemorial. The Indian traditional systems of medicines such as Ayurveda, Siddha, Unani, Tibetan and Homeopathy, talk about 2000 medicinal plant species. Around 70% of rural people and 40% of modern world directly or indirectly rely upon plant based herbal preparations in their day-to-day life (Pandey *et al.*, 2008; 2013). India is quiet unique in its biogeography by possessing extreme cold, dry cold deserts, temperate, alpine, subtropical, rain forests, wet evergreen humid tropics, arid and semi-arid, dry desert, tidal mangroves with rich in species, genetic and habitat diversity (Barbhuiya *et al.*, 2009).

The family Acanthaceae

Acanthaceae family comprised of herbs, shrubs and vines of 2500 species classified under 200 genera (Willis, 1966). Most of them are shrubs, tropical herbs or twining vines. Characteristic features of Acanthaceae are opposite pairs of leaves, presence of calcium carbonate crystals in vegetative parts, bisexual flowers, large and petaloid bracts, 4-5 petals and sepals, 2-4 stamens, staminodes, superior ovary with 2 fused ovules. The members of Acanthaceae are horticulturally important and cultivated as ornamentals (Amirul-Aiman *et al.*, 2013; Awan *et al.*, 2014).



Fig. A. Habit of *Justicia betonica* L.

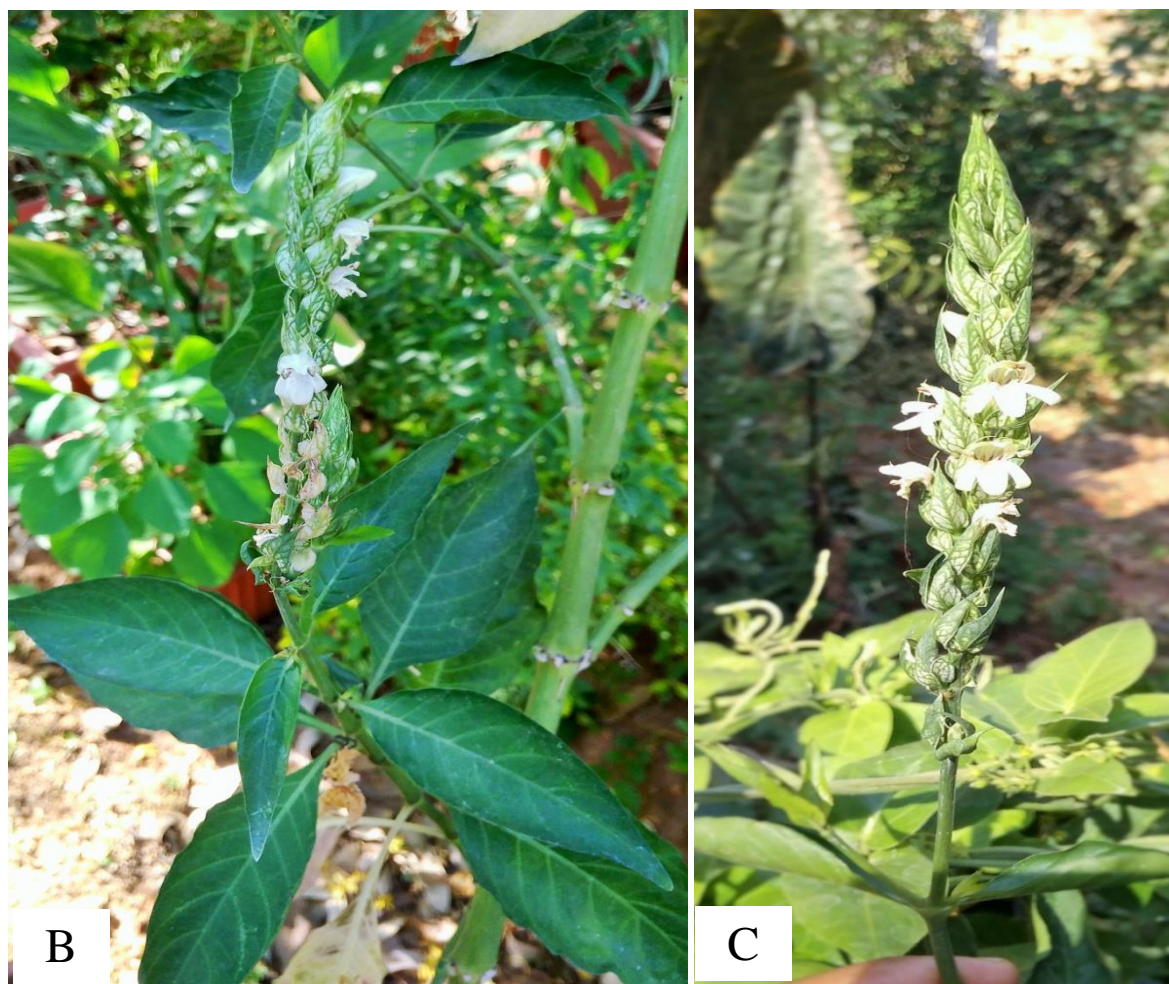


Fig. B. & C. Flowering twig of *J. betonica* L.

Presence of valuable secondary metabolites and biological activities of the family members are well documented. Medicinal properties of various genera of the family are well explored by folk, traditional and modern systems of medicines (Islam *et al.*, 2012). Several plant species of the family are over exploited and are near extinction such as *Anisotes ukambensis*, *Barleria aristata*, *Crossandra friesiorum*, *Dicliptera cordibracteata*, *Dyschoriste sallyae*, *Ecbolium tanzaniense*, *Justicia galapagana*, *Justicia kulalensis*, *Lepidagathis fimbriata*, *Nilgirianthus ciliates* etc.

Genus *Justicia*

Genus *Justicia* occupies major place and represents as largest genus with approximately 700 species of the family Acanthaceae with many unresolved species (Daniel, 2011). Genus *Justicia* possesses 1695 species with scientific names, out of which 376 are accepted species names and 32 scientific names of intraspecific. *Justicia* species are reported to occur in tropical to warm temperate regions of the Americas, India, Indonesia, Southeast Asia, Malaysia, Pakistan and Africa (Perveen and Qaiser, 2010).

Species of *Justicia* are widely explored in folk medicines to cure respiratory, gastrointestinal, inflammatory disorders, malaria, viral fever, epilepsy, rheumatism, headache, cancer, diabetes, mental disorders, arthritis and HIV (Correa and Alcantara, 2012). The phytochemical characterization of *Justicia* species reveals the presence of quinazoline as major compound with other important bioactive substances viz., Vasicine, Vasicinone and Vasicinol. These secondary metabolites are well studied for bronchodilator, hypotensive, abortifacient, anticancer, cytoprotective, antimycobacterial, hepatoprotective, antidiabetic, antispasmodic, anti-bleeding, male contraceptive and hallucinogenic properties (John *et al.*, 2013; Kavitha *et al.*, 2014).

***Justicia betonica* L.**

Systematic position

Kingdom: Plantae
Division: Tracheophyta
Class: Magnoliopsida
Order: Lamiales
Family: Acanthaceae
Sub-family: Nelsonioideae
Tribe: Ruellieae
Sub-tribe: Justiciinae
Genus: *Justicia*
Species: *betonica* L.

Synonyms

Adhatoda arenaria Nees, *Adhatoda betonica* (L.) Nees, *Adhatoda cheiranthifolia* Nees, *Adhatoda lupulina* (E. Mey.) Nees, *Adhatoda ramosissima* (Roxb.) Nees, *Adhatoda trinervia* (Vahl) Nees, *Adhatoda variegata* Nees, *Adhatoda variegata* Nees var. *pallidior* Nees, *Betonica frutescens* Bontekoe, *Dicliptera lupulina* Presl, *Dicliptera trinervia* (Vahl) Juss., *Ecbolium betonica* (L.) Kuntze, *Ecbolium trinervium* (Vahl) Kuntze, *Gendarussa arenaria* (Nees) Steud., *Gendarussa betonica* (L.) Nees ex Steud., *Gendarussa trinervia* Nees ex Steud., *Gendarussa variegata* Hochst. ex Nees, *Justicia antidota* Sm. ex T. Anders., *Justicia betonicifolia* Hort. Berol. ex Nees, *Justicia betonicoides* C. B. Cl., *Justicia cheiranthifolia* (Nees) C. B. Cl., *Justicia lupulina* E. Mey., *Justicia ochroleuca* Blume, *Justicia pallidior* (Nees) C. B. Cl., *Justicia pallidior* (Nees) C.B. Clarke var. *cooperi* C.B. Clarke, *Justicia pseudo-betonica* Roth., *Justicia ramosissima* Roxb., *Justicia trinervia* Vahl, *Justicia uninervis* S. Moore, *Justicia variegata* (Nees) Martelli, *Nicoteba betonica* (L.) Lindau, *Nicoteba trinervia* (Vahl) Lindau., *Rhytocalymma ochroleucum* (Blume) Bremek are the synonyms of this plant species (<https://indiabiodiversity.org/species/show/266692>, 2019; www.theplantlist.org; Ved *et al.*, 2016).

Vernacular Names

Global Names:

Australia: Sleeper weed
English: Paper plume, Squirrel's tail, Hill Justicia, White Shrimp Plant

Ethiopia:	Goppe Dhaliyaa
Indonesia:	Ekor Tupai, Om Rompien (Java); Jukut Buntut Seroh (Sundanese)
Kenya:	Kipkesio
Malaysia:	Ekor Tupai
Sri Lanka:	Sudupuruk (Singhalese)
Tanzania:	Akech (Luo)
Uganda:	Kwiniini omuganda

Indian Names:

Hindi:	Had-paata, Mokandar, Prameha-harati
Irula:	Vella balaga
Malayalam:	Paduthamara, Vellakurunji, Vellakurinji
Marathi:	Gulabi Adulasa
Sanskrit:	Sveta-sahacarah
Tamil:	Velimungil
Telugu:	Tellarantu

2. NATIVITY AND DISTRIBUTION OF *J. BETONICA*

The generic name '*Justicia*' honors James Justice (1698–1763), a famous Scottish horticulturist and the specific epithet '*betonia*' mean betony-like, resembling *Betonica*, a plant genus which come from a name *Vettonica*, medicinal plant from Vectones (Vettones), Spain (RHS, 2008). *Justicia betonica* is reported to be native of eastern and southern Africa (i.e. Kenya, Mozambique, Lesotho, Namibia, South Africa and Swaziland) and the Indian sub-continent (i.e. India and Sri Lanka) and widely naturalized in warmer coastal districts of eastern Australia and Pacific islands.

3. DESCRIPTION AND EMBRYOLOGY OF THE PLANT

Justicia betonica is a glabrous perennial shrub, grows up to the height of 2 m. Leaves are simple, opposite-decussate, lanceolate to ovate, sometimes elliptic, measures approximately 6-17.5 × 2.2-4 cm, leaf base acute-attenuate, tapering into petiole (2 cm long) with entire to crenate margin. Stem is terete, herbaceous, tumid and purple above the nodes. Inflorescence (6-25 cm long) is simple, rarely branched terminal spikes and peduncles long (3-8.5 cm). Bracts are 4-ranked, imbricate (overlapping), white to pale green, broadly ovate, acute at apex, usually scarious at margins, 3-nerved with purplish nerves and elliptic bracteoles. Flowers are hermaphrodite, 0.8-1.5 cm long, hypogynous, zygomorphic and usually on one side of the spike. Corolla bilipped infundibular, white with 2 pinkish ridges or spots in the corolla throat with upper lip hooded and limb pinkish mauve. Corolla tube is 5-8 mm, greenish white, finely velvety with scattered stalked glands (Narayanan, 1956; Khare, 2007).

Stamens are 2 in numbers, epipetalous, posses 3 types of epidermal hairs, two types are restricted to anther and third type is present on the filament. Anatomy of anther reveals the presence of outer wall layer epidermis, fibrous endothecium, single ephemeral middle layer and glandular dimorphic tapetum. Distinct variation reported in the cells of anther wall and

connective in terms of size, shape and number of nuclei. Microspore mother cells give rise to tetrahedral and decussate pollen tetrads. Endothecium absent, the pollen grains are dimorphic in size, tricolporate (63.1 ± 8.9 in polar view and 23.1 ± 5.1 in equatorial view), possess pseudocolpi and sheds at 2-celled stage (Narayanan, 1956; Rueangsawang *et al.*, 2013)

Gynoecium is bicarpellary, syncarpous, ana-campylotropous, unitegmic and tenuinucellate. Four ovules arranged in axial placentation on 2 longitudinal rows. Single archesporial cell act as megaspore mother cell, embryo sac development is Polygonum type. Antipodals and synergids are ephemeral, funiculus with distinct micropyle. The micropylar end has 4-nucleate stage (Narayanan, 1956).

Fruits are pubescent capsules, 2 lobed, ovoidly clavate (10-20 x 5-6 mm) with a short solid base. Flowering and fruiting reported throughout the year. Seeds 4-8 mm broad, are pale/dark brown in color, orbicular (spinulose) to suborbicular in shape, sub-compressed, rugulose-tuberculate, 3.25–4.48 mm width × 3.32–4.52 mm length, endosperm is cellular, testa is densely tuberculate and made of loosely arranged cells and comparatively large among the *Justicia* species (Khare, 2007; Ruengsawang *et al.*, 2012). Seed surface is uneven with fibrous thickenings and characterized by the presence of a jaculator or retinacula (Bhatnagar and Puri, 1970).

Justicia betonica is considered ecologically significant species due to its flowering throughout the year. The pollen and nectar glands were attracted by the bees, and the genus serves as host to many butterfly species, such as *Anartia fatima* (Bhalchandra *et al.*, 2014)

4. USES OF *JUSTICIA BETONICA* IN TRADITIONAL MEDICINES

Entire plant of *J. betonica* is boiled with the stem or root of *Plectranthus ternifolius*, whole plant parts of *Barleria strigosa*, *Lepidagathis fasciculata* and rhizome of *Smilax glabra* and taken orally 3 times a day to treat insect bites in Thailand (Hooker, 1897; Chuakul *et al.*, 1996). It is well acknowledged for gastrointestinal complaints (Kokwaro and Luo, 1998). The infusion made from the whole plant is used to cure stomach ache by Lou tribe of Tanzania (Kokwaro and Luo, 1998).

In India, the inflorescence extract of this plant is given orally to treat vomiting and constipation and used externally to wash hairs (Rao *et al.*, 2006). Leaves crushed and applied as poultice on abscesses to relieve pain and swelling (Khare, 2007). Nandi community of Kenya devours leaf and flower ash internally for the treatment of cough, diarrhea and orchitis (Jeruto *et al.*, 2008). Decoction of *J. betonica* leaves was used by Kipsigis people in Kericho County of Kenya to cure vomiting and headache due to plasmodial infection and malarial symptoms (Pacifica *et al.*, 2008). Fresh roots are homogenized in water and given orally to aid snake bite in human and cattles (Bekalo *et al.*, 2009). In Uganda the leaves of *J. betonica* are used against HIV/AIDS (Lamorde *et al.*, 2010). *J. betonica* leaves are boiled with the leaves of *Cannabis sativa* and *Tetradenia riparia* and used to control helminthes in calves and cattles. The plants are grown with Banana plantation in Uganda to extract bluish purple dye form the leaves and reported to possess more preponderance of dye compared to stem and root (Wanyama *et al.*, 2011). Nyakayojo sub country in southwestern Uganda uses *J. betonica* to treat malaria (Stangeland *et al.*, 2011). Gangabhavani and Ravishankar (2013) reported that *J. betonica* administered to lower cholesterol and used to treat paralysis, earaches, headaches, bruises, diarrhea, vomiting, constipation, pain and inflammation and malaria in India.

Two spoons of root paste are administered twice a day for 5 days to treat muscular pains by Pedabayalu Mandalam tribals of Visakhapatnam district of Andhra Pradesh (India) (Satyavathi *et al.*, 2014). Leaves are crushed, mixed with water and allowed to take bath to alleviate weakness during pregnancy and malaria. Root infusion is taken orally to treat hernia. Root is pounded in water and taken orally to cure worm infection (Tugume *et al.*, 2016). Luhya community of Kakamega East sub-County from Kenya pounds the aerial parts with cold water and boiled extract is administered for malaria. Due to its bitter taste resembling their native anti-malarial plant *Ajuga integrifolia*, *J. betonica* plants were introduced in the river beds of Kenya to control malaria (Mukungu *et al.*, 2016). Luhya community in Kakamega County of Western Kenya chews the whole plant of *J. betonica* to treat toothache and malaria (Odongo *et al.*, 2018).

5. PHYTOCHEMICAL INVESTIGATIONS CARRIED OUT ON *J. BETONICA*

Aqueous and methanolic extracts of various parts (leaf, stem and root) of *J. betonica* were investigated for the presence of polyphenolic contents using Folin-Ciocalteu assay. The total phenolics detected from aqueous extracts of leaf, stem and roots are 2.57, 2.11 and 9.31 mg GAE/gm respectively (Sini *et al.*, 2018). Simultaneously absolute methanolic extracts exhibited 2.22, 1.77 and 4.95 mg GAE/gm for leaf, stem and root of *J. betonica*. The standard phytochemical procedures using aqueous and methanolic extracts reveal that all plant parts possess alkaloids, flavonoids, glycosides, carbohydrates, gum and mucilage, proteins, fixed oils and fat, phenolics and tannins (Bbosa *et al.*, 2013). Alkaloids, fixed oils and fat are absent in the roots and the whole plant is devoid of saponins and sterols. Total phenolic content is reported to be least in *J. betonica* as compared with other *Justicia* species such as *J. adhatoda*, *J. beddomei*, *J. carnea*, *J. gendarussa*, *J. montana* and *J. wayanadensis* (Sini *et al.*, 2018).

Methanol extracts of leaf, stem and roots shows the presence of total phenolics and flavonoid compounds. Leaves of *J. betonica* reported to possess high amount of flavonoids (2.86 mg QE) than *J. adhatoda*, *J. beddomei*, *J. gendarussa*, *J. montana* and *J. wynaadensis* (John *et al.*, 2013).

Qualitative phytochemical analysis using standard procedures reveals that the ether extracts of *J. betonica* possess steroids and triterpenoids, alkaloids and saponins and these compounds are reported to be active against malaria parasite (Bbosa *et al.*, 2013).

Phytochemical characterization of the aerial parts of *J. betonica* yielded four triterpenoidal glycosides (justiciosides A–D), and structure of these compounds are detected by NMR spectroscopic analysis. The compounds are structurally named as (i) olean-12-ene-1b,3b,11a,28-tetraol 28-O-b-D-glucopyranosyl-(1→2)-b-D-glucopyranoside, (ii) olean-12-ene-1b,3b,11a,28-tetraol 28-O-b-D-glucopyranosyl-(1→2)-b-D-glucopyranosyl-(1→2)-b-D-glucopyranoside, (iii) 11a-methoxy-olean-12-ene-1b,3b,28-triol 28-O-b-D-glucopyranosyl-(1→2)-b-D-glucopyranoside and (iv) 11amethoxy-olean-12-ene-1b,3b,28-triol 28-O-b-D-glucopyranosyl-(1→2)-b-D-glucopyranosyl-(1→2)-b-D-glucopyranoside (Kanchanapoom *et al.*, 2004).

Lignans (Taiwanin E methyl ether, Chinensinaphthol A, B) isolated from methanolic extract of *J. betonica* showed antiplatelet aggregation and cytotoxicity against human cervical carcinoma (Day *et al.*, 1999). Justicidin A isolated from ethanolic extract of *J. betonica* was reported to possess cytotoxicity, antiviral, 'fish-killing properties, and induced apoptosis in

human hepatoma cells (Munakata *et al.*, 1965; Day *et al.*, 1999). Tuberculin, Cinnaphthalide A, 10H-Quindoline and Jusbetonin are reported to have antitumor activities (Day *et al.*, 1999; Lu *et al.*, 2008; Caprio *et al.*, 2000).

Methanol extracts of shade dried leaves of *J. betonica* was reported to possess Jusbetonin (indolo{3, 2 -b}quinoline alkaloid glycoside), and three other alkaloids (10H-quindoline, 6H-quinindoline and 5H, 6H-quinindolin-11-one). The structures of the compounds are established using 1D and 2D NMR (1H-1HCOSY, HMQC and HMBC) and HRFABMS on a JEOL JMS-700 mass spectrometer. The resulted jusbetonin was yellow amorphous powder and 5H, 6H-Quinindolin-11-one was identified from pink amorphous material (Subbaraju *et al.*, 2004).

6. PHARMACOLOGICAL ACTIVITIES OF *J. BETONICA*

Anti-*Plasmodium falciparum* activity

Ether extracts of *J. betonica* and *Aloe dawei* was screened for anti-*Plasmodium falciparum* activity by Bbosa *et al.* (2013). Chloroquine diphosphate was used as positive control. Extracts of *J. betonica* exhibited schizonts suppression activity per 200 WBC, EC₅₀ of 13.36 µg/ml (95% CI: 8.032 to 22.23).

Antibacterial activity

Petroleum ether, benzene, chloroform, ethyl acetate, methanol, aqueous and chloramphenicol extracts of *J. betonica* leaves were screened for antibacterial activity against *Aeromonas hydrophila*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Vibrio cholera* and *V. parahemolyticus*. Ten mg/ml of petroleum ether extract inhibits *S. typhi*. Maximum inhibition zone was observed against all tested microbes except *V. cholera* in benzene extract at the concentration of 10 mg/ml. Chloramphenicol extract at 3 µg/disc only found to inhibit the *V. chlorae* cultures (Sasikumar *et al.*, 2007).

Antioxidant activity

The methanolic extracts of whole plant of *J. betonica* were screened for antioxidant activity. The percentage inhibition of free radical formation was determined at concentrations of 250 µg/ml, 500 µg/ml, and 1000 µg/ml. Among various concentrations tested, 1671 µg/ml and 31.1% inhibition was observed at inhibitory concentration 50 (IC₅₀) (Odongo *et al.*, 2017).

Analgesic activity

The analgesic activity of alcoholic extract of *J. betonica* was carried out using Eddy's hot plate method in rats and acetic acid induced writhing in mice. Tramadol was used as standard drug for analgesic activity and maximum inhibition (88.86%) was observed with 300 mg/kg ethanol extract at 120 min. Maximum 10 ± 2.3 writhing with maximum 87.95% inhibition was observed with 300 mg/kg ethanolic extract (Gangabhavani and Ravishankar, 2013).

In vitro anti-inflammatory activity

The *in vitro* anti-inflammatory activity was carried out using carrageenan-induced paw oedema in rats and HRBC (human red blood cells) membrane stabilization method. Diclofenac

sodium was used as standard drug and the rats treated with ethanolic extract. Significant anti-inflammatory activity was reported at 150 mg/kg and 300 mg/kg and caused a noteworthy inhibition compared with standard drug Diclofenac sodium (Gangabhavani and Ravishankar, 2013).

7. PHARMACOGNOSTICAL INVESTIGATIONS

Leaf anatomy

Transverse sections of the leaves are dorsiventral. The epidermis is compact parenchymatous, single layered with cuticle and stomata. Hypodermis is collenchymatous, 7-8 layered; ground tissues are parenchymatous with the presence of crystals. Vascular bundles are closed, collateral and conjoint. Lamina is flat; mesophyll is divided into upper palisade and lower spongy parenchymatous layers. Leaves are amphistomatic, diacytic, the number of stomata was reported to be 35-45/mm². Venation is reticulate with prominent midrib, vein-islets are polygonal, 21-30 /mm² and veinlet termination is 17-25/mm². The spongy cells are 2-3 layered (Eapen *et al.*, 2019).

Fluorescence properties of leaf

The fluorescence properties of *J. betonica* leaves are analyzed with natural light and short and long wavelength of UV light. Characteristic color intensity with the various chemicals was recorded by Eapen *et al.* (2019). Alcoholic extract of leaf powder with 1N NaOH and leaf powder treated with 1N aq. KOH were appeared dark under 365 nm wavelength of UV (Eapen *et al.*, 2019).

Stem anatomy

Epidermis is double layered and covered with cuticle. Hypodermis is collenchymatous, 8-10 layered, cortex parenchymatous, xylem occupies major part in vascular bundles, medullary rays are uniseriate or biseriate and conspicuous. Starch grains, cystoliths and parenchymatous central pith also observed in this plant (Eapen *et al.*, 2019).

Root anatomy

Transverse section of root presents outer layers of thick walled cells with reddish brown contents followed by noticeable cork layers (6–8), and square or rectangular layers of cells. Sclereids are observed in the center to the cork and the major part is availed by xylem vessels (wood). Vessels were reported to be small in size and radially arranged. Xylem fibers, thick walled xylem parenchyma, uniseriate as well as biseriate medullary rays are also reported by Eapen *et al.* (2019)

Petal anatomy

Epidermal cells of petals possess sinuous anticlinal walls and cyclo-paracytic stomata. Simple elongated, multicellular trichomes as well as capitate glandular trichomes with long stalk and terminal multicellular head reported in the petal of *J. betonica*. These characters are reported to be species specific for *J. betonica* as compared with *J. comata*, *J. carnea*, *J. betonica* and *J. procumbens* (Amirul-Aiman *et al.*, 2014).

8. TISSUE CULTURE STUDIES IN *J. BETONICA*

Yacob *et al.* (2013) regenerated plantlets through petiole and internode derived callus of *J. betonica*. Murashige and Skoog's medium (MS) supplemented with 1.5 mg/L NAA and 0.5 mg/L BAP was found optimum for callus induction from petiole and internode explants. Petiole was found optimum for rhizogenesis when cultured on MS medium augmented with 1.5 mg/L NAA and 0.5 mg/L BAP. Genetic stability analyses of *in vitro* and *in vivo* plants were studied. Yacob *et al.* (2014) used leaf, stem and shoot explants to regenerate callus. Multiplication was reported on MS medium fortified with 1.5 mg/L NAA and 0.5 and 1.5 mg/L BAP and 2.0 mg/L NAA and 0.5 mg/L BAP). The plantlets were acclimatized with 80% of survival rate.

9. CONCLUSIONS

Justicia betonica plays significant role in folk as well as in veterinary medicines but, it is less explored plant species as compared to its traditional usefulness. The literature survey also indicates that there is a lack in phytochemical, pharmacological and biotechnological investigations. The folk reports demand further investigation as this species could serve mankind well in drug discovery research.

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