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Bioactive constituents and pharmacological profile of willow-leaved *Justicia*: A review

Priyanka Roy¹, Litty Joseph^{1,*}

¹Department of Pharmaceutical Sciences, Mahatma Gandhi University, Cheruvandoor - 686631, Kottayam, India

ABSTRACT: *Justicia gendarussa* Burm f., a member of Acanthaceae family, is indigenous to China and is widely dispersed throughout India, including the Andaman and Nicobar Islands. *J. gendarussa* is one of the most important plant species utilised in Ayurveda and Chinese medicine. Typically the plants are employed for different medicinal purposes. The plant possesses diverse biological activities like antiangiogenic, antibacterial, hepatoprotection, antifungal, antidepressant, sedative-hypnotic, anxiolytic, immunosuppressant, cytotoxicity, antioxidant and anthelmintic activities. Various chemical elements such as carbohydrates, alkaloids, polyphenolic chemicals, steroids, terpenoids, and saponins have been identified to contribute to these functions. The leaves were found to own most of the constituents like lupeol, friedelin, β -sitosterol and aromatic amines. The current review emphasises on *Justicia gendarussa* Burm's ethnobotanical significance, bioactive ingredients, and pharmacological profile.

1. INTRODUCTION

Traditional medicinal practices are an important part of most developing countries' basic healthcare delivery systems. India has a long history of traditional medicine and is a veritable pharmacy of therapeutic herbs. (Sivasakthi & Vijayalakshmi, 2014). According to the World Health Organization, around 3.5 billion people in Asia and Africa rely on plants as their primary source of healthcare since herbal remedies are relatively cheaper, available easily in abundance with negligible or minor side effects and have frequently been recommended to patients of all age groups (Christian et al., 2013).

More than 35000 species of plants in India have now been believed to have therapeutic properties and are used in various human societies around the world for treatment (Taid et al., 2014). The manifold healing properties of the natural products are reasonably described in traditional literatures on indigenous medications (Sonal et al., 2011). There is a continual need for prospective pharmaceuticals to treat a variety of conditions, which might be made easier by focusing on traditional remedies (Sivasakthi & Vijayalakshmi, 2014).

Numerous species of Genus *Justicia* are being used traditionally for ethnomedicinal resolves. *J. gendarussa* Burm. f. in the family of Acanthaceae (flowering plants) is synonymously called *Gendarussa vulgaris* Nees (Kirthikar & Basu, 2004) and well-known as Willow-leaved *Justicia* in English. Literatures on the plant suggest that the secondary metabolites derived from the plant like tannins, alkaloids, polyphenolics are of numerous

pharmacological aptitudes that may serve as the sources of inspiration for the isolation of novel drug compounds. This plant is used to treat arthritis, muscle pain, fever, headache, ear discomfort, hemiplegia, respiratory disorders like bronchitis, digestive problems, symptoms of vaginal discharges, dyspepsia, and eye diseases in Indian and Chinese traditional medicine.

2. METHODOLOGY

The study was started with the help of various articles and knowledge obtained from various sources including Scopus, EMBASE and PubMed. The records thus collected were then screened and sequentially arranged to divide those under different headings. Plant parts with similar pharmacological and therapeutic effects were combined and the isolated bioactive and phytochemical constituents were grouped for tailoring the study. This provided a clear path to the study and helped to organize the work much easier. Methods that had been used are depicted in the Prisma chart, Figure 1.

3. RESULTS AND DISCUSSION

3.1. Ethnobotanical records on the plant

Justicia gendarussa Burm.f is reported to have several medicinal uses and those vary from region to region (Figure 2). According to the Ayurvedic system, the plant can help with bronchitis, inflammations, vaginal discharges, dyspepsia, eye problems, tympanitis, and pyrexia because of its bitter, pungent, dry, and hot character. The Malays and Javanese employ the

* Corresponding author.

E-mail address: littyjimmyalappatt@gmail.com (Litty Joseph)

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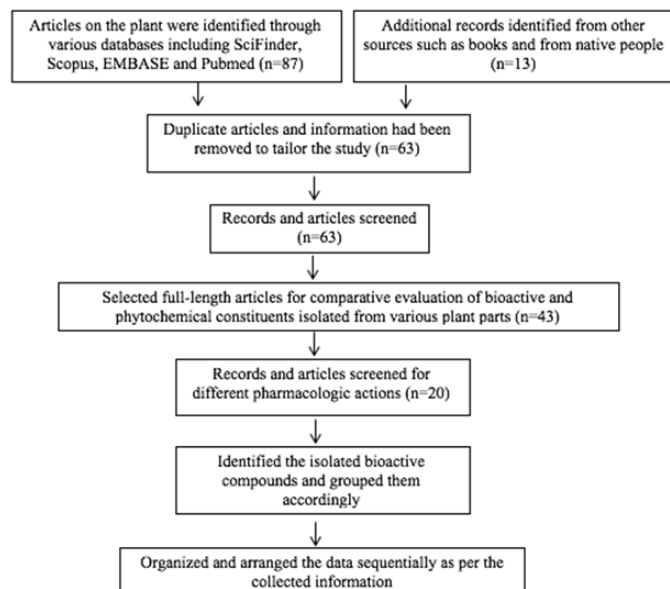


Figure 1. Prisma chart on the methodology of the study

plant as febrifuge and emetic respectively. An infusion of leaves is used orally for facial paralysis, hemiplegia, and cephalalgia, while an oil made from the leaves is beneficial in eczema. The fresh leaf juice when dropped into the ears and nostrils, gives relief from earache and hemicrania.



Figure 2. *Justicia gendarussa* Burm.f

In Madagascar, the plant is principally employed for rheumatism. Leaf and flower top decoctions are generally used as a drink or as fumigation. Root decoction boiled in milk is given for rheumatism, chronic indigestion, fever, jaundice and dysentery. In La Reunion, leaf decoction is employed as stimulant and emetic (Kirthikar & Basu, 1995).

Leaves, when scattered among clothes, preserve them from insect attack. A bath in water saturated leaves is very much efficacious in fever and rheumatism. Leaf juice prevents cough and colic in children. Glandular swellings of the neck and throat may be cured when the leaf mixed with oil is used. Leaves and tender stalks when put together with salt in a bag, warmed and externally applied, give relief from chronic rheumatism and other joint complaints (Nandakarni, 1976). The leaves and stems are likewise useful as emmenagogue, insecticide, in facial paralysis, otalgia, dysmenorrhoea, amenorrhoea, internal haemorrhages, ascites and debility. The bark is considered to be emetic (Vadihyaratnam, 2001).

3.2. Traditional uses of *J. gendarussa*

As per published literatures, following ethnobotanical uses of the plant and its various parts have been recorded (Table 1).

Leaves are proposed for use in the treatment of hemiplegia, cephalalgia, rheumatism, hemicrania, beriberi, as antiperiodic, insecticide (Subramaniam, 1999); for bone dislocation (Rao, 1981); pain from nerve displacement (Borthakur, 1976); antiperiodic, alterative (Nandi, 1986), facial paralysis (Mandal et al., 1996; P. Prasad & Abraham, 1984; Subramaniam, 1999), for the treatment of measles, small pox (J. Singh et al., 1992); rheumatic pains (Prathyusha et al., 2009; Silja et al., 2008; Tripathi et al., 1996); diaphoretic (Tripathi et al., 1996); in fractures (Rao, 1981; Tiwari et al., 1986); headache, bronchitis (Karuppaiah & Sekar, 2006); cough, cold (Samuel & Andrews, 2010); asthma (Raghupathy & Newmaster, 2009).

Leaf oil is used to treat paralysis (Tripathi et al., 1996). **Flowers** are given for the management of eye pain, cough (Raghupathy & Newmaster, 2009). **Bark** has emetic property (Subramaniam, 1999). **Juice** of the plant may act as haemostatic, antiseptic, as nasal drops in nasal bleeding, in dysentery, gargle in aphthae, in cuts and wounds (Jiaswal, 2010), in cancer, leucorrhoea, to check bleeding (Sur et al., 1990); in colic and ear ache (Nandi, 1986; Viswanathan, 1997).

Roots are employed as antiseptic, antidepressant and antipyretic (Subramaniam, 1999); as antivenom (S. Maiti & Mishra, 2000); in ulcerosis (Karuppusamy et al., 2002) leucorrhoea (Venkata & Venkata, 2005). The **whole plant** may be used as emmenagogue, emetic, diaphoretic (Nandi, 1986), (Subramaniam, 1999); vaginal discharges, tympanitis, dyspepsia, bronchitis, ophthalmia (Tripathi et al., 1996); snake bites, cold affections (Subramaniam, 1999); hypotensive, tonic, stoppage of bleeding on cuts and wounds, bone fracture (Jain, 1992); rheumatic pain (Udayan et al., 2006); healing of septic wounds (Mandal et al., 1996); stomach troubles, amenorrhoea (Nandi, 1986); paralysis (P. Prasad & Abraham, 1984).

Table 1Traditional uses of various parts of *J. gendarussa* Burm. f

Sl. no	Plant part	Uses	References
1.	Leaf, stem	Bronchitis, inflammations, vaginal discharges, dyspepsia, eye diseases, tympanitis and fever	Kirthikar and Basu (1995); Nandi (1986); Viswanathan (1997)
2.	Leaf	Febrifuge	Kirthikar and Basu (1995)
3.	Leaf	Emetic	Kirthikar and Basu (1995); Subramaniam (1999)
4.	Oil	Eczema	Kirthikar and Basu (1995)
5.	Leaf infusion	Facial paralysis, hemiplegia and cephalalgia	Mandal et al. (1996); P. Prasad and Abraham (1984); Subramaniam (1999)
6.	Fresh leaf juice	Earache, hemicrania	Nandi (1986); Viswanathan (1997)
7.	Whole Plant	Emmenagogue, emetic, diaphoretic	Nandi (1986), (Subramaniam, 1999)
8.	Leaf and flower top decoctions	Drink or as fumigation	Subramaniam (1999)
9.	Root decoction boiled in milk	Rheumatism, jaundice, chronic indigestion, fever, dysentery	Karuppaiah and Sekar (2006); Tripathi et al. (1996)
10.	Leaf decoction	Stimulant and emetic	Subramaniam (1999)
11.	Leaves (scattered among clothes)	Prevents insect attack	Subramaniam (1999)
12.	Water saturated with leaves	Fever and rheumatism	Subramaniam (1999); Venkata and Venkata (2005)
13.	Leaf juice	Cough and colic in children	Viswanathan (1997)
14.	Leaf mixed with oil	Cures glandular swellings of the neck and throat	Tripathi et al. (1996)
15.	Leaves and tender stalks	Chronic rheumatism and other joint complaints	Nandakarni (1976)
16.	Leaves and stems	Emmenagogue, insecticide, in facial paralysis, otalgia, dysmenorrhoea, amenorrhoea, internal haemorrhages and ascites	Kirthikar and Basu (1995)
17.	Bark	Emetic	Vadihyaratnam (2001)
18.	Shoot and leaves	Earache, rheumatism	Basak (1997); Nandi (1986)
19.	Flowers	Eye pain	Raghupathy and Newmaster (2009)
20.	Flowers, barks, leaves	Cough	Raghupathy and Newmaster (2009)
21.	Plant juice	Haemostatic, antiseptic, nasal drops in nasal bleeding, dysentery, gargle, cuts and wounds, cancer, leucorrhoea,	Jiaswal (2010); Nandi (1986); Viswanathan (1997)
22.	Roots	Antiseptic, antidepressant and antipyretic, antivenom, ulcerosis, leucorrhoea	S. Maiti and Mishra (2000); Subramaniam (1999); Venkata and Venkata (2005)

3.3. Reported chemical constituents

Phytochemical studies on aerial parts, stem and leaves disclosed the occurrence of glycosides, alkaloids, triterpenoids, phenolic and flavonoid components (Moorthy et al., 2012), reducing sugars, lactones, saponins, sterols and terpenoids (Krishna et al., 2010). These components were identified from various fractions; presence of steroids and triterpenoids in petroleum ether; alkaloids phenolic compounds, tannins, and carbohydrates in chloroform and methanol and the remaining compounds were present in the aqueous extracts (N. Prasad et al., 2017).

The petrol ether portion of a methanolic extracts of the whole plant yielded stigmaterol, lupeol and 16-hydroxy lupeol (Rakib et al., 2011). β -sitosterol was identified in the roots (Anjaneyulu et al., 1969).

Components such as luteolin have been found in blossoms. (Nair et al., 1965). 2-(2'-aminobenzylamino) benzyl alcohol, p-amino benzyl alcohol and ortho- methyl ethers were isolated from the leaves. Lupeol, -sitosterol, friedelin (Chakravarty et al., 1982) are also found in the leaves. Naringenin and

Kaempferol were identified in the alcohol extract of mature leaves Zahidah et al. (2017). The constituents and the respective plant parts are shown in Table 2.

3.4. MEDICINALLY ACTIVE COMPOUNDS ISOLATED FROM THE PLANT

3.4.1 Anti-HIV diphyllin glycosides

The identification of two anti-HIV compounds emerged from bioassay-directed separation of methanolic extracts of *Justicia gendarussa* stalk and bark. Justiprocumins A and B are two molecules that belonging to a new class of aryl naphthalide lignan (ANL) glycoside moieties (Hong-Jie et al., 2017). HPLC analysis of the fractions disclosed that ANL glycosides were present in the active fractions of the plant extract.

Chromatographic fractionalization of the methanol extract over silica gel column give the active fraction, named as F26, and was then exposed to preparative HPLC separation to generate 8 distinct fractions (F4 to F 48). Two ANL glycosides were elucidated from fractions F45 and F48 and each of them possess a methylene-dioxy and two methoxy group when the ^1H and

Table 2Various chemical constituents present in *J. gendarussa* Burm. f

Sl no:	Plant part	Chemical constituent	References
1.	Flowers and leaves	Luteolin, lupeol, β -sitosterol, friedelin	Chakravarty et al. (1982); Nair et al. (1965)
2.	Roots	β -sitosterol	Anjaneyulu et al. (1969)
3.	Aerial parts	Glycosides, alkaloids, triterpenoids, phenolic and flavonoid	Moorthy et al. (2012)
4.	Stem	Cardiac glycosides, flavonoids and phenolics, reducing sugars, lactones, saponins, sterols and terpenoids	Krishna et al. (2010)
5.	Plant	Tannins, alkaloids, saponins, flavonoids and phenolics, terpenoids, steroids, sterols and oils	P. Maiti (1968); Mukherjee and Ray (1986)
6.	Leaves	Steroids, triterpenoids, alkaloids, phenolic compounds, tannins, saponins, carbohydrates, glycosides,	N. Prasad et al. (2017)

^{13}C NMR data were compared to those of the known aryl naphthalide lignan compounds

The compounds 1 and 2, were isolated as a white powder of the same molecular formula $\text{C}_{35}\text{H}_{38}\text{O}_{17}$. Compound 1 and 2 can be differentiated only by the acetyloxy group position. 9-(1,3-benzodioxol-5-yl)-4-[(3-O-acetyl-6-deoxy-4-O-b-D-glucopyranosyl-b-Dglucopyranosyl) oxy] -6,7-dimethoxy-naphtho[2,3-c]furan-1(3H)-one and 9-(1,3-benzodioxol-5-yl)-4-[(2-O-acetyl-6-deoxy-4-O-b-D-glucopyranosyl-b-D-glucopyranosyl)oxy]-6,7-dimethoxy-naphtho [2,3-c]furan-1(3H)-one as compound 1 and 2 respectively. They were named as Justiprocumins A and B (Figure 3).

3.4.2 Gold nano-particles

Nanotechnology is an interdisciplinary field that combines atomic, molecular, and solid state physics with chemistry. Bioactive nano particle is capable of producing either beneficial or antagonistic effect on a living matter by interacting with any cell or tissue in the human body (P.K. Singh & Kundu, 2013).

The gold nanoparticle biosynthesis from plant extracts gained much significance owing to its relative stability, simplicity, non-toxic nature and cost effectiveness (Feroze et al., 2012). Because therapeutic herbs contains phenolics and flavonoids, they are a rich source of antioxidants, they are able to reduce metal ions and these antioxidant molecules are capable of producing gold nanoparticles by reducing gold ions Au^{3+} .

Methanol leaf extract of *J. gendarussa* was fractionated using immiscible solvents (diethyl ether, chloroform and ethyl acetate) sequentially by solvent extraction and partitioning technique (Feroze et al., 2012). A mixture of each phytochemical fraction and aqueous chloroauric acid produced gold nanoparticles. Particle size, distribution and stability of the nanoparticles were assessed as per standard protocols. The flavonoid and phenolic contents, antioxidant properties and cytotoxicity of the fractions were determined.

The cytotoxicity of various concentrations of diethyl ether fraction was examined in the Raw 264.7 cell line, and no reduction in survival was observed at the doses studied.

The diethyl ether fraction had more polyphenols and flavonoids, and produced its most gold nanoparticles in 15

minutes, as well as reduced gold nanoparticles to a greater extent with UV absorbance at 536 nm, spherical, triangle, truncated triangle and hexagonal shapes when compared to other fractions. The decreased proportion of gold nanoparticles (AuNPs) had a median size of 27 nm and was steady for 5 months at a pH beyond 7. The primary functional groups like alcohols, phenols, aromatic amines, aliphatic amines and carboxyl groups in this fraction were associated in the decline and capping of AuNPs.

The study revealed that this herb has awesome uses because of its natures such as non-toxic, environmental-friendly and companionable for future biomedical uses and large-scale synthesis.

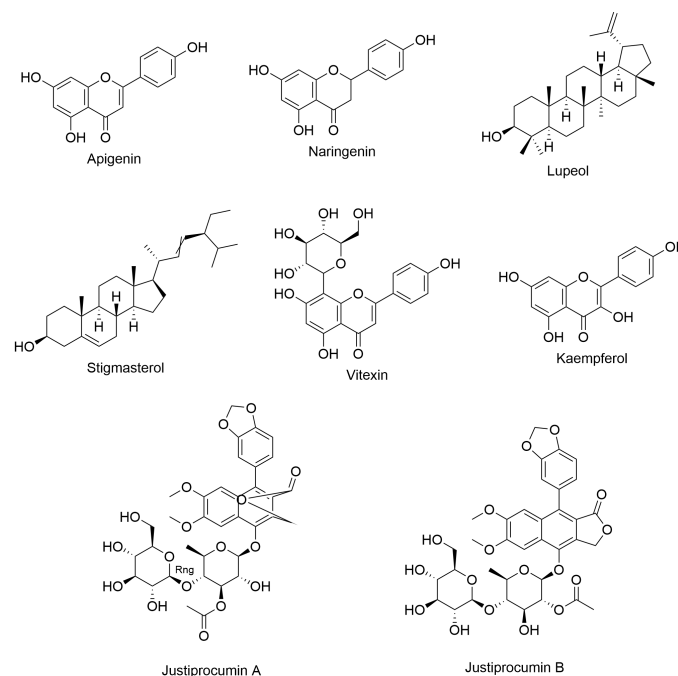


Figure 3. Isolated bioactive constituents from *J. gendarussa* Burm.f

3.4.3 Apigenin and Vitexin

Thin layer chromatography (TLC) and reverse phase high performance chromatography (RP-HPLC) were used to sepa-

rate and characterize the flavonoid components. Ultraviolet-Visible spectrophotometry, Fourier Transform-Infra Red spectroscopy (FTIR), Nuclear magnetic resonance NMR (^{13}C and ^1H), Liquid Chromatography-Mass Spectrometry (LC-MS), Differential Scanning Calorimetry (DSC) and Scanning Electron Microscopy (SEM) techniques were used to recognize the configurations and chemical bonds (Raghu & Agrawal, 2016a, 2016b).

In the method, the methanol extract of the leaves was partitioned with petrol ether, ethyl ether and ethyl acetate. The latter was concentrated, and column chromatography was performed (Table 3). TLC was executed with the compounds obtained from column chromatography. The HPLC analysis pointed out that compound-1 and compound-2 identified from TLC had the same retention time as the standard flavonoids, namely Apigenin and Vitexin. Finally, two bioactive flavonoids were successfully isolated and from the spectral data the compounds were confirmed as Apigenin and Vitexin (Figure 3).

3.4.4 Naringenin and Kaempferol

Young and mature leaves of *J. gendarussa* from four distinct localities were extracted in methanol. (Skudai, Batu Pahat, Mersing and Muar) was exposed to Flame Ionization Detector (GC-FID process) -Gas Chromatography (Zahidah et al., 2017). The outcome denoted that the mature leaves contain highest amounts of kaempferol and naringenin in comparison to the other. The mature leaves from the Skudai and Muar regions have the highest concentration of these components. It was observed that the flavonoids contents were affected by the location as well as plant part which can be correlated to differences in soil, ecological, physiological, geological and hereditary factors.

Naringenin was generated early in the flavonoid biosynthetic pathway, and it was activated by chalcone isomerase (CHI). Flavanol synthases then hydroxylated it to dihydrokaempferol, which was then transformed to kaempferol (FLS). The perfect conversion of naringenin into kaempferol occurs in mature leaves. This could be the reason why mature leaves were found to contain more amounts of these flavonoids.

3.4.5 Stigmasterol, lupeol and 16-hydroxylupeol

Stigmasterol, lupeol and 16-hydroxylupeol were identified by a series of processes including solvent-solvent partitioning, repeated chromatographic separation and purification from the petrol-ether fraction of methanol extract of the whole plant (Rakib et al., 2011). This fraction was chromatographed using Sephadex (LH-20) and was eluted using solvents such as n-hexane: dichloromethane:methanol mixture (2:5:1) followed by dichloromethane and methanol (9:1; 1:1) and then methanol (100%). The structures were elucidated from the NMR data examination, similarity with available values and co-TLC with standards.

Stigmasterol was obtained as needle like crystalline mass from n-hexane: dichloromethane: methanol (2:5:1) fraction. Lupeol was seen as whitish solid. Preparative TLC eluted

with methanol-dichloromethane (1:9) and 20% ethyl acetate in toluene gives 16-hydroxy lupeol (Figure 3).

3.5. Proven pharmacological actions

3.5.1 Anti-HIV activity

AIDS is one of the universal causes of fatality. The importance and demand for new anti-HIV drugs are of great concern as the resistance and side effects are increasing day by day. Two compounds were isolated as described in 3.3.1. Anti-HIV screening was performed in order to detect the HIV replication inhibitors followed by confirmation of activity using broad spectrum HIV strains (Hong-Jie et al., 2017).

Justiprocumin B was tested against 4 HIV strains: BAL, SF-162, LAV0.04 and 89.6 using human PBMC assay. This revealed potent inhibition on all four HIV-1 isolates with IC_{50} values of 14 to 21 nM that was comparable with Zidovudine (IC_{50} -77- 95 nM). Further evaluation demonstrated that the compound was a powerful inhibitor of both Nucleoside reverse transcriptase inhibitor and Non-nucleoside reverse transcriptase inhibitor resistant HIV-1 (Table 4).

3.5.2 Antibacterial activity

Advancement of technology and therapeutic knowledge, the public has become increasingly aware of the importance of high-quality medical care. Antibiotics are used disproportionately in lives stock feed to prevent contagious illnesses in aquaculture and livestock, paving the door for resistance. They propagate across multiple servers and cause drug-resistant genes to be transferred between bacteria, resulting in the formation of multidrug-resistant microorganism (Pai-Wei et al., 2015). As a result, there is a critical necessity for novel antibiotics that are effective against multidrug resistant microorganism.

Leaves of *J. gendarussa* subjected to successive extraction using solvents like Methanol, Chloroform and Petroleum ether were evaluated for antibacterial activity against bacterial cultures of *Pneumocystis pneumonia* and *Pseudomonas vulgaris* using the disc diffusion assay (D. Kumar et al., 2017). The remarkable bactericidal action was observed against *P. pneumonia* and *P. vulgaris* with methanol and chloroform extracts and petroleum ether extract displayed highest activity against *P. vulgaris*. Methanol and chloroform extracts demonstrated highest zone of inhibition against *P. pneumonia*.

Another study revealed antibacterial action of the plant against eight bacteria, including both gram+ve and gram-ve organisms like *S. flexneri*, *K. pneumoniae*, *E.coli*, *B. subtilis*, *P. vulgaris*, *S. mutans*, *M. luteus*, and *S. aureus* using disc diffusion method. *J. gendarussa* leaf extracts were fractionated with increasing polarity solvents. Gentamycin was used as the standard for comparison.

Methanolic extract had a good zone of inhibition (12mm) with *B. subtilis* and *E. coli*. The antibacterial action could be attributable to various phytochemicals found in the plant as discussed in section 3.2.

Table 3
Isolated constituents from *J. gendarussa* Burm.f

Sl. No.	Isolated constituents	Extract/ fraction	Plant part	Use	References
1.	Justiprocumins A and B	Methanol	Stem and bark	Anti -HIV	Hong-Jie et al. (2017)
2.	Gold nano-particles	Methanol	Leaf	Antioxidant	Feroze et al. (2012) ; P.K. Singh and Kundu (2013)
3.	Apigenin, Vitexin	Ethyl acetate	Leaf	Bioactive flavonoids	Raghu and Agrawal (2016a, 2016b)
4.	Naringenin and Kaempferol	Methanol	Mature leaves	Flavonoid	Zahidah et al. (2017)
5.	Stigmasterol, lupeol and 16-hydroxy lupeol	Petroleum ether fraction	Whole plant		Rakib et al. (2011)

3.5.3 Antifungal activity

Fungal infections are caused by microorganisms that invade the epithelial tissue. The fungal kingdom comprises molds, yeasts, rusts and mushrooms. Fungi are heterotrophic and obtain nutrients from the surroundings ([Myers, 2006](#)). Fungi are sometimes ramblingly responsible for allergic or toxic complaints among people due to the production of mycotoxins or allergens ([Paola et al., 2011](#)).

Antifungal action of the extracts were assessed against *M. gypseum* and *C. albicans* using agar well diffusion method.

Nystatin was used as the positive control. Zone of inhibition of 7mm were observed with *Microsporum gypseum* followed by *Candida albicans* with 2.4mm. The extract and Nystatin have a MIC of 0.5 mg/mL and 100 g/mL respectively ([Table 4](#)).

3.5.4 Antidepressant activity

Depression is the most common psychiatric disease, affecting about 21% of the global population. Due to the major side effects of existing medicines such as sexual dysfunction, cardiac toxicity, weight gain, and sleep disturbances, there has been a significant focus in the medicinal application of natural products on neurological condition over the last decade ([V. Kumar et al., 2015](#)).

The aerial parts of *J. gendarussa* were macerated using ethanol and water in the ratio of 30:70 and subjected to antidepressant activity in *Albino* Wistar rats using the forced swimming method at a dose levels of 250 mg/kg and 500 mg/kg of the extract with Imipramine (30 mg/kg) as the reference standard ([Mythili & Jothimanivannan, 2017](#)).

The duration of immobility shown by the animal after forcing them to swim in an open cylindrical container was tested. The tested extract presented substantial reduction ($p < 0.05$ and $p < 0.001$) in immobility when it is compared with control group. The high dose group revealed an effect comparable with that of Imipramine. The results indicated that the plant has the potential to be used as an adjuvant in the management of depression and other bipolar disorders.

3.5.5 Cytotoxic activity

A compound is regarded as cytotoxic if it hampers with cell proliferation, attachment, morphology or if it has a negative impact on the cell growth rate, or causes cell death ([Niles et al., 2008](#)). Cytotoxic agents are of prime importance in the management of various cancers where they can kill tumor cells specifically. Anticancer agents from natural sources are safer and patient compliant and the development of newer therapeutic agents are of great significance.

Methanol extracts of *J. gendarussa* leaves from numerous places in Johor, Malaysia and flavonoids such as kaempferol and naringenin detected by GC-FID, as well as a mixture of these flavonoids, were tested for cytotoxic activity in breast cancer (MDA-MB-231 and MDA-MB-468) and normal CHO (Chinese Hamster Ovary). Tamoxifen showed significant cytotoxicity against CHO and MDA-MB-231, with IC_{50} values of 8 g/mL and 12 g/mL, respectively. Mersing extract showed highest cytotoxicity to the breast cancer cell line, with IC_{50} values of 23 g/mL and 40 g/mL.

Among the flavonoid compounds, Kaempferol exhibited better cytotoxic activity against MDA-MB-468 with IC_{50} values of 23 μ g/mL and MDA-MB-231 cell line showed 34 μ g/mL.

Cytotoxic activity of the extract was further tested on human cancer cell lines such as HT-29 (Colon adenocarcinoma), BxPC-3 (Pancreatic cancer) and HeLa (Cervical cancer) cell line. The extract (Mersing) also revealed highest cytotoxicity against BxPC-3 with an IC_{50} value of 16 μ g/mL. The findings unveiled that the cytotoxicity shown by the leaf extract was owing to the presence of flavonoid compound, namely kaempferol in *J. gendarussa*.

3.5.6 Antianxiety activity

Anxiety is one of the most debilitating multifaceted disorders affecting more than 450 million people worldwide. Nowadays, the usage of benzodiazepines for the management of anxiety is limiting due to their unwanted side effects which pave the attention towards plant based remedies.

The alcoholic extract of the defatted *J. gendarussa* was subjected to preliminary anti-anxiety screening to ascertain its traditional claim for the management of anxiety disorders.

Table 4Pharmacological actions of various extracts of *J. gendarussa* Burm.f

Sl no:	Pharmacological action	Extract/ fraction	Plant part	References
1.	Anti-HIV	Methanol	Barks and stems	Hong-Jie et al. (2017)
2.	Antibacterial	Methanol, Chloroform and Petroleum ether	Leaves	D. Kumar et al. (2017)
3.	Antifungal	Methanol	Leaves	Paola et al. (2011)
4.	Cytotoxic	Methanol	Leaves	Hong-Jie et al. (2017)
5.	Antianxiety	Ethanol	Defatted aerial parts	Subramanian et al. (2013)
6.	Sedative and hypnotic	Ethanol	Leaves	Charney et al. (2001)
7.	Anthelmintic	Methanol	Crude stem and leaf extract	Monika et al. (2012)
8.	Anti-hyperuricemic	Ethanol	Leaves	Katrin et al. (2011)
9.	Anti-inflammatory	Ethanol	Aerial parts	Jothimanivannan et al. (2010)
10.	Anti-nociceptive	Ethanol	Aerial parts	Jothimanivannan et al. (2010)
11.	Hepatoprotective	Methanol	Leaves	Krishna et al. (2009)
12.	Antiangiogenic	Ethanol and aqueous	Leaves	Periyanyagam et al. (2009)
13.	Anti-arthritis	Ethanol	Leaves	Jaijesh et al. (2009)
14.	Antioxidant	Methanol, Water	Leaves	Krishna et al. (2009)
15.	Immunosuppressant	Ethanol	Leaves	Arokiyaraj et al. (2007)

The extracts were screened for anxiety activity (Elevated plus maze and light dark models) using male Swiss albino mice (Subramanian et al., 2013).

Anxiogenic and anxiolytic agents acting at the Benzodiazepine-GABA receptor were greatly responsive to elevated plus maze (EPM). Increased open arm exploration caused by a drug indicates that it is an efficient anxiolytic and closed arm exploration reveals anxiogenic property. It was observed that the mice spent most of their allotted time in the open arms.

The light-dark method is an analogous method to EPM that helps in the discrimination of anxiogenic and anxiolytic drugs. When compared to the dark area, the duration spent in the light area is longer, indicating anxiolytic capacity. The ethanol extract revealed the light area exploration of the animals. The effects produced in both the models were comparable with that produced using standard Diazepam at a dose of 2mg/kg.

The findings of the two *in vitro* anxiolytic assays suggested that the extract could improve the exploration period in the open arm and light area, demonstrating its anti-anxiety potential. This could be due to polyphenolic constituents, tannins, and saponins in the plant, which can reverse CNS disorders (Table 4).

3.5.7 Sedative and hypnotic activity

Sleeplessness, often known as insomnia, is a consistent struggle in falling or staying asleep that can interfere with daily activities and cause serious mental and emotional problems. Most patients rely on long-term use of benzodiazepines analogs to treat this condition (Elisabetsky et al., 1999). Copious medicinal herbs are renowned as active central nervous system drugs having hypothetical prospective of alleviating chronic conditions like depression, anxiety, headaches or epilepsy, that do not retort well to conventional treatment procedures (Yoshiaki and C, 1998). Indian traditional medicine employs *Justicia gendarussa* for the refreshing, tranquillizing and mood

elevating properties.

Ethanol extract of *Justicia gendarussa* was evaluated for its sedative-hypnotic potential using Traction test and Thiopental-induced sleep in male albino mice (250mg/kg and 500mg/kg) using Diazepam as the standard (Subramanian et al., 2014). Benzodiazepines bind to the GABA receptor type-ionophore complex (GABAA) and lessen the onset and length of barbiturate-induced sleep, as well as decreasing empirical action, indicating their usage as a sedative (Charney et al., 2001).

In the Traction test, crude extracts (250 and 500 mg/kg) had a sedative effect, as seen by the relatively longer time for the mice to awaken from their sleep. The extent of thiopental-induced sleeping time was used to assess hypnosis caused by the extract at 250 and 500 mg/kg, *p.o.* The extract reduced the time it took for Thiopental to produce sleep, and it was comparable to Diazepam (3 mg/kg). The presence of flavonoid and terpenoid components in the ethanol extract could explain its sedative and hypnotic effects.

3.5.8 Anthelmintic activity

Selection and appropriate assessment of remedial herbs could uncover its anthelmintic property that is now being researched and could lead to a treatment that is sustainable and eco-friendly (Egual & Giday, 2009).

Adult earthworms (*Pheretima posthuma*) were used to assess the anthelmintic properties of crude methanol extracts of *J. gendarussa* because of the physiological and anatomical similarities to parasitic worms in the human body. Earthworms were examined at dosages of 10, 20, 30, 40, and 50 mg/mL to see if they were debilitated or die. For comparison, albendazole was employed as the reference. The anthelmintic efficacy of the leaf and stem extracts was dosage dependent, with the highest inhibition of motility and the shortest paralysis period at 50 mg/mL (Monika et al., 2012).

The leaf extract (50 mg/mL) induced earthworm paralysis and death at 35.33 and 70.67 minutes, respectively, whereas

the stem extract caused immobility and died at 41.33 and 89.33 minutes. At 17 minutes and 48 minutes, albendazole (10 mg/mL) caused paralysis and death, accordingly. The anthelmintic activity of the plant may be due to the presence of stigmaterol, lupeol, and 16-hydroxylupeol in the methanol extract (Rakib et al., 2011).

3.5.9 Anti-hyperuricemic activity

Hyperuricemia is an ailment where uric acid level in blood is higher than the normal and is caused by the increased production or decreased uric acid excretion in the body. This leads to the deposit of uric acid crystal in the bowel and may lead to increased risks of gout, cardiovascular disorders, nephrolithiasis etc (Joseph et al., 2002).

The hypouricemic property of ethanol extract of *J. gendarussa* leaves was investigated in potassium oxonate-induced hyperuricemic model using male *Sprague-Dawley* rats. Allopurinol was used as the reference standard. The extract at doses of 1.3, 2.6 and 5.2g/kg of body weight presented dose dependent inhibition of 56.53%, 74.41 % and 95.14% of uric acid levels. Allopurinol at 0.18g/kg showed a reduction of 100.04%. As a result of the findings, plant extract appears to be a viable alternative for the management and cure of hyperuricemia (Katrin et al., 2011).

3.5.10 Anti-inflammatory activity

Inflammation, acute or chronic, involves protective mechanisms to eliminate causes of cell injury, pathogenic infectious agents and dead cells from the body which contribute to the maintenance of homeostasis (Jothimanivannan et al., 2010). Extracts of the leaves and aerial parts showed anti-inflammatory potential on various models.

Carrageenan-induced rat paw edema and cotton pellet granuloma in male Wistar *albino* rats were used to test the anti-inflammatory assays (Jothimanivannan et al., 2010). Chronic inflammatory phases can be evaluated using cotton pellet induced granuloma model. When compared to the control group, ethanol extract (250 mg/kg and 500 mg/kg) significantly reduced the formation of granulomatous tissue. Wet and dry cotton pellet granuloma inhibition was seen at 500mg/kg doses of 52 and 45 percent, respectively. Aceclofenac showed maximum inhibition (57.25%) of granuloma formation.

Carrageenan and Formalin induced rat paw oedema and cotton pellet induced granuloma in male *albino* rats were also investigated using the leaf alcoholic extract of *J. gendarussa* (Shikha et al., 2010). The former revealed inhibition of 56.92%, 61.53% and 75.38% at doses of 125, 250 and 500mg/kg respectively. Standard drug Indomethacin disclosed an inhibition of 84.61%.

Different concentration of extract (125, 250 and 500mg/kg) was able to reverse the formalin induced paw edema with inhibition of 55.55%, 70.4% and 74.22% respectively. Indomethacin exhibited 86.66% inhibition. The extract at these doses also revealed concentration dependent increase in percentage inhibition on cotton pellet induced granuloma formation.

Besides alkaloids, sitosterols and saponins, *J. gendarussa* is known to contain flavonoids like Vitexin and Apigenin which may have a positive relation with the proposed anti-inflammatory activity of the extract.

3.5.11 Anti-nociceptive activity

Nociception or algesia is the sensation of pain towards a stimulus. Cessation of pain could be accomplished by the use of either narcotic or non-narcotic agents that act on central or peripheral nervous systems respectively. Traditional healers claim the use of *J. gendarussa* in alleviating pain.

Ethanol extract of *J. gendarussa* (aerial portions) displayed analgesic effect *in vivo* studies. The acetic acid induced writhing and hot plate method were used to test its nociceptive activity in male *albino* mice (Jothimanivannan et al., 2010). After being exposed to thermal stimuli in a hot plate method, the latency time for jumping and paw licking was measured, and the animals treated to 250mg/kg and 500mg/kg of the test showed dosage related increases in latency of response. The findings are compatible to those of normal Pentazocine, demonstrating the ethanol extract's central analgesic activity.

Administration of the two doses of extract reduced the acetic acid induced writhes in animals and the effect was found to be dose dependent with an inhibition of 33% at 500 mg/kg and the of standard Aspirin was exhibited 67%. which This results in the assumption that the extract may also involve in the peripheral analgesic property. The analgesic property shown by the ethanol extract was reported to be due to the presence of flavonoid components in the extract as revealed from phytochemical screening of the same

3.5.12 Hepatoprotective activity

The liver is the biggest organ in the human body and is responsible for the purification of external xenobiotics as well as drug metabolism. Maintenance of a healthy liver is crucial for prevention of acute and chronic liver diseases (Bonorajan et al., 2014). Methanol leaf extracts of *J. gendarussa* revealed as hepatoprotective *in vivo* in both mice and rats (Krishna et al., 2009).

Methanolic extract (200 mg/kg and 400 mg/kg) was assayed against hepatotoxic mice using silymarin as a reference. Evaluation of serum biochemical parameters revealed restoration of the liver enzymes in comparison to the normal and positive control groups.

Superoxide Dismutase, Glutathione Reductase, Catalase, and Lipid Peroxidation, as well as total bilirubin (TB) levels, demonstrated considerable hepatoprotective action against the vehicle and CCl₄ induced mice. The extract was found to reverse hepatotoxicity by reversing biochemical indicators.

Wistar *albino* hepatotoxic rats treated with 150, 300 and 500 mg/kg of the leaf extract of *J. gendarussa* elevated the biochemical markers of hepatotoxicity (Krishna et al., 2010). Decline in the SGPT and SGOT enzymes was observed at 300mg/kg dose level and the percentage reduction was 58.45% and 30.71% respectively. The percentage reductions in total and

direct bilirubin at 300mg/kg were 73.71% and 31.70% and that for standard Silymarin was 71.64% and 48.78% respectively.

A concentration related reduction in the ALP levels was noticed at 150 and 300 mg/kg with maximum effect at 300mg/kg, which declined at the dose of 500mg/kg. Thus, the test revealed moderate hepatoprotection that can be credited to its free radical scavenging ability.

3.5.13 Antiangiogenic activity

Inhibition of neovascularization is a crucial approach for managing of solid tumors, which ultimately relies on hindrance of the blood stream to neoplasm, causing ischemia and cell death in tumor mass. Agents that can cause reversal of angiogenesis are potential anticancer drugs. (Periyanayagam et al., 2009).

The angiogenesis inhibiting properties of alcoholic and water extract of *J. gendarussa* leaves were tested using the chick chorio-allantoic membrane (CAM) assay (Periyanayagam et al., 2009). The study's control and vehicle were β -1,4 galactan sulphate and agarose pellets, accordingly. The extract had no impact on the vascular network at doses lower than 10 g/mL.

The sample was capable of inhibiting blood vessel formation in the CAMs at concentrations larger than 10 g/mL, and the reduction progressed in a dose-dependent way. Ethanol extract at 50 μ g/mL and aqueous extract at 100 μ g/mL, presented avascular zones, indicating the absence of new vessels, proving antiangiogenic property.

3.5.14 Anti-arthritis activity

Rheumatoid arthritis is one of the most wrecking autoimmune disorders that affect the joints and the tissues like muscles, skin, and blood vessels that surround them. The anti-arthritis efficacy of ethanol extracts of *Justicia gendarussa* leaves was tested in male Wistar rats utilizing FCA and Collagen induced arthritic (CIA) models against conventional aspirin (Paval et al., 2009). Collagen induced model shares numerous pathological and clinical features as that of rheumatoid arthritis. Paw volume and hematological parameters of the treatment groups were compared and the results revealed highest inhibition of paw volume by 47% in CIA and that of aspirin was 38%.

One of the major complications accompanying rheumatoid arthritis is anemia, which may be attributed to the changes in the bone marrow and gastrointestinal blood loss. The animals in the treated group reversed the arthritis induced anemia which persisted in induced and aspirin treated groups. C-reactive protein (CRP) and ceruloplasmin quantities were reported to be much less with the extract and aspirin received groups, indicating that the extract has anti-inflammatory properties. This could be explained by flavonoids and β -sitosterols in the plant, which are already identified to have anti-inflammatory action.

An analysis of the differences of anti-arthritis property of *Justicia gendarussa* with *Withania somnifera* on FCA and CIA induced models suggested that the *J. gendarussa* displayed maximum activity with 48% inhibition and *W. somnifera* exposed 45% and 43% inhibition of paw volume in the

respective models (Paval et al., 2009).

3.5.15 Antioxidant activity

Free radicals show a decisive part in the advance of cell injury in innumerable human diseases including tumour, aging, parkinson's, alzheimer's and hardening of arteries due to the imbalances in the free radical scavenging and oxidative stress. Antioxidants are important in the prevention of these diseases. The antioxidant activities of chemicals originating from plants (Alam & Bristi, 2013; Paval et al., 2009) which are important in regard to their nutritious significance and role in healthiness and illness, are attracting more attention. The extracts of *J. gendarussa* revealed antioxidant property in different methods.

In one of the studies, methanol extract of stem was fractioned by means of petrol ether; chloroform and methanol and assayed using reduction of ferric ions, DPPH and hydrogen peroxide radical assays (Krishna et al., 2010). Methanol fraction showed moderate free radical scavenging potential. All other fractions displayed weak DPPH scavenging.

Methanol and water fraction of *Justicia gendarussa* leaves were screened for antioxidant property using reduction of ferric ions, DPPH and hydrogen peroxide assays and the study revealed moderate scavenging of free radicals by methanol extract in all three methods. This was thought to be due to the occurrence of phenolic and flavonoid components (Krishna et al., 2009).

3.5.16 Immunosuppressant activity

Advances in the immunosuppressant drugs over the past decades resulted in dramatic developments in the organ transplantation processes and diminishing graft rejections (Rathe et al., 2012). Regulation of immune response by the use of plant products is of great concern in this century. The role of immune system in the protecting the human body from numerous infections is vital. The search for immunomodulatory plants is increasing due to the uncontrollable side effects of existing drugs.

Four medicinal plant species were taken from the Kolli hills of Tamil Nadu and tested for their immunosuppressive action: *J. gendarussa* (leaf), *Plumbago indica* (root), *Aloe vera* (leaf), and *Aegle marmelos* (leaf) (Arokiyaraj et al., 2007). The dry methanol extract was extracted by n-hexane, ethyl acetate, chloroform, 96 percent alcohol, benzene, acetone, and water. The efficacy of crude methanol extracts of the plants to suppress PMBC development was tested using a Lymphocyte proliferation assay using 3H thymidine uptake.

Leaf extract of *J. gendarussa* at a dose of 100 μ g/mL exhibited maximum suppressive result of 85%. Among the sequentially fractioned methanol extract, aqueous fraction of *J. gendarussa* revealed a strong immunosuppressive effect on mitogen stimulated lymphocyte at a concentration 50 μ g/mL, revealing its inhibitory action on non-specific cellular immune response.

4. CONCLUSION

Regardless of the astonishing innovations of the modern medicine, the traditional system of medicine still serves as a prospective health care modality in the developing countries. The contemporary reports advocate that there is a renewed concern towards traditional system even in the developed countries too, due to the acute adverse effects of chemotherapy and radiotherapy (Ayele, 2018). According to the WHO, conventional medicine is the summation of information, practices, and abilities based on beliefs, concepts, and practices that are indigenous to various cultures and nations.

Justicia gendarussa is an interesting plant specimen with a long history of traditional medicinal use, as evidenced by study. This article explicates the ethnobotanical reports, traditional uses, phytochemical constituents and pharmacological actions of *J. gendarussa*. The ethnobotanical reports, conventional applications, phytoconstituents, and bioactivity of *J. gendarussa* are discussed in detail in this report. The mechanism of action could be revealed by studies at molecular level. Anti-HIV, antioxidant, antidepressant, sedative-hypnotic, analgesic, anti-inflammatory, anxiolytic, antiangiogenic, cytotoxic, anti-arthritis, hepatoprotective, antibacterial, antifungal, and anthelmintic effects have been discovered for this plant, which can be linked to bioactive elements found in plants such as flavonoids, lignan glycosides, phenolic compounds, alkaloids, steroids, carbohydrate, carotenoids and terpenoids. Studies at the molecular level may reveal the mechanism of action. Based on the findings of this study, it can be stated that the plant *J. gendarussa* is a promising candidate for the emergence of novel therapeutic leads.

CONFLICTS OF INTEREST

The authors state that they have no competing interests.

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ORCID

Priyanka Roy 0000-0002-9683-6245

Litty Joseph 0000-0002-7703-5039

AUTHOR CONTRIBUTIONS

LJ - Review concept and design; PR - Searching literature; PR- Collection and/or assembly of data; LJ - Data analysis and interpretation; PR - Manuscript drafting; LJ - Critical revision of the article, Proof reading and final approval of the article.

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