



# ***Rauvolfia tetraphylla* L. (Apocynaceae) - A Comprehensive Review on Its Ethnobotanical Uses, Phytochemistry and Pharmacological Activities**

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Received: 17 Jan 2019 / Accepted: 19 Mar 2019 / Published online: 1 Apr 2019

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## **Abstract**

Plants are an indispensable source of various needs of human beings such as food, construction tools, flavoring agents, and medicine. *Rauvolfia tetraphylla* L. is endangered and is one of the important plants of Apocynaceae family being cultivated for its immense medicinal properties. The present review highlights ethnobotanical uses and pharmacological potential of *R. tetraphylla*. The plant *R. tetraphylla* is widely used in traditional medicine and often it is used as an adulterant or substitute of *Rauvolfia serpentina*. The plant is used as a remedy for snake and other poisonous bite, blood pressure, diabetes, piles, malaria, wound, helminthiasis, hypertension, vomiting, insomnia, skin diseases, mental disorders, cough, fever and others. A vast number of secondary metabolites such as reserpine, reserpiline, yohimbine, ajmaline, lankanescine, raivotetraphyllines, alstonine, deserpidine, aricine, isoreserpine, sarpagine and others have been detected in the plant among which reserpine is the most important one. *R. tetraphylla* is shown to exhibit a range of pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, cytotoxic, platelet antiaggregant, cardioprotective, sedative, antihypertensive, insecticidal, allelopathic and antiparasitic activities. Isolated constituents are shown to be pharmacologically active as they have displayed activities such as antimicrobial and antipsychotic activity. In conclusion, the plant *R. tetraphylla* is a critically endangered medicinal species with tremendous ethno medicinal importance and an important source of valuable alkaloids, hence, it is very important to conserve the species for its multifold beneficial applications.

## **Keywords**

*Rauvolfia tetraphylla* L., Apocynaceae, Ethnobotany, Phytochemicals, Pharmacological activity.

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## 1. INTRODUCTION

Since time immemorial plants have been exploited by mankind for various purposes such as timber, medicine, food and source of dyes. In certain kinds of formulations, plants have been extensively used to get rid of several ailments by traditional practitioners all over the world as well as by indigenous medicinal systems such as Ayurveda, Siddha, Unani and Traditional Chinese medicine (TCM). Plant based medicines are the primary source of treatment for people living in remote places and having no access for modern medicine. The knowledge of indigenous medicinal practitioners on plants and their healing properties is passed from generation to generation. Plants produce a great number of secondary metabolites (for e.g. alkaloids, terpenes and polyphenolic compounds), many of which, are known to possess therapeutic applications. Plant derived chemicals have found distinct place in modern therapy as they have been considered very important leads for modern drug discovery. Compounds such as vincristine, vinblastine, digitalin, digoxin, atropine, camptothecine, morphine, codeine, reserpine, quinine and artemisinin are from plant origin. Plants, either singly or in polyherbal formulations, are being used traditionally worldwide to combat several ailments including microbial diseases, snake bite, skin diseases, diabetes, inflammation and cancer. A vast knowledge on the therapeutic role of plants and their bioactive principles is gathered due to many studies being carried out on medicinal and pharmacological properties of plants [1-12].

The plant genus *Rauvolfia* L. belongs to the family Apocynaceae and encompasses herbs or shrubs with leaves in whorls of 3 or 4. The genus *Rauvolfia* is pantropical in distribution and contain variety of alkaloids. The genus includes two very important medicinal species viz. *Rauvolfia serpentina* (L.) Benth. ex Kurz (an Indian species commonly known as sarpagandha in India) and *Rauvolfia tetraphylla* L. Both the species contain bioactive alkaloids having certain biological activities. The roots of *R. serpentina* is widely used in medicine. *R. tetraphylla*

differs from *R. serpentina* in having leaves in whorls of 4 and short corolla tube. *R. tetraphylla* is often used as a substitute of *R. serpentina* for medicinal purpose. *R. tetraphylla* is a critically endangered species and is known by names viz. Wild snake root, Devil pepper, Four-leaf devil pepper and Be Still tree in English, Barachandrika in Hindi, Papataku in Telugu, Pampukaalaachchedi in Tamil, Patalagarudi in Oriya, Vanasarpagandha in Sanskrit and Dodda chandrike in Kannada. The plant is native to West Indies and now naturalized in many countries such as India, Pakistan, Sri Lanka, Bangladesh, Nepal, and Myanmar and often cultivated in gardens. In India, the plant is distributed in various states such as Karnataka, Madhya Pradesh, Orissa, West Bengal, Bihar, Andhra Pradesh, Kerala and Tamil Nadu. *R. tetraphylla* is one of the well-known plants being widely used in traditional medicine. The plant contains alkaloids such as reserpine, rauvolsine, ajmalicine, ajmaline, canescine, pseudoyohimbine, and yohimbine. *R. tetraphylla* contains a number of chemicals and several studies carried out on the plant have revealed various pharmacological activities [13-24]. In this review, an attempt is made to compile information available on traditional uses and pharmacological potential of *R. tetraphylla* by rigorous literature survey that followed searching journal articles, standard flora and various search engines such as PubMed, Science Direct and Google scholar.

## PLANT DESCRIPTION

*Rauvolfia tetraphylla* L. (Figure 1) synonym *Rauvolfia canescens* L. is a pubescent, ever-green shrub with woody stem and reaching a height of 4-6 feet. Leaves are unequal, 5-9 x 3-4 cm, elliptic-ovate, acute at apex, pubescent and usually found in whorls of 4. Flowers are cream colored, about 5mm across, found in terminal corymbose cymes. Calyx lobes are short, ciliate and round. Corolla is white in color, approximately 3mm long, lobes and tubes are short. Drupes are ovoid, 2-seeded, 5-10mm across, smooth, jointed to the top, purple when ripe. Flowering occurs throughout the year [19, 25, 26].



**Figure 1: *Rauvolfia tetraphylla* L. (photograph by Prashith Kekuda)**

## 2. ETHNOBOTANICAL USES OF *RAUVOLFIA TETRAPHYLLA*

Plants are an integral component of traditional medicine. Throughout world, whole plants or certain parts of the plants have been widely used to cure several diseases and disorders either in single or polyherbal formulations. Plant based medicines are cheaper, usually devoid of side effects (that are associated with the use of modern medicines like antibiotics and anticancer agents), confer multifold beneficial effects and are locally available [27-35]. The plant *R. tetraphylla* possesses a range of ethnobotanical applications in various countries across the world as indicated by several studies. The latex from the plant is reported to be cathartic, emetic, and expectorant besides its use in the treatment of dropsy. The juice prepared from the fruit is used as a substitute for ink. Leaf juice is used in eye troubles and decoction prepared from leaves is used for toothache. Root is reported to have antihypertensive, sedative, hypnotic effect. Paste prepared from roots is useful in stomach pain and snake bite [14,36,37,38]. *R. tetraphylla* is one among the various ethnomedicinal plants being used by Amerindian communities of Caribbean basin [39]. The roots of the plant are used in nervous disorders and insomnia in Gopalganj district of Bihar, India [40].

Whole plant as well as different parts viz. root, fruit and leaves of *R. tetraphylla* are widely used in traditional medicine (in various forms such as paste, powder, decoction and juice), particularly snake bite. In Bagota, Colombia, the plant is used as an antidote and for blood pressure [41]. The leaves of *R. tetraphylla* are used ethnomedicinally by Peasant community of San Jacinto, Northern Colombia to relieve tension [42]. Roots are used in high blood pressure and mental disorders in Coimbatore district, Tamil Naud, India [43]. The leaves and roots

are used against nervous disorders, anxiety, excitement, cough and fever in Barpeta district, Assam, India [44]. Roots are used for treating nervous disorders in Villupuram district, Tamil Nadu, India [45]. The Nyishi tribe of Arunachal Pradesh, India uses roots of the plants as anthelmintic, antidote to snake bite and to cure vomiting, insomnia, insanity, blood pressure, malaria [46]. The leaves are used traditionally for treating eczema in Tamil Nadu, India [47]. The leaves and fruits are used against snake bite in Thiruvannamalai district, Tamil Nadu, India [48]. In Odisha, India, the leaves and roots are used to treat stomachache [49]. Roots and whole plant of *R. tetraphylla* are used to increase uterine contraction, as anthelmintic and to treat skin diseases and hypertension in South West Bengal, India [50].

In Midnapore district, West Bengal, India, the roots are used as sedative, hypnotic and for hypertension. Leaf juice is used for the purpose of removal of opacities of cornea of eyes [51]. The whole plant and root or *R. tetraphylla* are used in the treatment of impotency and to increase libido in Greater Naogaon district, Bangladesh [52]. The plant is used as an antidote (to poison) in Rajshahi district, Bangladesh [53]. In Chuadanga and Jhenaidah districts of Bangladesh, different parts viz. leaf, stem and root are used in snake bite and as snake repellent [54]. In Vizianagaram district of Andhra Pradesh, India the plant is used to treat blood pressure [55]. In Chittagong, Bangladesh, the leaves and roots are used against high blood pressure [56]. Various parts viz. seed, leaf and root of *R. tetraphylla* are used to treat snake bite in Kancheepuram district, Tamil Nadu, India [57]. The roots are used to treat mental disorders and high blood pressure in Erode district, Tamil Nadu, India [58]. More information on the plant part, form and ethnomedicinal uses is presented in Table 1.

**Table 1: Ethnomedicinal uses of *R. tetraphylla***

Geographical area	Part used	Ailment	Form	Reference
Chittoor district, Andhra Pradesh, India	Root	Head sore	Root paste	Ganesh and Sudarsanam [59]
Anuppur district, Madhya Pradesh, India	Root	Stomach pain, intestinal worm	Root extract	Malaiya [60]
Vizianagaram district, Andhra Pradesh, India	Root	Blood pressure	Root bark decoction	Babu et al. [61]
Paschim Medinipur, West Bengal, India	Root	Loose motion, snake bite	Root extract	Manna and Manna [62]
Koraput district, Odisha, India	Root	Snake bite	Root paste	Kumar et al. [63]
Salem district, Tamilnadu, India	Whole plant	Snake bite	Paste	Alagesaboopathi [64]
Ranga Reddy district, Telangana, India	Whole plant	Skin diseases	Paste	Ramakrishna et al. [65]
Bhadrak district, Odisha, India	Whole plant	Skin diseases	Juice	Panda et al. [66]
South Western Ghats, Kerala, India	Root	Snake bite	Paste	Sulochana et al. [67]
Latehar district, Jharkhand, India	Root	Blood pressure, chronic wound	Powder, paste	Marandi and Britto [68]
Kancheepuram District, Tamil Nadu, India	Whole plant	Skin diseases	Paste	Muthu et al. [69]
Kanyakumari wildlife sanctuary, Tamil Nadu, India	Fruit	Intestinal worms	Roasted fruits	Rani and Jeeva [70]
Coimbatore and Ooty District, Tamil Nadu, India	Root	Skin diseases, snake bites, as antidote for insect bites, to destroy parasites	Powder	Kumar et al. [71]
Eastern Ghats, Andhra Pradesh, India	Root bark	Blood pressure	Decoction	Rao et al. [72]
Chittagong, Bangladesh	Root	Diarrhea and dysentery	Water extract	Morshed and Nandni [73]
Madumalai wildlife sanctuary, Tamil Nadu, India	Root bark	Snake and other poisonous bite	Decoction	Rani et al. [74]
Odisha, India	Root	Malaria	Paste	Singh et al. [75]
Salem district, Tamil Nadu, India	Whole plant	Snake and scorpion bite	Paste	Alagesaboopathi et al. [76]
West Rarrh region, West Bengal, India	Root	Snake bite, diabetes mellitus	Juice	Ghosh [77]
Sundargarh, Orissa, India	Root	Snake bite	Powder, paste	Girach et al. [78]
Birbhum district, West Bengal, India	Root	Snake bite, diabetes	Juice	Sarkar et al. [79]
Eastern Ghats, Tamil Nadu, India	Root, leaf	Piles, sterility	Juice	Vaidyanathan et al. [80]
Sundargarh district, Orissa, India	Root	Snake bite	Paste	Prusti and Behera [81]

### 3. PHYTOCHEMICALS IN RAUVOLFIA TETRAPHYLLA

Plants produce a range of chemicals that can be divided into two categories viz. primary and secondary metabolites. These chemicals are known as phytochemicals and most of the secondary metabolites (with complex chemical composition), for e.g., alkaloids, terpenes and polyphenolic compounds, exert multifold effects on the health of human beings besides conferring resistance to plants that produce them against insects, pathogens and herbivores. Secondary metabolites in plants are restricted in distribution within the plant kingdom i.e. some metabolites are present in only one plant species or in a related group of species. Metabolic pathways such as shikimic acid pathway, malonic acid pathway and mevalonic acid pathway are responsible for synthesis of secondary metabolites in plants [82-90]. Due to advancements in chromatographic and spectral methods, a vast number of plant secondary metabolites have been identified and their structures are proposed [91-97]. *R. tetraphylla* is shown to contain a myriad of secondary metabolites, particularly alkaloids. Reserpine is an important alkaloid found in *R. tetraphylla*. With the help of chromatographic and spectral analyses, many secondary compounds have been isolated from different parts of *R. tetraphylla* and their structures have been elucidated. Klohs et al. [98] isolated reserpine from roots of *R. tetraphylla*. Stoll and Hofmann [99] isolated two alkaloids canescine and pseudoyohimbine from the roots of *R. tetraphylla*. Two ester alkaloids, named as raunescine and isoraunescine, have been isolated from *R. tetraphylla* [100]. An alkaloid designated as Recanescine (that lack the methoxyl group in the C-II position of reserpine) with sedative and hypotensive property was isolated from *R. tetraphylla* [101,102]. An alkaloid designated as heterophyllin was isolated

from roots of *R. tetraphylla* [103]. Raunescine (renamed as canembine), an alkaloid, was isolated from roots of *R. tetraphylla* [104]. An alkaloid pseudoreserpine possessing hypotensive and sedative activity was isolated from the root of *R. tetraphylla* [105]. Djerassi et al. [106] elucidated the structures of two alkaloids from root of *R. tetraphylla* viz. tetraphylline and tetraphyllicine. Belikov [107] isolated alkaloids viz. ajmaline, ajmalicine, arieine, reserpiline,  $\alpha$ -yohimbine, sarpagine and reserpine from roots and epigeal parts of *R. tetraphylla*.

A new sarpagine-type alkaloid, named as N ( $\alpha$ )-Demethylaccedine, was isolated from stem bark of *R. tetraphylla* [108]. By HPTLC analysis, reserpine and ajmalicine were identified in root of *R. tetraphylla* [109]. Quercetin was identified in the leaves of *R. tetraphylla* by HPTLC analysis [110]. Alkaloids viz. 10-methoxytetrahydroalstonine, isoreserpiline,  $\alpha$ -yohimbine, reserpiline were isolated from chloroform fraction of *R. tetraphylla* leaves by pH-zone-refining fast centrifugal partition chromatography [111]. Six indole alkaloids viz. isoreserpiline, 10-methoxytetrahydroalstonine, 11-demethoxyreserpiline, 10-demethoxyreserpiline,  $\alpha$ -yohimbine and reserpiline have been identified from leaves of *R. tetraphylla* [112]. A new labdane diterpene characterized as 3 $\beta$ -hydroxy-labda-8(17),13(14)-dien-12(15)-olide (Figure 2) was isolated from air-dried stems and branches of *R. tetraphylla* [113]. The study by Panda et al. [114] revealed a varying concentration of reserpine in different parts of *R. tetraphylla*. Root was shown to contain high concentration of reserpine followed by stem and leaf. More information on the chemicals/phytochemical groups identified in *R. tetraphylla* is presented in Table 2 and Table 3. Structures of some secondary metabolites in *Rauvolfia tetraphylla* are shown in Figure 2-7.

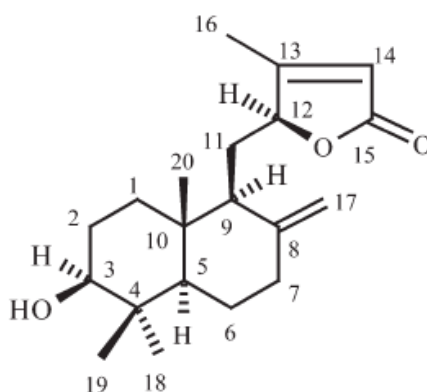


Figure 2: Structure of labdane diterpene [113]

**Table 2: Phytochemicals identified in *R. tetraphylla***

Part	Method	Phytochemicals identified	Reference
Callus	TLC, HPLC	Reserpine	Anitha and Kumari [18]
Leaf	HPTLC	Yohimbine	Kumar et al. [115]
Stem	HPTLC, MS	Reserpine	Paul [116]
Aerial parts	IR, HPLC, NMR	Rauvotetraphyllines A–E, nortetraphyllicine, raucaffricine, peraksine, alstonine, sarpagine, 3-hydroxysarpagine, dihydroperaksine, 10-hydroxydihydroperaksine	Gao et al. [117]
Aerial parts	IR, HPLC, NMR	Rauvotetraphyllines F–H, 17-epi-rauvotetraphylline F and 21-epi-rauvotetraphylline H	Gao et al. [118]
Root bark, leaves	UV, IR, NMR, MS	Ajmaline, yohimbine, a-yohimbine, aricine, isoreserpine, corynanthine, deserpidine, reserpiline, isoreserpiline, lankanescine	Arambewela and Madawela [119]
Root	HPLC–ESI-QToF-MS/MS, HPTLC	Reserpine	Bindu et al. [120]
Leaf	TLC, HPLC, NMR, IR, GC-MS	18,19-secoyohimban, curan-17-oic acid, reserpiline	Behera and Bhatnagar [121]
Leaf	HPTLC	10-methoxy tetrahydroalstonine, reserpiline, $\alpha$ -yohimbine, isoreserpiline	Gupta et al. [122]
Leaf	HPTLC-MS	3-Isoreserpine, ajmalicine, ajmaline, yohimbine, reserpine	Nandhini and Bai [123]
Leaf	GC-MS	8-Octadecenoic acid, Pentadecanoic acid, 2,(2-Carboxyvinyl)pyridine, Heptadecanoic acid, 3(2H)-Phenanthrenone	Chandra and Vignesh [124]

**Table 3: Phytochemical groups identified in *R. tetraphylla* by preliminary analysis**

Part	Phytochemical group identified	Reference
Leaf	Terpenoids, tannins, flavonoids, alkaloids, phenols	Satyanarayana et al. [110]
Leaf, stem	Alkaloids, tannins, steroids, saponins, cardiac glycosides	Vaghasiya et al. [125]
Leaf, stem, root	Alkaloids, tannin, steroid	Panda et al. [114]
Leaf	Alkaloids, steroids, tannins, phenols, saponins, flavonoids	Kavitha et al. [126]
Leaf, fruit	Alkaloids, flavonoids, tannins, saponins	Behera et al. [127]
Leaf	Alkaloids, tannins, flavonoids, saponins, terpenes	Patel et al. [128]
Leaf	Alkaloids, flavonoids, saponins, tannins and phenols	Thinakaran et al. [129]
Root	Tannins, flavonoids, saponins, glycosides, phenols, terpenoids, steroids	Kapoor [130]
Leaf	Steroid, alkaloid, phenolic compounds, catechin, tannin	Haniffa and Kavitha [131]
Leaf	Alkaloid, flavonoid, steroid, saponin, tannin	Nandhini and Bai [132]
Leaf	Tannins, flavonoids, alkaloids, terpenoids	Archana and Jeyamanikandan [133]
Leaf	Steroids, alkaloids, phenolic compounds, tannins, catechin	De Britto et al. [134]
Leaf, fruit	Steroids, alkaloids, flavonoids, phenolics, glycosides	Vinay et al. [135]
Leaf	Saponins, terpenoids, flavonoids, coumarin, alkaloids	Senthilmurugan et al. [136]
Leaf	Alkaloids, flavonoids, glycosides, saponins, tannins, steroids	Singh et al. [137]
Leaf	Tannins, flavonoids, cardiac glycosides	Shyma et al. [138]
Leaf	Flavonoids, alkaloids, phytosterols, glycosides, saponins, tannins, triterpenoids	Ashokkumar et al. [139]
Leaf	Alkaloids, glycosides, tannins, phenols, saponins	Chandra and Vignesh [124]



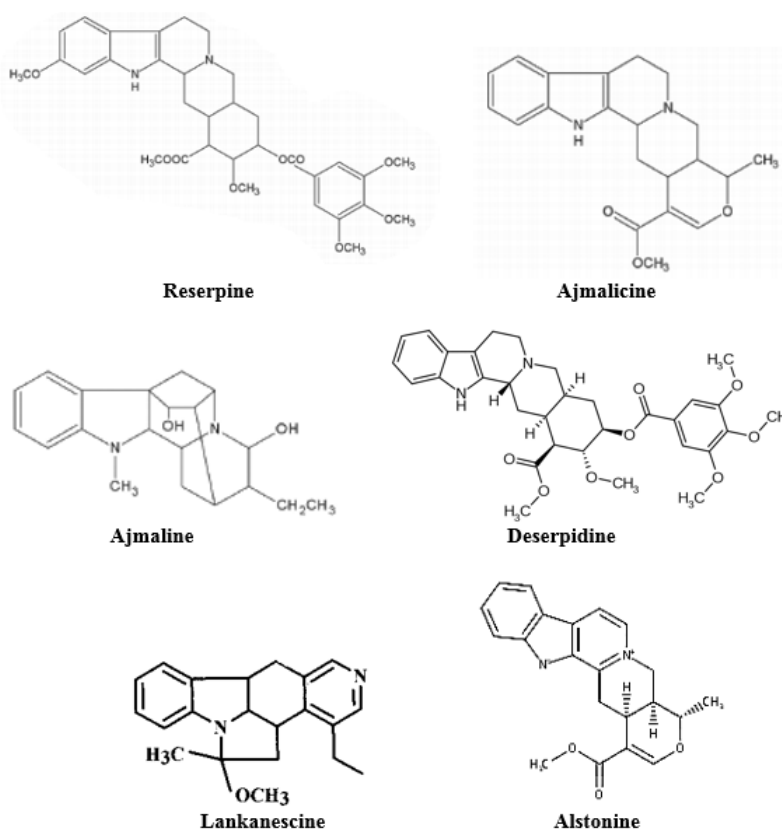


Figure 3: Structures of some *Rauvolfia* alkaloids

#### 4. PHARMACOLOGICAL ACTIVITIES OF *RAUVOLFIA TETRAPHYLLA*

Various parts of *R. tetraphylla* are shown to exhibit a range of pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, cytotoxic, antihemolytic, antihypertensive, anxiolytic, insecticidal, allelopathic, platelet antiaggregant, cardioprotective, antipsychotic and antiparasitic activity. A brief description on pharmacological potentials of the plant is given here.

##### Antibacterial activity

Whole plant as well as different parts such as root, leaf and fruit of *R. tetraphylla* were shown to be effective antibacterial agents. Ethanol extract from *R. tetraphylla* was shown to reveal marked inhibition of gram positive and gram negative bacteria [140]. Aqueous extract of *R. tetraphylla* leaves exhibited concentration dependent inhibition of test bacteria viz. *Escherichia coli* and *Klebsiella pneumoniae* [129]. Ethanol extract of leaves were effective in causing concentration dependent inhibition of bacteria. *Salmonella typhimurium* and *Micrococcus luteus*

were inhibited to highest and least extent, respectively [132]. Reserpine, isolated from leaves of *R. tetraphylla*, was found to display inhibitory activity against gram positive and gram negative bacteria [141]. Leaf extract of *R. tetraphylla* displayed inhibition of *Staphylococcus aureus* and *Enterobacter faecalis* with marked activity against *S. aureus* [136]. Methanolic leaf extract was shown to be effective against *S. aureus* and *K. pneumoniae* in a dose dependent manner [133]. Compounds viz. 10-Methoxytetrahydroalstonine, reserpiline, isoreserpiline, demethoxyreserpiline, serpentine and  $\alpha$ -yohimbine from *R. tetraphylla* were effective in causing inhibition of nalidixic acid sensitive and resistant strains of *Escherichia coli*. Further, these compounds were also capable of acting synergistically with nalidixic acid [142]. More information on antibacterial activity of *R. tetraphylla* is presented in Table 4. Silver nanoparticles, synthesized using leaf extract of *R. tetraphylla*, were shown to be effective against *S. aureus*, *E. coli*, *P. aeruginosa* and *B. subtilis* [143].

**Table 4: Antibacterial activity of *R. tetraphylla***

Part	Extract	Method	Activity against	Reference
Root bark	Methanol, hexane, ethyl acetate, ethanol extracts	Agar well diffusion assay	Gram positive and gram negative bacteria	Rao et al. [144]
Leaf, stem, root	Methanol, petroleum ether, chloroform, benzene extracts	Agar well diffusion assay	Gram positive and gram negative bacteria	Rohela et al. [145]
Leaf (in-vitro cultured)	Ethanol extract	Disk diffusion assay	Gram positive and gram negative bacteria	Nandhini and Bai [123]
Leaf	Methanol extract	Disk diffusion assay	<i>Aeromonas hydrophila</i>	Haniffa and Kavitha [131]
Fruit	Petroleum ether, chloroform, ethyl acetate extracts	Agar well diffusion assay	Gram positive and gram negative bacteria	Alagesaboopathi [146]
Leaf	Methanol extract	Agar well diffusion assay	<i>S. aureus</i> , <i>S. typhi</i> and <i>S. paratyphi A</i>	Mahida and Mohan [147]
Leaf	Methanol extract	Disk diffusion assay	<i>E. coli</i> , <i>S. aureus</i> , <i>Shigella dysenteriae</i> , <i>S. boydii</i>	De Britto et al. [134]
Leaf	Diethyl ether extract	Disk diffusion assay	<i>E. coli</i> , <i>S. aureus</i>	Shyma et al. [138]
Leaf	Alcohol, chloroform extracts	Broth microdilution assay	Gram positive and gram negative bacteria	Shariff et al. [148]
Callus	Alcohol, benzene, chloroform, methanol extracts	Broth microdilution assay	Gram positive and gram negative bacteria	Shariff et al. [148]
Leaf	Methanol, aqueous, ethyl acetate, chloroform extracts	Disk diffusion assay	Gram positive and gram negative bacteria	Ashokkumar et al. [139]
Whole plant	Ethanol extract	Disk diffusion assay	<i>S. aureus</i> , <i>E. coli</i>	Gorai et al. [149]
Leaf	Aqueous, alcohol, chloroform extracts	Disk diffusion assay	Gram positive and gram negative bacteria	Patel et al. [128]
Leaf	Ethanol extract	Disk diffusion assay	Gram positive and gram negative bacteria	Abubacker and Vasantha [141]
Leaf	Aqueous, methanol extracts	Agar well diffusion assay	Gram positive and gram negative bacteria	Kavitha et al. [126]
Aerial parts	Methanol extract	Disk diffusion assay	Gram positive and gram negative bacteria	Vaghasiya [150]
Leaf	Ethanol extract	Disk and well diffusion assays	<i>E. coli</i> , <i>B. cereus</i> , <i>S. typhi</i>	Chandra and Vignesh [124]

#### Antifungal activity

Several studies conducted on antifungal potential of *R. tetraphylla* revealed its effectiveness against a panel of human and phytopathogenic fungi including dermatophytes and seed-borne fungi. The study

carried out by Kumaran and Kannabiran [151] revealed mycotoxic effect of ethanol extract obtained from roots of *R. tetraphylla* against the growth of *Colletotrichum capsici*. Aqueous leaf extract of *R. tetraphylla* was effective in causing



antifungal activity against *Fusarium indicus* and *Aspergillus flavus* dose dependently [129]. Ethanol extract from leaves was effective against human pathogenic fungi viz. *C. albicans*, *M. canis*, *T. rubrum*

and *Cryptococcus* sp [132]. More information on antifungal potential of *R. tetraphylla* is given in Table 5.

**Table 5: Antifungal activity of *R. tetraphylla***

Part	Extract	Method	Activity against	Reference
Leaf (in vitro cultured)	Ethanol extract	Disk diffusion assay	<i>Candida albicans</i> , <i>Cryptococcus</i> sp., <i>T. rubrum</i> , <i>M. canis</i>	Nandhini and Bai [123]
Root	Aqueous, ethanol, ethyl acetate extracts	Poisoned food technique	<i>Alternaria solani</i> and <i>Fusarium moniliforme</i>	Sangvikar [152]
Leaf	Ethanol extract	Poisoned food technique	<i>Aspergillus niger</i> and <i>Penicillium</i> spp	Suresh et al. [140]
Leaf	Methanol extract	Well diffusion assay	<i>A. niger</i> , <i>A. flavus</i> and <i>Rhizopus indicus</i>	Kavitha et al. [126]
Leaf and callus	Alcohol, chloroform extracts	MIC determination	<i>Aspergillus ochraceous</i> , <i>A. flavipes</i> , <i>Fusarium verticilloides</i> and <i>Penicillium</i> sp	Shariff et al. [148]
Root	Ethanol extract	Conidial germination inhibition assay and poisoned food technique	<i>Colletotrichum capsici</i>	Kumaran et al. [153]
Root	Methanol-water extract	Poisoned food technique	<i>Aspergillus flavus</i>	Shukla et al. [154]

#### Anti-inflammatory activity

Rao et al. [144] investigated anti-inflammatory activity of various solvent extracts of root bark of *R. tetraphylla* by carrageenan induced rat paw edema model. Among extracts, hydro-alcoholic and methanol extract displayed significant reduction in paw edema when compared to hexane and ethyl acetate groups.

#### Allelopathic activity

Mandal et al. [155] studied allelopathic effect of aqueous root extract of *R. tetraphylla* in *Cicer arietinum* seeds. Treatment of extract (100mg/ml) was shown to promote germination of seeds, vigour index, and seedling weight. Besides, an increase in total sugar, soluble protein, amino acid, DNA and RNA content was also observed. The study of Sangvikar and Wadje [156] revealed the positive effect of various solvent extracts of *R. tetraphylla* on seedling emergence of maize.

#### Anxiolytic activity

Singh et al. [137] evaluated anxiolytic activity of ethanol extract of *R. tetraphylla* leaves by elevated plus maze model in mice. The extract was shown to exhibit significant anxiolytic activity. Extract treatment resulted in a significant increase in the time spent on open arm, open arm entries % and % time spent.

#### Antihypertensive activity

Gadhvi et al. [157] evaluated antihypertensive activity of *R. tetraphylla* in rats. Sodium chloride was administered in animals to elevate systolic, diastolic and mean arterial blood pressure. Administration of methanolic root extract resulted in significant decrease in systolic pressure.

#### Antihemolytic activity

Maheshu et al. [158] evaluated antihemolytic activity of methanol extract of *R. tetraphylla* leaves using cow RBC. The extract was shown to cause concentration dependent inhibition of hemolysis of RBC with IC<sub>50</sub> value of 135µg extract/ml.

#### Anti-venom activity

Rajesh et al. [159] screened aqueous and methanol extracts of *R. tetraphylla* root for venom detoxifying activity in mice against crude venom obtained from Indian cobra. Both extracts did not protect the mice from the lethal dosage of cobra venom.

#### Cytotoxic activity

The study of Kakad and Dhembare [160] revealed cytotoxic activity of leaf extract of *R. tetraphylla* against chick embryo fibroblast cell line with cell viability of 50.54%. Behera et al. [127] evaluated cytotoxic potential of *R. tetraphylla* by brine shrimp lethality assay. Solvent extracts of leaf and fruit displayed dose dependent mortality of shrimps.

Kavitha et al. [161] revealed cytotoxic nature of fruit of *R. tetraphylla* by using *Allium cepa* chromosome aberration assay. It was observed that the fruit extract had a significant effect on the mitotic index and induced chromosomal aberrations in a dose dependent manner. In a study, five indole alkaloids viz. Rauvotetraphyllines A-E (Figure 4) isolated from aerial parts of *R. tetraphylla* were screened for in vitro cytotoxicity against cancer cell lines HL-60,

SMMC-7721, A-549, MCF-7, and SW-480 by MTT method. All 5 compounds were inactive and showed  $IC_{50}$  values of  $>40\mu M$  [117]. Similarly Rauvotetraphyllines F-H, 17-*epi*-rauvtetraphylline F and 21-*epi*-rauvtetraphylline H (Figure 5) isolated from the aerial parts of *R. tetraphylla* were shown to be inactive against cancer cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines with an  $IC_{50}$  value of  $>40\mu M$  [118].

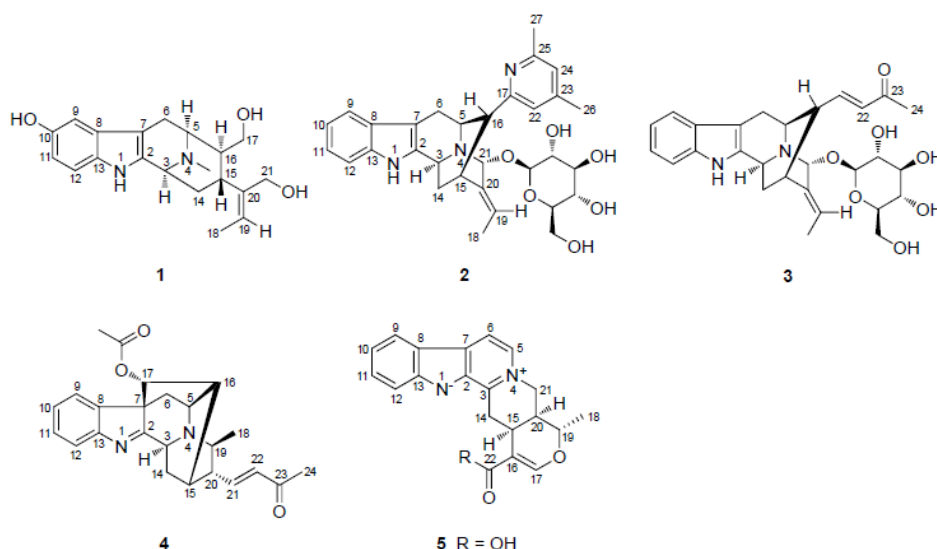


Figure 4: Structures of Rauvotetraphyllines A-E (1-5)

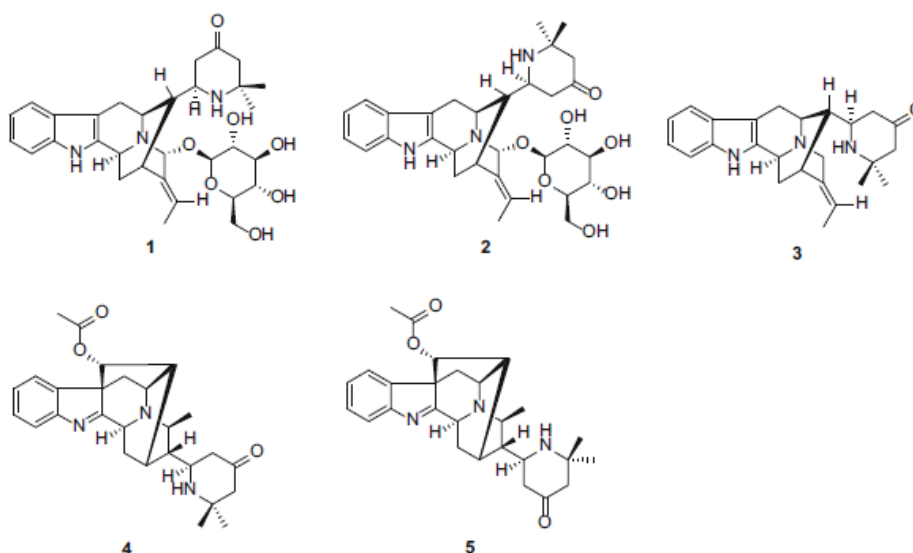


Figure 5: Rauvotetraphyllines F-H (1, 3, 4), 17-*epi*-rauvtetraphylline F (2) and 21-*epi*-rauvtetraphylline H (5)

#### Mutagenic activity

Tamboli and Pandit [162] determined mutagenic effect of leaf extract of *R. tetraphylla* (3, 10, 33, 100, 330, 1000, 3330 and 5000  $\mu g/plate$ ) by Ames test

using *S. typhimurium* (TA98, TA100, TA1535, TA1537) and *E. coli* (WP2uvrA) tester strains. It was observed that the methanolic extract did not showed biologically significant fold increase in revertant

colonies of tester strains indicating nonmutagenic nature of leaf extract.

#### Sedative activity

Madawala et al. [163] determined sedative activity of crude extract obtained from *R. tetraphylla* root bark by rat hole board technique using rats. Extract administration resulted in a significant and dose dependent decrease in the locomotory activity, number of rears and number of head dips in rats.

Further, a decrease in faecal boluses was also observed.

#### Antioxidant activity

Various parts viz. leaf, root and fruit of *R. tetraphylla* are shown to exhibit antioxidant activity. Methanol extract obtained from the fruits of *R. tetraphylla* was effective in scavenging DPPH radicals in a dose dependent manner [164]. More information on antioxidant potential of *R. tetraphylla* is presented in Table 6.

**Table 6: Antioxidant activity of *R. tetraphylla***

Part	Extract	Activity	Reference
Leaf	Methanol extract	Metal chelating, DPPH scavenging, superoxide scavenging, total antioxidant and reducing power assays	Nair et al. [165]
Leaf	n-hexane, methanol, dichloromethane extracts	Total antioxidant activity	Vinay et al. [135]
Root	methanol extract	Total antioxidant activity	Vinay et al. [135]
Leaf	Methanol extract	DPPH assay	Archana and Jeyamanikandan [133]
Leaf	methanol extract	DPPH assay, reducing power assay	Shyma et al. [138]
Leaf, fruit	Hexane, chloroform, acetone, methanol extracts	DPPH scavenging assay, nitric oxide scavenging assay	Behera et al. [127]
Leaf	Methanol extract	DPPH scavenging, ABTS scavenging, reducing power assays	Maheshu et al. [158]
Leaf	Ethanol extract	DPPH assay	Chandra and Vignesh [124]

#### Insecticidal activity

Methanolic extract of roots of *R. tetraphylla* was shown to exhibit insecticidal activity in terms of larvicidal activity against the larvae of *Musca domestica*. The activity observed was dose dependent. The protein and nucleic acid content in larvae was considerably reduced [130].

#### Antiulcer activity

Mitra et al. [166] evaluated antiulcer activity of *R. tetraphylla* leaves against ethanol induced gastric ulcer in rats. The extract failed to exhibit antiulcer activity in rats.

#### Platelet antiaggregant activity

Villar et al. [167] revealed the platelet antiaggregant activity of aqueous extract obtained from *R. tetraphylla* roots against platelet aggregation induced by thrombin with an IC<sub>50</sub> value of 0.893mg/ml.

#### Enzyme inhibitory activity

Methanol extract obtained from leaves of *R. tetraphylla* was shown to exhibit inhibitory activity against  $\alpha$ -amylase. An inhibition of 87% of amylase activity was observed in the study [133].

#### Antipsychotic activity

Six indole alkaloids viz.  $\alpha$ -yohimbine, reserpiline, 10-methoxytetrahydroalstonine, isoreserpiline, an isomeric mixture of 11-demethoxyreserpiline and 10-demethoxyreserpiline, isolated from leaves of *R. tetraphylla* (Figure 6) were screened for antipsychotic activity by amphetamine induced hyper active mouse model. Among alkaloids, four alkaloids viz. 11-demethoxyreserpiline, 10-demethoxyreserpiline,  $\alpha$ -yohimbine and reserpiline displayed significant antipsychotic activity in a concentration dependent manner [112].

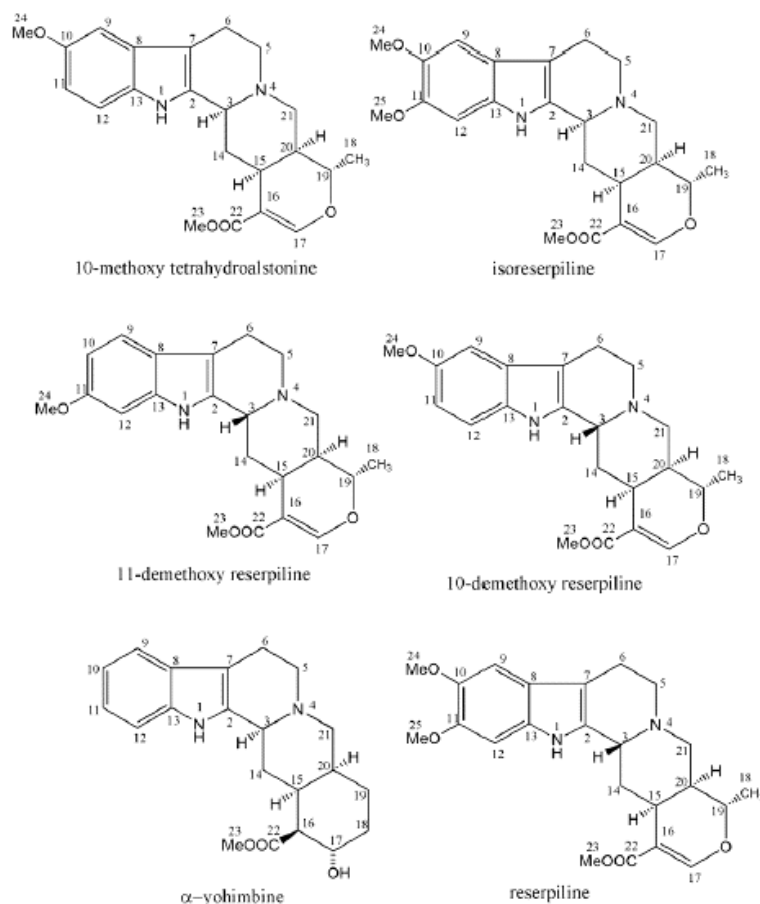


Figure 6: Structures of alkaloids having antipsychotic activity [112]

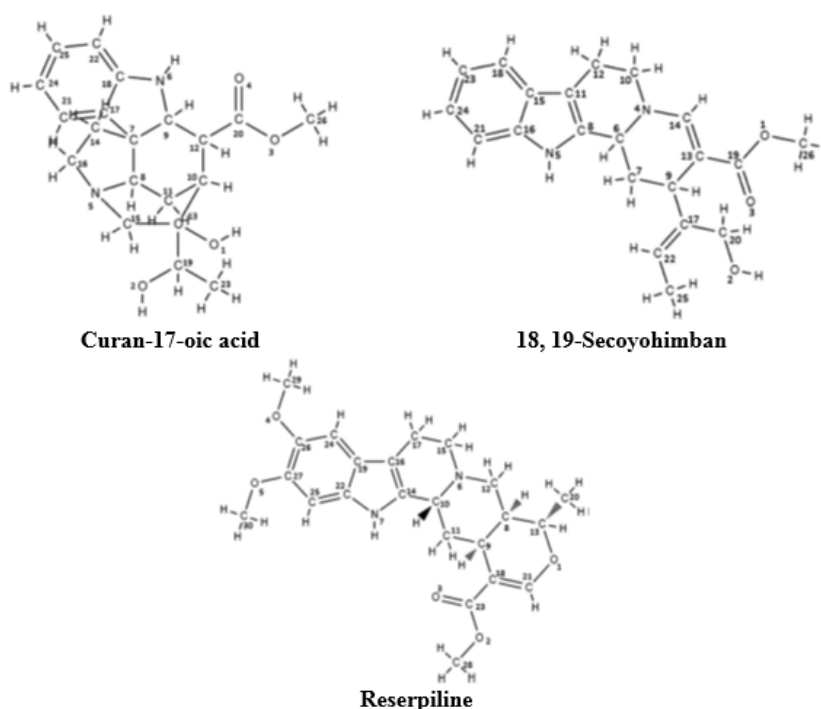
#### Cardioprotective activity

Nandhini and Bai [168] evaluated cardioprotective activity of *R. tetraphylla* in rats induced with myocardial infarction by isoproterenol hydrochloride. Oral administration of leaf extract revealed significant reduction in the isoproterenol induced rise in the biochemical levels and prevented the fall of GSH levels indicating cardioprotective activity. Restoration of endogenous antioxidants, controlling of lipid peroxide formation and preservation of histo-architecture of myocytes was observed in extract treated animals.

#### Antiparasitic activity

Antifilarial activity of solvent extracts of *R. tetraphylla* leaves was evaluated against *Setaria cervi*. Among extracts, methanol extract revealed significant activity with 89.28% reduction at 10mg/ml concentration. Extract was also inhibited glutathione-S-transferase (GST) enzyme activity

significantly [169]. Mandal and Nandi [170] investigated the control of *Meloidogyne incognita* by ethanol extract from different parts viz. fruit, root and leaf of *R. tetraphylla*. At 2mg/ml concentration, the extracts caused marked mortality in the juveniles of *M. incognita* after 6 hours exposure. Foliar application of root and fruit extracts to tomato promoted the plant growth and also reduced nematode infestation. Behera and Bhatnagar [121] isolated three alkaloid compounds viz. 18, 19-secoyohimban, curan-17-oic acid and reserpiline from leaves of *R. tetraphylla* (Figure 7) and investigated there in vitro antifilarial activity against bovine filarial parasite *S. cervi* which is supported by in silico docking analysis on GST enzyme of *Wuchereria bancrofti*. All three alkaloids were effective in causing antifilarial activity as well as causing inhibition of GST enzyme.



**Figure 7: Structures of antifilarial alkaloids [121]**

### Immune enhancing activity

Yogeshwari et al. [171] studied the effect of diet containing *R. tetraphylla* against *Aphanomyces invadans* infection in *Labeo rohita*. The innate immune response and disease resistance in fishes was studied. Fishes that were fed with diet containing *R. tetraphylla* revealed a significant increase in WBC, biochemical parameters, phagocytic, and lysozyme activity, the respiratory burst activity, complement activity, and myeloperoxidase activity. Moreover, the diet also boosted the antioxidant activity significantly indicating the ability of diet to enhance innate immunity and confers disease resistance.

### 5. CONCLUSION

An intensive literature survey conducted in this review highlighted potential utilization of *R. tetraphylla* ethnomedicinally in different parts of the country. Besides, a vast literatures have also revealed pharmacological activities such as antimicrobial, anxiolytic, antioxidant and anti-inflammatory activities. *R. tetraphylla* is shown to possess similar commercial and therapeutic properties as that of *R. serpentina*. The presence of various chemicals such as reserpine, quercetin, and others, particularly alkaloids, might have been responsible for bioactivities of the plant. *R. tetraphylla* seems to be a potential candidate for developing novel therapeutic agents. The species is

turning to an extremely endangered because of indiscriminate collection and inadequate plantation. Hence, it is very much more important to conserve this species and to grow this species at bigger scale for its possible utilization.

### ACKNOWLEDGEMENTS

Authors express sincere thanks to Principal, S.R.N.M.N College of Applied Sciences, and N.E.S, Shivamogga for constant encouragement. Authors also thank Mr. Sudarshan S.J, Research scholar, Pondicherry University, Pondicherry for providing useful literatures on the plant.

### SOURCES OF FUNDING

None

### CONFLICTS OF INTEREST

None declared

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