

A comparative review of phytochemistry, applications in medicine and pharmacological potential of antimicrobial and anti-inflammatory activity between two species of Genus *Rauvolfia*: *Rauvolfia serpentina* and *Rauvolfia tetraphylla*

ABSTRACT

Aims: This review article aims to provide a comparative analysis of phytochemistry, medicinal applications and pharmacological potential of antimicrobial and anti-inflammatory activity of *Rauvolfia serpentina* and *Rauvolfia tetraphylla*

Scope of review: Review data from published literature and assess about phytochemicals, therapeutic applications and efficacy of *Rauvolfia serpentina* and *Rauvolfia tetraphylla* against microorganisms and inflammation comparatively.

Methodology: Research papers and review articles on *Rauvolfia serpentina* and *Rauvolfia tetraphylla* were collected and analyzed about phytochemicals, medicinal uses, antibacterial and inflammatory properties.

Summary of findings: *Rauvolfia serpentina* and *Rauvolfia tetraphylla* have many similarities in its phytochemistry and they have been used to treat similar diseases and illness conditions.

Conclusion: According to the findings, due to endangered status of *Rauvolfia serpentina*, *Rauvolfia tetraphylla* can be used as an ideal substitute and can be used for developing novel

Keywords: *Rauvolfia serpentina*, *Rauvolfia tetraphylla*, phytochemistry, antimicrobial activity, anti-inflammatory activity, alkaloids

1 INTRODUCTION

The connection between nature and humanity can be defined as a deeply intertwined relationship (Ayele *et al.*, 2024). Humans have been dependents on plants and trees for essential resources such as food, shelter, clothing and also medicine for their survival since time immemorial (Gurib-Fakim, 2006). In fact, plants have been considered as gifts of gods and worshipped by Hindus as a matter of gratitude, since they were vital for the existence of mankind (Wani *et al.*, 2020).

Medicinal plants are rich in bioactive compounds which are produced from either primary or secondary metabolism. These compounds are mainly alkaloids, tannins, terpenoids, carbohydrates, steroids and flavonoids. (Yadav and Agarwala, 2011). These metabolites are known as phytochemicals. Secondary metabolites are non-nutritive to the plant but they enhance the ability of the plant to withstand unfavorable external environment conditions and defense against various pathogenic bacteria, fungi, viruses and insects. However, these phytochemicals also play a major role in the healthcare system in humans in different ways due to its diverse functions that have pharmaceutical value (Bitwell *et al.*, 2023) as well as therapeutic effect on humans (Yadav and Agarwala, 2011).

Antimicrobials (antibiotics, antifungals, antivirals and antiparasitics) are medicines that are utilized to treat and prevent infections in humans and even other animals and plants. Due to misuse and overuse of such drugs antimicrobial resistance has emerged as a global concern affecting the healthcare system and economy (World Health Organization, 2023). Bacterial resistance is one of the growing issues in the world and has led to rapid increase of multidrug resistant bacteria (MDR). Therefore, novel finding of new drugs from plants have become a trend nowadays (Samaraweera *et al.*, 2023).

Inflammation is a crucial reaction that occurs in the body which is a defense mechanism that protects against infections, burn, toxic chemical allergens and various harmful stimuli (Dharmadeva *et al.*, 2018). When inflammation is not controlled properly, it damages the tissue. Anti-inflammatory drugs such as NSAIDs (Non-steroidal anti-inflammatory drugs) come into play to regulate excessive inflammation and prevent tissue damage but they can cause dangerous side effects such as congestive heart failure and increased blood pressure. Therefore, it is important to discover new anti-inflammatory agents that have minimum side effects. Plants possess phytochemicals like flavonoids, terpenoids and polyphenols that may be effective for inflammation (Senadeera *et al.*, 2021).

Rauvolfia serpentina and *Rauvolfia tetraphylla* are plants that are classified under the same genus *Rauvolfia*. They have been utilized as a folklore medicine, since they possess an immense medicinal property that could be a treatment for hypertension, cardiovascular diseases, snakebites, digestive system, metabolic and central nervous system disorders (Kumar *et al.*, 2022). These two species are very important among other species of genus *Rauvolfia* since they contain crucial active biological compounds within themselves that have certain biological functions. *Rauvolfia tetraphylla* is often used as a substitute for *Rauvolfia serpentina* in therapeutic purposes (Mahalakshmi *et al.*, 2019).

2 PLANT MORPHOLOGY

2.1 *Rauvolfia serpentina* plant morphology (Figure 1 and Figure 2)

Rauvolfia serpentina is a small, woody, evergreen shrub that has about 60 cm of maximum height which is identified as a critically endangered species (Kumari *et al.*, 2013; Sulaiman *et al.*, 2020). This plant possesses cylindrical stems and barks are pale in appearance. Leaves are lanceolate or elliptical shaped that are pale green in colour and whorls of three. This plant contains light coloured viscous latex. *Rauvolfia serpentina* carries fruits that are small and fleshy which is green in colour that turns into purple-black when

ripened. The roots are tuberous and usually 3-7 inches long and they are lens-shaped and clustered (Perera *et al.*, 2023). Roots are greyish to yellow in colour (Srivastava *et al.*, 2021).



Figure 1: Plant *Rauvolfia serpentina*



Figure 2 : Flowers and fruits of *Rauvolfia serpentina*

2.2 *Rauvolfia tetraphylla* plant morphology (Figure 3)

Rauvolfia tetraphylla is a small tree shrub that has about 6 feet (~2 meters) of height (Rao *et al.*, 2012). It is whorled in 4, unequally-sized, 5-9 x 3-4 cm, elliptic-ovate shaped leaves. They are dark green in colour and their apex is acute and the base is round. Leaves have a characteristic odor and smooth textured. Both surfaces are pubescent. Stems are round, rough and hairy, green on the outer surface and internally creamy yellow. Roots are sub cylindrical shaped, curved, 8-15 cm long and have about 0.5-2 cm thickness. Outer surface is usually grayish-brown to reddish-brown in colour (Jakaria *et al.*, 2016). Flowers are creamy in colour and flowering can be observed throughout the year. This plant possesses 2 seeded ovoid shaped drupes which turns into purple in colour when ripened (Mahalakshmi *et al.*, 2019).



Figure 3: Plant *Rauvolfia tetraphylla*

3 PHYTOCHEMISTRY

3.1 Phytochemistry of *Rauvolfia serpentina*

Rauvolfia serpentina is considered an ethnomedicinally valued plant due to its richness of many phytochemicals. Many studies have been conducted extensively and explored various types of chemicals within different parts of the plant. Alkaloids, tannins, flavonoids, phenols are some of the key secondary metabolites found in *Rauvolfia serpentina* (Kumari *et al.*, 2013; Perera *et al.*, 2023).

3.1.1 Alkaloids

More than 50 types of alkaloids have been explored in this plant (Lobay, 2015). They are categorized as; Indole alkaloids, Indolenine alkaloids, Oxindole alkaloids and Pseudo indoxyl alkaloids (Srivastava *et al.*, 2021). Out of these, indole alkaloids are the most crucial type. Reserpine, Ajmaline, Ajmalicine and Yohimbine are some of the identified alkaloids within this plant (Srivastava *et al.*, 2006; Agrawal, 2019).

3.1.1.1 Reserpine

Reserpine is one of the most crucial and prevalent alkaloids present in *Rauvolfia serpentina*. The amount of reserpine in roots are higher compared to leaves and stems of the plant (Lobay, 2015). This alkaloid has a higher neurotherapeutic effect and it is also used as a natural tranquillizer. It is effective in treating and management of cardiovascular diseases, hypertension and neurological diseases. The antihypertensive effect of Reserpine in central nervous system and peripheral nervous system is demonstrated by binding to catecholamine storage vesicles in nerve cells, disrupting the normal catecholamine and serotonin storage leading to decrease in their levels. This impacts on autonomic nervous system to reduce neurotransmitters from adrenergic neurons and activate the central parasympathetic system (Kumari *et al.*, 2013).

3.1.1.2 Ajmaline

Ajmaline is derived from roots as a class I antiarrhythmic agent utilized in diagnosing “Brugada Syndrome” and to differentiate the subtypes of patients having this syndrome. Ajmaline is given intravenously for such patients during the “Ajmaline test”, causing sodium channels to be blocked instantly (Kumari *et al.*, 2013).

3.1.1.3 Ajmalicine

Ajmalicine reduces blood pressure and even prevents strokes by acting on smooth muscles. It maintains the normal cerebral blood flow (Kumari *et al.*, 2013).

3.1.1.4 Yohimbine

It is an alpha blocker that treats erectile dysfunction by dilating the blood vessels and increasing the blood flow to the penis by relaxing the smooth muscles in tunica media (Kumari *et al.*, 2013; Perera *et al.*, 2023).

3.1.1.5 Serpentine

Serpentine is derived from oxidation of Ajmalicine by peroxidase enzyme. It is a type II topoisomerase inhibitor that shows antipsychotic effects. It also possesses antihypertensive activity which is similar to that of Ajmaline (Kumari *et al.*, 2013).

3.1.2 Tannins

Tannins possess stringent properties which speeds up the wound healing and has anti-inflammatory properties (Kumari *et al.*, 2013).

3.1.3 Flavonoids

Flavonoids have anticancerous activity and also prevents oxidative damage of cells thanks to its antioxidative properties and free radical scavenging activity (Kumari *et al.*, 2013). Kaempferol which is a flavonoid isolated in *Rauvolfia serpentina* leaves possesses both anti-oxidative activity as well as potent anti-inflammatory properties (Thors *et al.*, 2008; Gupta and Gupta, 2015).

3.1.4 Phenols

Phenols are secondary metabolites that prevent the growth of pathogens and pests. Hence, it has a potent to be utilized as an antimicrobial agent. *Rauvolfia serpentina* shows antidiabetic and hypolipidemic effects due to presence of total phenolic compounds in higher amounts (Kumari *et al.*, 2013).

3.1.5 Saponins

Saponins are characterized by formation of foams in aqueous solutions, hemolytic activity, bitterness and cholesterol binding properties. They possess the anticoagulating activity of red blood cells therefore, this plant is utilized to treat preventing of bleeding and wound healing due to its higher amounts of Saponins in the plant (Kumari *et al.*, 2013).

3.2 Phytochemistry of *Rauvolfia tetraphylla*

Rauvolfia tetraphylla contains an innumerable number of phytochemicals especially alkaloids. Most crucial alkaloid found in this plant is also Reserpine. In addition to alkaloids, many phytochemical groups have been discovered such as tannins, phenols, steroids, saponins, flavonoids and terpenoids within different parts of the plant (Mahalakshmi *et al.*, 2019).

3.2.1 Alkaloids

Root of the plant *Rauvolfia tetraphylla* contains approximately 30 different types of alkaloids. Some of them are Reserpine, Reserpinine, Ajmalicine, Ajmalidine, neo Ajmaline, Deserpidine, Sarpagine, Rescinnamine, Serpentine, Alloyohimbine, Corynathine, Chandrine, Iscajmaline, Yohimbine, Thambine, Serpinine, Reserpoxidine, Reserpinine, Reserpiline, Rauwolscine, Raunoline, Raunatine and Papaverine (Jakaria *et al.*, 2016).

3.2.1.1 Reserpine

Reserpine is abundantly present in roots whereas leaves and stems contain comparatively lower amounts (Mahalakshmi *et al.*, 2019). Reduced blood pressure and acts as a tranquilizer (Jakaria *et al.*, 2016).

3.2.1.2 Serpentine

It acts as a weak hypotensive agent (Jakaria *et al.*, 2016).

3.2.1.3 Sarpagine

An alkaloid that has a fleeting effect on blood pressure (Jakaria *et al.*, 2016).

3.2.1.4 Yohimbine

Acts as a hypotensive agent, a cardiovascular depressant and a hypnotic agent. Alpha- yohimbine (α -yohimbine) is one of the indole alkaloids that possesses antipsychotic activity (Isoreserpiline and 10-methoxy tetrahydroalstonine are other indole alkaloids that has the antipsychotic activity found in *Rauvolfia tetraphylla*) (Jakaria *et al.*, 2016).

3.2.1.5 Ajmaline

Used in for the treatment of arrhythmic heart diseases. Also used to provoke intestinal movement and respiration (Jakaria *et al.*, 2016).

3.2.1.6 Recanescine

Another alkaloid isolated from *Rauvolfia tetraphylla* that lacks methoxyl group in the C-II position of Reserpine. It possesses sedative and hypotensive properties (Mahalakshmi *et al.*, 2019).

3.2.1.7 Pseudoreserpine

Isolated from roots of the plant that has sedative and hypotensive properties (Mahalakshmi *et al.*, 2019).

3.2.2 Flavonoids

Flavonoids are found in treating acute inflammation. Additionally, it has the inhibition ability against phospholipase A2, protein kinase C and protein Tyrosine kinases (Merlin *et al.*, 2020).

3.2.3 Terpenoids

Influence key signaling pathways which are associated with inflammatory responses such as nuclear transcription factor – Kappa B activation (NF – Kappa B) (Merlin *et al.*, 2020).

3.2.4 Other phytochemical groups

Many studies have been conducted and isolated tannins, saponins, cardiac glycosides, glycosides, steroids, phenols, triterpenoids, coumarin, catechin, fixed oils, fats, gums are mucilages within various parts of the plant (Jakaria *et al.*, 2016; Mahalakshmi *et al.*, 2019).

4 MEDICINAL USE

4.1 Medicinal use of *Rauvolfia serpentina*

Rauvolfia serpentina has a wide range of therapeutic effects. It is mainly effective in treatment of hypertension, anxiety, epilepsy, insomnia, psychotic disorders like schizophrenia and insanity. It is also used as a sedative and a hypnotic drug. Root extract of the plant is valued for intestinal disorders like diarrhea, dysentery and also as anathematic. Cholera, cholic and fever have been treated when combined with other plant extracts. It was believed that roots stimulate the uterine contractions and they were recommended during child birth. The juice of the leaves has been utilized as a remedy for the opacity of cornea. Gastrointestinal and circulatory disorders can be treated with juice of *Rauvolfia serpentina* juice and extract obtained from the roots. Juice acquired from tender leaves and root extract have been used to treat liver discomfort, stomach pain, dysentery and to eliminate intestinal worms. It is also used for the treatment of cancers (Malviya and Sason, 2016).

4.2 Medicinal use of *Rauvolfia tetraphylla*

Rauvolfia tetraphylla has a significant therapeutic effect with many medicinal applications. The whole plant is used as a remedy for scorpion bites, snake bites and insect bites and as well as an antidote and sedative agent. This plant is used as a substitute for *Rauvolfia serpentina* as a treatment for circulatory disorders. It can be also utilized as an antimicrobial agent. *Rauvolfia tetraphylla* is also utilized to treat skin infections.

Leaves are used to treat eye problems, tooth infections, cough, cold, and skin diseases. Root paste with milk or honey is used to treat mental illnesses and hypertension. To increase uterine contractions during labor, roots are used. Stomach aches, diarrhea, diabetes mellitus, malaria and muscle and joint pains are also treated with roots of the plant. Moreover, the roots have the ability to enhance libido and treat impotence (Erectile dysfunction). The resin is used as an antitussive agent. It also accelerates defecation and cures hydropsy. As a remedy for intestinal worms *Rauvolfia tetraphylla* fruits are used (Pandith and Devi, 2024).

5 COMPARATIVE ANALYSIS OF ANTIMICROBIAL ACTIVITY BETWEEN *RAUVOLFIA TETRAPHYLLA* AND *RAUVOLFIA SERPENTINA*

5.1 Antimicrobial activity of *Rauvolfia serpentina*

Rauvolfia serpentina aqueous leaf extract (RSALE) and encapsulated gold nanoparticles (R-AuNPs) were utilized to determine its antibacterial activity using disc diffusion method and obtained Minimum Inhibitory Concentration (MIC) against *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Bacillus subtilis* and *Proteus vulgaris*. Compared to RSALE and Amoxicillin (control), R-AuNPs gave significantly good antibacterial activity for all tested organisms except *Proteus vulgaris*. The MIC values that were obtained suggested that lower doses of R-AuNPs were significantly more effective than RSALE and Amoxicillin (Alshahrani *et al.*, 2021).

Methanol extract of *Rauvolfia serpentina* and few other plant species (*Tagetes erecta*, *Brassica nigra* and *Ocimum tenuiflorum*) were subjected to evaluate the antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* by disc diffusion method. The test was performed from leaves with different ages. Out of those four plants *Rauvolfia serpentina* exhibited the best antibacterial activity for the tested strains. However, *Salmonella typhi* had not shown any inhibition zone for all tested plants. Results demonstrated that along the advancement of age of *Rauvolfia serpentina* leaves, the antibacterial activity also increased (Rashid *et al.*, 2014).

Antimicrobial activity was determined from methanol, chloroform, hexane and aqueous extract of *Rauvolfia serpentina* roots against *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae* by agar well diffusion method and determined the Minimum Inhibitory Concentration (MIC). Methanol extract showed more effective antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus*, chloroform extract showed activity against *Enterococcus faecalis* and *Aspergillus niger* and aqueous extract demonstrated activity against *Streptococcus pneumoniae*. Another study demonstrated that methanol extract possessed the best antimicrobial activity against all tested organisms compared to aqueous, chloroform and hexane extracts. Methanol extract also exhibited the lowest MIC against *Staphylococcus aureus* (Owk and Lagudu, 2016).

5.2 Antimicrobial activity *Rauvolfia tetraphylla*

Hexane, ethyl acetate, methanolic and 70% ethanol root bark extract of *Rauvolfia tetraphylla* were tested for its antibacterial activity, comparing with the standard chloramphenicol (30µg/cup) by using cylinder plate assay. All tested extractions at every concentration (50µg/cup, 100µg/cup and 150µg/cup) except hexane, showed significantly good antibacterial activity against *Streptococcus pneumoniae*, *Bacillus cereus*, *Bacillus pumilis*, *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Streptomyces marienensis*. However, hexane extract on dose 50µg/ml had not shown any antibacterial activity against *Streptococcus pneumoniae*, *Enterobacter aerogens* and *Streptomyces mariensis* (Rao *et al.*, 2012).

Leaf and callus extract of *Rauvolfia tetraphylla* obtained from solvents such as benzene, chloroform, absolute alcohol, methanol and petroleum ether were subjected to test against bacterial species like *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas solanacearum*, *Xanthomonas vesicatoria* and fungal species like *Aspergillus ochraceous*, *Aspergillus flavipes*, *Fusarium verticilloides* and *Penicillium sp.*, using broth microdilution method. Out of these solvents both absolute alcohol and chloroform found to be more effective against both bacteria and fungi (Shariff *et al.*, 2006).

Rauvolfia tetraphylla methanolic extract against *Salmonella typhimurium*, *Escherichia coli*, *Citrobacter freundii*, *Proteus vulgaris*, *Enterococcus faecalis* and *Staphylococcus aureus* bacterial strains were tested using well diffusion method and observed for Minimum Inhibitory Concentration (MIC). The highest inhibition zone with lowest MIC was shown by *Staphylococcus aureus* and lowest inhibition zone was shown by *Salmonella typhimurium* whereas highest MIC was shown by *Escherichia coli*. *Proteus vulgaris* did not show any inhibition zone (Negi *et al.*, 2014).

The ethyl acetate extract of the leaves obtained from *Rauvolfia tetraphylla* were tested against *Shigella dysenteriae* and *Salmonella typhi* using anti-bacterial assay-agar disk diffusion method had shown a good inhibitory activity. The zone of inhibition was increased with the increasing of plant concentration (Krishnasamy *et al.*, 2023).

Evaluation of antimicrobial activity of aqueous and methanolic extracts of *Rauvolfia tetraphylla* leaf against bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Leuconostoc lactis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and fungi (*Aspergillus niger*, *Aspergillus flavus*, *Rhizopus indicus* and *Mucor indicus*) through the well diffusion method. *Streptococcus pyogenes* exhibited the highest zone of inhibition in methanol extract compared to aqueous extract. Except *Mucor indicus*, all other fungal strains showed some antifungal activity for methanolic extraction whereas all four fungal strains did not show any antifungal activity for aquatic extraction (Kavitha *et al.*, 2012).

6 COMPARATIVE ANALYSIS OF ANTI-INFLAMMATORY ACTIVITY BETWEEN RAUVOLFIA TETRAPHYLLA AND RAUVOLFIA SERPENTINA

6.1 Anti-inflammatory activity of *Rauvolfia serpentina*

In the evaluation of chemical composition of *Rauvolfia serpentina*, flavonoids were found to be present (1.65±0.12 mg/100 g). Since flavonoids act as antioxidants they provide anti-inflammatory activity (Bhardwaj and Yadav, 2016). Gupta and Gupta (2015) used *Rauvolfia serpentina* leaves to isolate and extract flavonoids and determine its antioxidant potential through DPPH-scavenging method (Radical scavenging activity showed 62.5% in leaves of *Rauvolfia serpentina*). They succeeded in finding the structure of a flavonoid called Kaempferol (3,5,7,4'-tetrahydroxy flavones) using chromatographic and spectrometry techniques. Kaempferol is a natural antioxidant that has anti-inflammatory activity (Gupta and Gupta, 2015). It inhibits the activity of fatty amide hydrolase enzyme (FAAH) acting as a very good anti-inflammatory agent (Thors *et al.*, 2008).

6.2 Anti-inflammatory activity of *Rauvolfia teraphylla*

Rao *et al.* (2012) utilized Carrageenan induced rat paw oedema model, to evaluate the anti-inflammatory activity of *Rauvolfia tetraphylla* by measuring the thickness of rat paws and it was found that the plant has potent anti-inflammatory effect. In this study, root bark extract prepared from solvents; methanol, hexane, ethyl acetate and 70% ethanol were used at several concentrations (200 mg/kg, 400 mg/kg, 800 mg/kg for 70% ethanol; 100 mg/kg, 200 mg/kg, 400 mg/kg for other solvents) to be orally treated for rats. The results showed that all the dose concentration of both hydro-alcoholic (70% ethanol; 200 mg/kg, 400 mg/kg, 800 mg/kg) and methanolic (100 mg/kg, 200 mg/kg, 400 mg/kg) extract exhibited significantly better reduction (P<0.001) compared to drug vehicle treated control group. Also, ethyl acetate and hexane root bark extract

exhibited significant reduction activity ($P < 0.001$). This finding supports the folklore use of *Rauvolfia tetraphylla* as a treatment for inflammations and to further improvements and research in medicine related to inflammations (Rao *et al.*, 2012).

According to Merlin *et al.* (2020) results obtained from aqueous root extract of *Rauvolfia tetraphylla* assessed by heat induced egg Albumin denaturation method carries potentially good anti-inflammatory activity that could provide somewhat relief for inflammation and to be further developed into an efficient anti-inflammatory drug, thanks to its synergistic effect of phytochemicals such as flavonoids, phenols and terpenoids (Merlin *et al.*, 2020).

Using protein (Bovine serum Albumin) denaturation assay, anti-inflammatory activity of crude extracts (Chloroform, hexane, ethyl acetate and methanol) of fruit and leaf *Rauvolfia tetraphylla* were evaluated and optimized by utilizing Response Surface Methodology (RSM) by Nallasamy *et al.* (2024), aiming to identify a potential and safer anti-inflammatory drug as an alternative to existing conventional anti-inflammatory drugs. Ethyl acetate fruit extract showed the best anti-inflammatory effect and its optimized extract possessed the promising effectiveness to treat inflammations in safer manner instead of using conventional anti-inflammatory drugs. This study also validated the traditional use of *Rauvolfia tetraphylla* for inflammatory conditions (Nallasamy *et al.*, 2024).

7 CONCLUSION AND FUTURE DIRECTIONS

From the above study we conclude that *Rauvolfia serpentina* and *Rauvolfia tetraphylla* have many similarities in its phytochemistry and they have been used for treating almost same diseases and illness conditions such as hypertension, circulatory disorders, psychotic disorders, skin and eye diseases and elimination of intestinal worms. According to the findings, due to the endangered status of *Rauvolfia serpentina*, *Rauvolfia tetraphylla* can be used as a perfect substitute candidate. In vitro antimicrobial properties and anti-inflammatory of both plants suggest that they can be utilized in drug development to address antimicrobial resistant and to produce anti-inflammatory drugs with mild side effects in the future, but further in vitro and in vivo studies are required to evaluate their efficacy.

8 REFERENCE

1. Ayele, A.H., Seid, A., Mekonnen, A.B., Adnew, W.W. and Yemata, G. (2024) 'Ethnobotanical study of the traditional use of medicinal plants used for treating human diseases in selected districts of West Gojjam zone, Amhara Region, Ethiopia', *Phytomedicine Plus*, 4(3).
2. Gurib-Fakim, A. (2006) 'Medicinal plants: Traditions of yesterday and drugs of tomorrow', *Molecular Aspects of Medicine*, 27(1), pp. 1–93.
3. Wani, A.M., Sahoo, G. and Gupta, S. (2020) 'Sacred Trees of India: Traditional Approach towards Plant Conservation', *International Journal of Current Microbiology and Applied Sciences*, 9(1), pp. 2606–2613.
4. Yadav, R.N.S. and Agarwala, M. (2011) 'Phytochemical analysis of some medicinal plants', *Journal of Phytology*, 3(12), pp. 10–14.
5. Bitwell, C., Indra, S.S., Luke, C. and Kakoma, M.K. (2023) 'A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants', *Scientific African*, 19, p. e01585.
6. World Health Organization. Antimicrobial resistance [Internet]. 2023 [cited 2024 Mar 29]. Available from: <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>
7. Samaraweera, T., Samaraweera, T., Senadeera, N. and Ranaweera, C.B. (2023) 'Evaluation of Antibacterial Activity of Endemic *Jeffreyia zeylanica* Plant Found in Sri Lanka', *South Asian Journal of Research in Microbiology*, 16(1), pp. 1–9.

8. Dharmadeva, S., Galgamuwa, L.S., Prasadinie, C. and Kumarasinghe, N. (2018) 'In vitro anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method', *AYU International Quarterly Journal of Research in Ayurveda*, 39, p. 239-242.
9. Senadeera, S.P.N.N., Fernando, K.S.K., Wickramasekara, W.L.L.N., Fernando, M.Y.S., Ranaweera, C.B., Rajapaksha, W. and Silva, A.R.N. (2022) 'In vitro Anti-inflammatory Activity of Endemic *Artocarpus nobilis* Thw Found in Sri Lanka', *Asian Plant Research Journal*, 8(4), pp. 116–122.
10. Kumar, S., Kumari, D. and Singh, B. (2022) 'Genus *Rauvolfia*: A review of its ethnopharmacology, phytochemistry, quality control/quality assurance, pharmacological activities and clinical evidence', *Journal of Ethnopharmacology*, 295.
11. Mahalakshmi, S.N., Achala, H.G., Ramyashree, K.R. and Prashith, K.T.R. (2019) '*Rauvolfia tetraphylla* L. (Apocynaceae) - A Comprehensive Review on Its Ethnobotanical Uses, Phytochemistry and Pharmacological Activities', *International Journal of Pharmacy and Biological Sciences*, 9(2), pp. 664–682.
12. Kumari, R., Rathi, B., Rani, A. and Bhatnagar, S. (2013) '*Rauvolfia serpentina* L. Benth. ex Kurz: Phytochemical, pharmacological and therapeutic aspects', *International Journal of Pharmaceutical Sciences Review and Research*, 23(2), pp. 348–355.
13. Sulaiman, C.T., Jyothi, C.K., Unnithan, J.K., Prabhukumar, K.M. and Balachandran, I. (2020) 'Identification of suitable substitute for Sarpagandha (*Rauvolfia serpentina* (L.) Benth. ex Kurz) by phytochemical and pharmacological evaluation', *Beni-Suef University Journal of Basic and Applied Sciences*, 9(42), pp. 2–11. <https://doi.org/10.1186/s43088-020-00069-5>.
14. Perera, D.S., Perera, R.P. and Vidhyajini, R.V. (2023) 'Neuro-therapeutic effect of *Rauvolfia serpentina* (Sarpagandha/ Ekaweriya); A review', *Journal of Advances in Experimental Therapeutics and Neurotherapeutics*, 1(2), pp. 7–12.
15. Srivastava, M., Kesharwani, S., Kesharwani, R., Patel, D.K. and Singh, S.N. (2021) 'A review on potential bioactive chemical from *Rauvolfia serpentina*: Reserpine', *International Journal of Research in Ayurveda and Pharmacy*, 12(1), pp. 106–109.
16. Rao, B.G., Rao, P.U., Rao, E.S., Rao, T.M. and Praneeth. D, V.S. (2012) 'Evaluation of in-vitro antibacterial activity and anti-inflammatory activity for different extracts of *Rauvolfia tetraphylla* L. root bark', *Asian Pacific Journal of Tropical Biomedicine*, 2(10), pp. 818–821.
17. Jakaria, M.D., Tareq, S.M., Ibrahim, M. and Bokhtearuddin, S. (2016) '*Rauvolfia tetraphylla* L. (Apocynaceae): A Pharmacognostical, Phytochemical and Pharmacological Review', *Journal of Chemical and Pharmaceutical Research*, 8(12), pp. 114–120.
18. Lobay, D. (2015) '*Rauvolfia* in the Treatment of Hypertension', *Integrative Medicine: A Clinician's Journal*, 14(3), pp. 40–46.
19. Srivastava, A., Tripathi, A.K., Pandey, R., Verma, R.K. and Gupta, M.M. (2006) 'Quantitative determination of Reserpine, Ajmaline, and Ajmalicine in *Rauvolfia serpentina* by Reversed-Phase High-Performance Liquid Chromatography', *Journal of Chromatographic Science*, 44(9), pp. 557–560.
20. Agrawal, S.N. (2019) '*Rauvolfia Serpentina*: A Medicinal Plant of Exceptional Qualities', *Alternative Medicine & Chiropractic Open Access Journal*, 2(2), pp. 1–5.
21. Thors, L., Belghiti, M. and Fowler, C.J. (2008) 'Inhibition of fatty acid amide hydrolase by kaempferol and related naturally occurring flavonoids', *British Journal of Pharmacology*, 155(2), pp. 244–252.
22. Gupta, J. and Gupta, A. (2015) 'Isolation and Extraction of flavonoid from the leaves of *Rauvolfia serpentina* and evaluation of DPPH-scavenging antioxidant potential', *Oriental Journal of Chemistry*, 31, pp. 213–225.
23. Merlin, F., Ratnasooriya, W., and Pathirana, R. (2020) 'Investigation of anti-inflammatory activity of *Rauvolfia tetraphylla* using in vitro protein denaturation assay', *International Journal of Scientific and Research Publications*, 10(5), pp. 963–967.

24. Malviya, A. and Sason, R. (2016) 'The phytochemical and pharmacological properties of Sarpagandha: *Rauwolfia serpentina*', *International Journal of Research in AYUSH and Allied Systems*, 3(1), pp. 473–478.
25. Pandith, A.H. and Devi, N.R. (2024) 'A review of *Rauwolfia tetraphylla* L.: Phytochemistry and Medicinal uses', *Medalion Journal: Medical Research, Nursing, Health and Midwife Participation*, 5(1), pp. 1–8.
26. Alshahrani, M.Y., Rafi, Z., Alabdallah, N.M., Shoaib, A., Ahmad, I., Asiri, M., Zaman, G.S., Wahab, S., Saeed, M. and Khan, S.A. (2021) 'A Comparative Antibacterial, Antioxidant, and Antineoplastic Potential of *Rauwolfia serpentina* (L.) Leaf Extract with Its Biologically Synthesized Gold Nanoparticles (R-AuNPs)', *Plants*, 10(11), p. 2278.
27. Rashid, Md.M.O., Amin, M.R., Chowdhury, M.M., Rahman, Md.A. and Shamim, Md. (2014) 'Comparative Study on the Antibacterial Activity of Four Medicinal Plants Leaves of Different Ages', *International Journal of Pharmaceutical Science Invention*, 3(3), pp. 26–32.
28. Owk, A.K. and Lagudu, M.N. (2016) 'In-vitro Antimicrobial Activity of Roots of *Rauwolfia serpentina* L. Benth Kurz', *Notulae Scientia Biologicae*, 8(3), pp. 312–316.
29. Shariff, N., Sudarshana, M.S., Umesh, S. and Hariprasad, P. (2006) 'Antimicrobial activity of *Rauwolfia tetraphylla* and *Physalis minima* leaf and callus extracts', *African Journal of Biotechnology*, 5(10), pp. 946–950.
30. Negi, J.S., Bisht, V.K., Bhandari, A.K., Bisht, D.S., Singh, P. and Singh, N. (2014) 'Quantification of reserpine content and antibacterial activity of *Rauwolfia serpentina* (L.) Benth. ex Kurz', *African Journal of Microbiology Research*, 8(2), pp. 162–166.
31. Krishnasamy, L., Chandramughi, V.P., Ramesh, T., Jayanthi, K. and Bharadwaj A.M. (2023) 'Green synthesis, Characterization of Silver Nanoparticles and Antimicrobial Activity using *Rauwolfia tetraphylla* L.', *Research Journal of Biotechnology*, 18(4), pp. 126–132.
32. Kavitha, R., Deepa, T., Kamalakannan, P., Elamathi, R. and Sridhar, S. (2012) 'Evaluation of phytochemical, antibacterial and antifungal activity of *Rauwolfia tetraphylla*', *International Journal of Pharmaceutical Sciences Review and Research*, 12(1), pp. 141-144.
33. Bhardwaj, N. and Yadav, M. (2016) 'Evaluation of the chemical composition of *Rauwolfia serpentina* and *Leucas aspera* - A comparative study', *World Journal of Pharmacy and Pharmaceutical sciences*, 5(10), pp. 914–920.
34. Nallasamy, L., Swaminathan, A., Krishnamoorthy, D., Murugavela, G.S. and Selvaraj, S.L. (2024) 'Optimization of Anti-Inflammatory Activity of *Rauwolfia tetraphylla* L. Crude Extracts Using Response Surface Methodology', *Indian Journal of Pharmaceutical Education and Research*, 58(3), pp. 934–943.