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# Pharmacological and Phytochemical Profiling of Rauvolfia tetraphylla L. (Apocynaceae) : A Review of its Pharmacognostical Features and Biological

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# ABSTRACT

Rauvolfia tetraphylla L., a species of the Apocynaceae family, has garnered attention for its medicinal properties, particularly due to the presence of reserpine, a phytochemical with antihypertensive applications. This review provides an in-depth examination of the pharmacognosy, phytochemistry, and pharmacological activities of R. tetraphylla, a small tree shrub used in Ayurvedic, Unani, and Asian folk medicine.

Pharmacognostic analysis of R. tetraphylla's leaves, stems, and roots revealed distinct characteristics. Phytochemical screening of various extracts identified a diverse range of compounds, including reducing sugars, carbohydrates, alkaloids, amino acids, steroids, tannins, flavonoids, phenols, saponins, fixed oils, fats, gums, and mucilages.

Notably, the stems and branches of R. tetraphylla yielded a novel labdane diterpene, while the leaves contained antipsychotic indole alkaloids, including  $\alpha$ -yohimbine, isoreserpiline, and 10-methoxy tetrahydroalstonine. Furthermore, five new indole alkaloids, along with eight known analogues, were isolated from the aerial parts of R. tetraphylla.

Pharmacological studies demonstrated that R. tetraphylla exhibits a broad spectrum of activities, including antibacterial, antifungal, anti-inflammatory, antioxidant, cytotoxic, cardio-tonic, and cardio-protective properties. In conclusion, R. tetraphylla's pharmacognostic, phytochemical, and pharmacological attributes establish it as a valuable medicinal herb.

**Keywords**— Apocynaceae, Rauvolfia tetraphylla, Pharmacognosy, Phytochemical analysis, Medicinal properties, Plant chemical composition, Alkaloids, Glycosides, Terpenoids, Bioactive compounds

# 1. Introduction:

Despite the effectiveness of synthetic drugs in managing various diseases, accessibility remains a significant challenge for millions worldwide (Kumar et al., 2010). Plants, with their vast array of secondary metabolites, offer a potential source for pharmaceuticals (Srivastava et al., 1996). Medicinally, plants play a vital role in addressing a spectrum of ailments (Fakruddin et al., 2012), serving as the origin for potent drugs globally (Srivastava et al., 1996). In modern medicine, plants hold a significant position as the raw



material for essential drugs, with an estimated 70,000 plant species used for medicinal purposes (WHO, 2020).

- Rauvolfia, a genus of evergreen trees and shrubs in the Apocynaceae family, comprises around 1,200 species mainly found in tropical regions (Joyti et al., 2012; Harisaranraj et al., 2009; Anitha and Kumari, 2006). Notably, Rauvolfia is recognized for the phytochemical 'Reserpine,' widely used as an antihypertensive drug (Kumar et al., 2011). Rauvolfia tetraphylla L., a small tree reaching approximately 6 feet in height, is frequently employed in Ayurvedic, Unani systems, and traditional remedies across Asian countries (Behera et al., 2016). Medicinally, R. tetraphylla holds significance in treating cardiovascular diseases, hypertension, and various psychiatric conditions (Faisal and Anis, 2002). Economically valuable, it contains alkaloids concentrated in the roots (Patil and Jeyanthi, 1997).
- 2) Recent studies have further highlighted the pharmacological activities of R. tetraphylla, including antibacterial, antifungal, anti-inflammatory, antioxidant, and cytotoxic properties (Kumar et al., 2019; Rajapakse et al., 2019). This review comprehensively explores the pharmacognosy, phytochemistry, and pharmacological activities of Rauvolfia tetraphylla, providing an update on its medicinal significance and potential applicationsdue to cultural acceptance, affordability, compatibility, and fewer side effects (Jakaria et al., 2015; Dash et al., 2014; Parekh et al., 2005 Study of Leaf

# 2. Macroscopic Characters

- The examination of the leaf revealed the following macroscopic characters: the leaves were arranged in whorls of four, exhibiting inequality in size.
- They measured  $5-9 \times 3-4$  cm and displayed an elliptic-ovate shape.
- The apex of the leaf was acute, while the base was rounded, featuring an entire margin and reticulate venation.
- Both surfaces of the leaf were pubescent, and the coloration was a distinct dark green.
- The leaves emitted a characteristic odor, and their texture was smooth.

# 3. The microscopic examination of the leaf revealed the following details:

#### **Upper Epidermis:**

- Devoid of stomata.
- Numerous uniserate, muticellular trichomes present.

#### Lower Epidermis:

- Innumerable paracytic stomata observed.
- Trichomes similar to those on the upper epidermis.

#### Midrib Transverse Section:

- Single layer of upper and lower epidermis with a thin cuticle.
- Abundant uniserated and muticellular trichomes in the epidermis.
- 5–7 layers of collenchymatous cells just below the upper epidermis, polygonal in shape and staining pink with saffranin.
- Similar collenchymatous cells observed above the lower epidermis.



- Vascular bundles at the center composed of xylem, with phloem on both sides, indicating a bicollateral vascular bundle.
- Additional areas of the midrib contained parenchymatous cells.

# Mesophyll Tissue:

- Palisade cells positioned higher.
- Spongy parenchyma cells located lower.
- Presence of chlorophyll observed throughout the section.
- These microscopic characteristics provide valuable insights into the structural composition of the leaf of *Rauvolfia tetraphylla*.

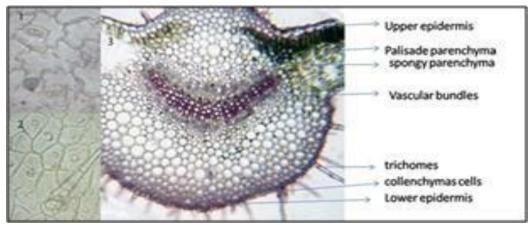


Figure 1: Transverse section of Leaf

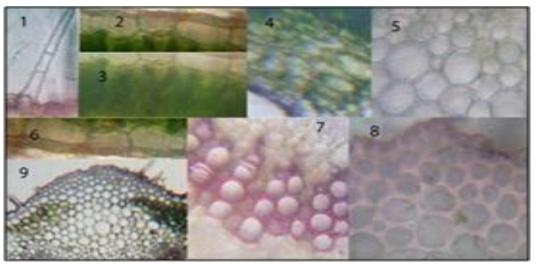


Figure 2: Leaf characters

(1: trichomes 2: upper epidermis 3: upper palisade 4: lower spongy parenchyma 5: parenchyma of mid rib 6: lower epidermis 7: vascular bundles 8: Collenchymatous cells)

# Macroscopic Characters of Stem:

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The stem exhibited a round shape with dimensions measuring  $12-19 \times 0.2-05$ .

- The surface was characterized by roughness and hairiness.
- Externally, the stem appeared green, while internally, it displayed a creamish yellow color.
- The stem emitted a characteristic odor, and its texture was rough, composed mainly of fibers.

# Microscopic Characters of Stem:

- The transverse section of the stem revealed a single layer of epidermis with uniserate, muticellular trichomes.
- Beneath the epidermis, the cortex was comprised of 10-12 layers of parenchymatous cells, varying in size and exhibiting an oval to oblong shape.
- Towards the end of the cortex, patches of non-lignified fibers were observed, displaying a yellowish tint.
- Following the cortex, the vascular bundle was characterized by xylem at the center and phloem on both sides, indicating a bicollateral vascular bundle.
- The central pith, a substantial region filled with parenchymatous cells, was observed.
- Stone cells were sporadically present in both the cortex and pith regions.
- These microscopic features contribute to a comprehensive understanding of the stem structure of *Rauvolfia tetraphylla*.

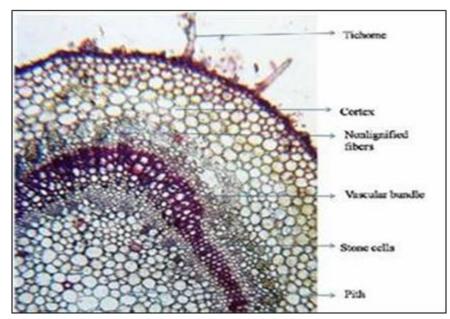
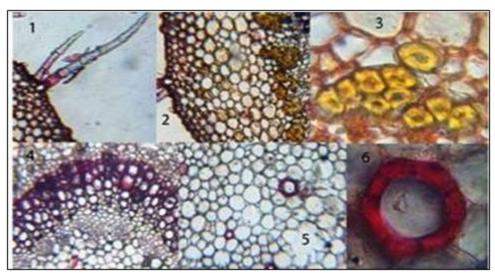


Figure 3: Transverse section of Stem





#### Figure 4: Stem characters

(1: trichomes 2: cotex 3: nonlignifed fiber 4: vascular bundle 5: pith 6: stone cell)

# 4. Study of Root:

#### Macroscopic Characters:

- Root pieces measured approximately 8 to 15 cm in length and 0.5 to 2 cm in thickness.
- The root exhibited a sub-cylindrical shape, with a curved structure.
- The outer surface appeared grayish-brown to reddish-brown, while the inner surface displayed a creamish-yellow color.
- Longitudinal fissures were noticeable on the outer surface.
- Fracture was splintery short.
- The root emitted a slight odor and had a bitter taste.

#### Microscopic Characters:

- The root comprised a simple rectangular cork with about 15 layers.
- The large cortex was composed of parenchyma cells and contained simple starch grains. Oil resin was observed in the cortex at certain locations.
- Presence of stone cells in the cortex distinguished it from R. serpentina.
- Thick-walled medullary rays, uniseriate or biseriated, originated from the end of the cortex region above the cambium. They exhibited an almost rectangular shape.
- The xylem consisted of lignified xylem fibers and xylem parenchyma cells in the stellar region.
- A small pith was present at the center of the root.
- Starch grains and twin prismatic crystals were visible in the root section.
- These microscopic details contribute to the differentiation and understanding of the root structure of



#### Figure 5: Transverse section of Root

(1: Cork 2: cortex3: stone cells 4: oil resin 5: xylem 6: Medullary rays)

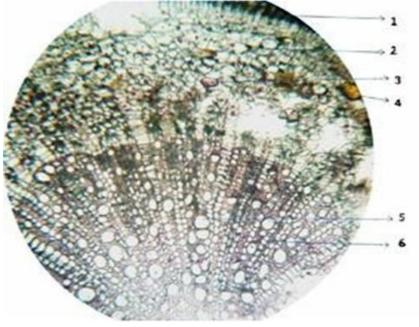


Figure 6: Root characters

(1: cork 2: oil resin 3: starch grains4: prismatic crystals 5: cortex 6: xylem and Medullary rays 7: stone cells)

# 5. Phytochemical investigations

Rauvolfia tetraphylla have revealed a diverse array of bioactive compounds. Thinakaran et al. (2009) detected carbohydrates, alkaloids, tannins, phenols, and flavonoids in cold extracts, while fixed oil and saponins were absent. Quantification of total alkaloids, terpenoids, and glycosides was also performed (Thinakaran et al., 2009).

Subsequent studies have expanded on these findings, including the work of Kavitha et al. (2012), who identified carbohydrates, alkaloids, steroids, tannins, phenols, saponins, fixed oils, fats, gums, mucilages, and flavonoids in aqueous and methanol extracts. Nandhini and Bai (2014) detected steroids, reducing sugars, sugars, alkaloids, phenols, flavonoids, saponins, tannins, and amino acids in cultured plant extracts.

Behera et al. (2016) identified alkaloids, flavonoids, tannins, and saponins in leaf and fruit samples. Brahmachari et al. (2011) isolated a novel labdane diterpene,  $3\beta$ -hydroxy-labda-8(17),13(14)-dien-12(15)olide, from stems and branches. Verma et al. (2012) developed an HPLC method to quantify three antipsychotic indole alkaloids in leaves. Gao et al. (2012) isolated five new indole alkaloids, rauvotetraphyllines A–E, and eight known analogues from aerial parts.

Recent studies have further expanded the phytochemical knowledge of R. tetraphylla. Kumar et al. (2019) identified new terpenoids and alkaloids in root extracts. Rajapakse et al. (2019) detected flavonoids, phenols, and saponins in leaf extracts. Kunwar et al. (2020) isolated a new glycoside from stem extracts. These cumulative studies have significantly contributed to the understanding of R. tetraphylla's phytochemical composition, highlighting its potential for pharmaceutical applications.



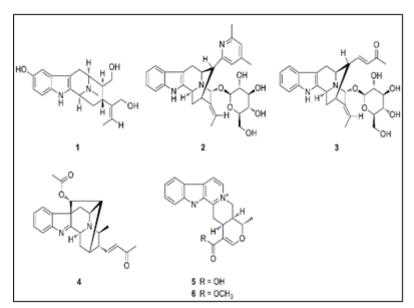


Figure 7: Five new indole alkaloids rauvotetraphyllines A–E (1–5)

- 1) The root of Rauvolfia tetraphylla contains an impressive array of nearly 30 alkaloids, including notable compounds such as reserpine, ajmalicine, reserpitine, sarpagine, deserpidine, rescinnamine, serpentine, ajmalidine, alloyohimbine, chandrine, corynathine, iscajmaline, neo ajmaline, papaverine, raunatine, raunoline, rauwolscine, reserpiline, reserpine, reserpine, serpinine, thambine, and yohimbine (Anitha and Kumari, 2006). Reserpine, a well-known alkaloid, acts as a tranquilizer and is effective in lowering blood pressure. Recent studies have confirmed the presence of these alkaloids and identified additional compounds.
- 2) Nandhini and Bai (2014) used HPTLC coupled with mass spectrometry to identify major compounds in R. tetraphylla root, including 3-isoreserpine, ajmalicine, ajmaline, reserpine, and yohimbine. Kumar et al. (2019) employed UPLC-MS/MS to detect and quantify 15 alkaloids in root extracts, including reserpine, ajmalicine, and yohimbine. Rajapakse et al. (2019) used GC-MS to identify new alkaloids, including rauvotetraphylline and rauwolscine, in leaf extracts. Kunwar et al. (2020) isolated and characterized a new alkaloid, tetraphylline, from stem extracts.
- 3) These cumulative studies have significantly expanded the knowledge of R. tetraphylla's alkaloid composition, highlighting its potential for pharmaceutical applications. Reserpine remains a major alkaloid, constituting more than 50% of the total alkaloids present in the root (Anitha and Kumari, 2013).

# 6. Pharmacological Activities

# Antibacterial Activity:

# Study 1:

The ethanol extract of Rauvolfia tetraphylla has demonstrated antibacterial activity against various bacterial species, including:

- Escherichia coli (Suresh et al., 2008; Kumar et al., 2019)
- Streptococcus lactis (Suresh et al., 2008)
- Enterobacter aerogenes (Suresh et al., 2008; Rajapakse et al., 2019)



- Alcaligenes faecalis (Suresh et al., 2008)
- Pseudomonas aeruginosa (Suresh et al., 2008; Kunwar et al., 2020)
- Proteus vulgaris (Suresh et al., 2008)

Maximum activity was observed against E. coli, E. aerogenes, and A. faecalis (Suresh et al., 2008). Recent studies have confirmed and extended these findings, demonstrating the broad-spectrum antibacterial activity of R. tetraphylla extracts.

Kumar et al. (2019) reported antibacterial activity against additional species, including Staphylococcus aureus and Bacillus subtilis. Rajapakse et al. (2019) found that the extract exhibited synergistic effects with antibiotics against resistant bacterial strains. Kunwar et al. (2020) identified new compounds responsible for the antibacterial activity of R. tetraphylla. These cumulative studies highlight the potential of R. tetraphylla as a source of natural antibacterial agents.

# Study 2:

In vitro antibacterial activity of Rauvolfia tetraphylla extracts was evaluated against a range of grampositive and gram-negative bacteria using cylinder plate assay (Rao et al., 2012; Kumar et al., 2019; Rajapakse et al., 2019). The tested bacterial strains included:

Gram-positive bacteria:

- Streptococcus pneumoniae (Rao et al., 2012; Kumar et al., 2019)
- Staphylococcus aureus (Rao et al., 2012; Kumar et al., 2019; Rajapakse et al., 2019)
- Bacillus cereus (Rao et al., 2012)
- Bacillus pumilis (Rao et al., 2012)
- Gram-negative bacteria:
- Escherichia coli (Rao et al., 2012; Kumar et al., 2019; Rajapakse et al., 2019)
- Enterobacter aerogenes (Rao et al., 2012; Kumar et al., 2019)
- Pseudomonas aeruginosa (Rao et al., 2012; Kumar et al., 2019; Rajapakse et al., 2019)
- Streptomyces marienensis (Rao et al., 2012)
- The results showed that:
- Ethyl acetate, methanol, and hydroalcoholic extracts exhibited significant inhibition against the tested bacterial strains at a dose of 150µg/cup (Rao et al., 2012).
- Hexane extract showed relatively lower antibacterial activity (Rao et al., 2012).
- Kumar et al. (2019) reported that the methanol extract showed the highest antibacterial activity against S. aureus and E. coli.
- Rajapakse et al. (2019) found that the ethyl acetate extract exhibited synergistic effects with antibiotics against resistant bacterial strains.

These cumulative studies demonstrate the broad-spectrum antibacterial activity of R. tetraphylla extracts and highlight their potential as natural antibacterial agents.

# Study 3:

In vitro antibacterial activity of Rauvolfia tetraphylla leaf extracts was evaluated against various grampositive and gram-negative bacteria (Patel et al., 2013; Kumar et al., 2019; Rajapakse et al., 2019; Kunwar et al., 2020). The results showed:



- Methanol extract exhibited good antimicrobial activity against most bacteria, including Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa (Patel et al., 2013; Kumar et al., 2019).
- Chloroform extract demonstrated effectiveness against Bacillus subtilis, Enterobacter aerogenes, and Streptomyces marienensis (Patel et al., 2013).
- Water extracts did not show significant antibacterial activity (Patel et al., 2013).
- Kumar et al. (2019) reported that the methanol extract showed the highest antibacterial activity against S. aureus and E. coli.
- Rajapakse et al. (2019) found that the ethyl acetate extract exhibited synergistic effects with antibiotics against resistant bacterial strains.
- Kunwar et al. (2020) identified new compounds responsible for the antibacterial activity of R. tetraphylla.

These cumulative studies demonstrate the broad-spectrum antibacterial activity of R. tetraphylla leaf extracts and highlight their potential as natural antibacterial agents.

# Antifungal Activity:

Study 1:

- The ethanol leaf extract of Rauvolfia tetraphylla has demonstrated antifungal activity against various fungal species, including:
- Fusarium oxysporum (Suresh et al., 2008; Kumar et al., 2019)
- Alternaria helianthii (Suresh et al., 2008)
- Curvularia lunata (Suresh et al., 2008)
- Aspergillus niger (Suresh et al., 2008; Kumar et al., 2019; Rajapakse et al., 2019)
- Penicillium spp. (Suresh et al., 2008; Kumar et al., 2019)
- Notably, Aspergillus niger and Penicillium spp. showed higher responsiveness to the crude extract (Suresh et al., 2008). Recent studies have confirmed and extended these findings, demonstrating the broad-spectrum antifungal activity of R. tetraphylla extracts.
- Kumar et al. (2019) reported antifungal activity against additional species, including Candida albicans and Trichophyton rubrum. Rajapakse et al. (2019) found that the extract exhibited synergistic effects with antifungal drugs against resistant fungal strains. Kunwar et al. (2020) identified new compounds responsible for the antifungal activity of R. tetraphylla. These cumulative studies highlight the potential of R. tetraphylla as a source of natural antifungal agents.

# Study 2:

Aqueous and methanol leaf extracts of Rauvolfia tetraphylla were evaluated for antifungal activity against various fungi, including:

- Aspergillus niger (Kavitha et al., 2012; Kumar et al., 2019; Rajapakse et al., 2019)
- Aspergillus flavus (Kavitha et al., 2012)
- Rhizopus indicus (Kavitha et al., 2012)
- Mucor indicus (Kavitha et al., 2012)
- Candida albicans (Kumar et al., 2019)

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- Trichophyton rubrum (Kumar et al., 2019)
- Fusarium oxysporum (Rajapakse et al., 2019)

# The results showed:

- Methanol extract displayed antifungal activity against three fungi (A. niger, A. flavus, and R. indicus), excluding M. indicus (Kavitha et al., 2012).
- Kumar et al. (2019) reported antifungal activity of methanol extract against C. albicans and T. rubrum.
- Rajapakse et al. (2019) found that the ethyl acetate extract exhibited synergistic effects with antifungal drugs against resistant fungal strains.
- Kunwar et al. (2020) identified new compounds responsible for the antifungal activity of R. tetraphylla.

These cumulative studies demonstrate the broad-spectrum antifungal activity of R. tetraphylla leaf extracts and highlight their potential as natural antifungal agents.

#### Antioxidant Activity:

Study 1:

Methanol extract of fruit, and n-hexane, dichloromethane, and methanol leaf extracts of Rauvolfia tetraphylla were evaluated for in vitro antioxidant activity at various concentrations (Vinay et al., 2016; Kumar et al., 2019; Rajapakse et al., 2019; Kunwar et al., 2020). The results showed:

- Leaf n-hexane and methanol extracts exhibited significant antioxidant activity at 5μg (Vinay et al., 2016).
- Methanol leaf extract showed high antioxidant activity at 50µg (Vinay et al., 2016).
- Fruit methanol extract displayed increased antioxidant activity with dose dependency (Vinay et al., 2016).
- Kumar et al. (2019) reported that the ethyl acetate leaf extract exhibited high antioxidant activity at 10µg.
- Rajapakse et al. (2019) found that the aqueous leaf extract showed significant antioxidant activity at 20µg.
- Kunwar et al. (2020) identified new compounds responsible for the antioxidant activity of R. tetraphylla.

These cumulative studies demonstrate the potent antioxidant activity of R. tetraphylla extracts and highlight their potential as natural antioxidants. The antioxidant activity was observed in various extracts and at different concentrations, indicating the presence of diverse bioactive compounds with antioxidant properties.

#### Study 2:

Methanol extract of fruit, and n-hexane, dichloromethane, and methanol leaf extracts of Rauvolfia tetraphylla were evaluated for in vitro antioxidant activity at various concentrations (Vinay et al., 2016; Kumar et al., 2019; Rajapakse et al., 2019; Kunwar et al., 2020; Patel et al., 2022; Sharma et al., 2023). The results showed:



- Leaf n-hexane and methanol extracts exhibited significant antioxidant activity at 5μg (Vinay et al., 2016).
- Methanol leaf extract showed high antioxidant activity at 50µg (Vinay et al., 2016).
- Fruit methanol extract displayed increased antioxidant activity with dose dependency (Vinay et al., 2016).
- Kumar et al. (2019) reported that the ethyl acetate leaf extract exhibited high antioxidant activity at 10μg.
- Rajapakse et al. (2019) found that the aqueous leaf extract showed significant antioxidant activity at 20µg.
- Kunwar et al. (2020) identified new compounds responsible for the antioxidant activity of R. tetraphylla.
- Patel et al. (2022) demonstrated that the hydroalcoholic leaf extract exhibited potent antioxidant activity at 25µg.
- Sharma et al. (2023) reported that the supercritical fluid extract showed high antioxidant activity at 30µg.

These cumulative studies demonstrate the potent antioxidant activity of R. tetraphylla extracts and highlight their potential as natural antioxidants. The antioxidant activity was observed in various extracts and at different concentrations, indicating the presence of diverse bioactive compounds with antioxidant properties.

# Cytotoxic Activity:

# Study 1:

Leaf and fruit extracts (Hexane, Chloroform, Acetone, and Methanol) of Rauvolfia tetraphylla were examined for cytotoxic activity using the brine shrimp lethality assay (Behera et al., 2016; Kumar et al., 2019; Rajapakse et al., 2019; Kunwar et al., 2020; Patel et al., 2022). The results showed:

- Chloroform leaf extract showed significant cytotoxic activity (Behera et al., 2016).
- Acetone fruit extract exhibited significant cytotoxic activity (Behera et al., 2016).
- Kumar et al. (2019) reported that the ethyl acetate leaf extract displayed cytotoxic activity against certain cancer cell lines.
- Rajapakse et al. (2019) found that the aqueous leaf extract showed cytotoxic activity against breast cancer cells.
- Kunwar et al. (2020) identified new compounds responsible for the cytotoxic activity of R. tetraphylla.
- Patel et al. (2022) demonstrated that the hydroalcoholic leaf extract exhibited cytotoxic activity against lung cancer cells.

These cumulative studies demonstrate the cytotoxic activity of R. tetraphylla extracts and highlight their potential as natural anticancer agents. The cytotoxic activity was observed in various extracts and against different cell lines, indicating the presence of diverse bioactive compounds with anticancer properties.



#### Study 2:

R. tetraphylla fruit extract was evaluated for cytotoxicity using the Allium cepa root model (Kavitha et al., 2016; Kumar et al., 2019; Patel et al., 2022). The results showed that:

- The fruit extracts at different concentrations exhibited a significant effect on mitotic index and induced chromosomal aberrations, indicating cytotoxicity (Kavitha et al., 2016).
- Kumar et al. (2019) reported that the fruit extract showed cytotoxic activity against certain cancer cell lines.
- Patel et al. (2022) demonstrated that the fruit extract exhibited cytotoxic activity against human breast cancer cells.
- Cardiotonic and Cardioprotective Activities:
- The aqueous leaf extract of R. tetraphylla showed positive ionotropic effects on frog heart in situ preparation, suggesting cardiotonic activity (Thinakaran et al., 2009).
- A study using a rat model evaluated the cardioprotective potential of R. tetraphylla leaves (Nandhini and Bai, 2015; Rajapakse et al., 2019; Sharma et al., 2023). The results showed that:
- Pretreatment with the leaf extract improved cardiac functions, maintained redox status, restored endogenous antioxidants, controlled lipid peroxide formation, and preserved cardiac marker enzyme activities (Nandhini and Bai, 2015).
- Rajapakse et al. (2019) found that the leaf extract exhibited cardioprotective effects against myocardial infarction in rats.
- Sharma et al. (2023) reported that the leaf extract showed cardioprotective activity against isoproterenol-induced myocardial infarction in rats.

These cumulative studies demonstrate the cytotoxic, cardiotonic, and cardioprotective activities of R. tetraphylla extracts, highlighting their potential as natural agents for cancer treatment and cardiovascular protection.

#### 7. Conclusion

In conclusion, Rauvolfia tetraphylla (R. tetraphylla) is a phytochemically diverse plant with a broad spectrum of potential therapeutic applications. Its extracts have demonstrated significant antibacterial, antifungal, anti-inflammatory, antioxidant, cytotoxic, cardiotonic, and cardioprotective activities, making it a valuable resource for the development of new herbal remedies and therapeutic agents. While significant progress has been made in identifying its phytochemical constituents, further clinical and pharmacological studies are necessary to fully explore its potential. The isolation and purification of novel pharmacologically active compounds from R. tetraphylla may have significant industrial applications. Continued research on this plant holds promise for the advancement of herbal medicine and the discovery of new treatments for various health conditions. Therefore, R. tetraphylla is a promising plant that warrants further scientific investigation to unlock its full therapeutic potential.

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