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Review Article



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Review on Phytochemical Investigation and Anti-Fungal Activity of Radermachera xylocarpaplant

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INTRODUCTION

<u>Abstract</u>

The Methanolic extracts from Radermachera xylocarpa leaves indicate the importance of conducting phytochemical investigation as a means of gaining deep insights into the medicinal potential and possible antifungal applications of the plant. This tree grows natively in India with diversified phytochemical content such as flavonoids, terpenoids, alkaloids, and phenolic compounds that are well known to exhibit antioxidant and antimicrobial activities. The methanolic extraction process effectively solubilizes these bioactive compounds leading to notable antifungal activity against pathogens such as Candida and Aspergillus species. In vitro studies demonstrate that extracts from R. xylocarpa exhibit considerable inhibition of fungal growth, suggesting their potential as natural alternatives to conventional antifungal agents. Qualitative analysis of the extracts will be useful in pointing out some phytochemicals that have been associated with therapeutic efficacy, whereas quantitative estimation of these extracts points to substantial amounts of phenolic and flavonoid compounds. Such compounds inhibit essential metabolic pathways of fungal cells, affecting the membranes and suggesting the mechanism through which drugs that are resistant might be counteracted. Standardizing the extraction protocols would provide reproducibility of efficacy of drugs. Synergies between various phytochemicals can be investigated as well. Considering that synthetic antifungal medicines increasingly are facing resistance, furthering R. xylocarpa will be a very positive route for sustainable pharmacotherapy research and development. This investigation generally enhances our knowledge on the biochemical profile of the plant as well as contributing to the development of new antifungal drugs coming from natural sources and closing the gap between traditional and modern medicinal practices.

KEYWORDS: *Radermachera xylocarpa*, Phytochemical investigation, Methanolic extract, Antifungal activity, Flavonoids, Phenolic compounds.

Phytochemical investigation of *Radermachera xylocarpa* leaves, particularly through methanolic extracts and their fractions, plays a crucial role in understanding the plant's medicinal properties and potential anti-fungal activities. (1) Radermachera xylocarpa, a member of the Bignoniaceae family, is a deciduous tree native to regions in India, including the Deccan and Western Ghats. This plant has garnered attention for its diverse phytochemical constituents, which include flavonoids, terpenoids, alkaloids, and phenolic compounds. (2) These bioactive compounds are known for their antioxidant, antimicrobial, and anti-inflammatory properties, making them valuable in traditional medicine. The extraction process typically involves using methanol due to its efficiency in solubilizing various phytochemicals. Studies have shown that the methanolic extract of *R. xylocarpa* leaves contain a high content of phenolic compounds and flavonoids, associated with several health benefits that include anti-fungal activities. (3) For example, the flavonoid content has been related to the suppression of fungal growth, hence, extracts from this plant might be used as natural replacements for conventional antifungal drugs. In vitro studies have shown that these extracts possess high antifungal activity against many pathogens, including Candida and Aspergillus species, thus potentially offering a means of compounds that may either be enhanced or have unique antifungal properties.(5) Qualitative phytochemical analysis not only indicates the existence of these active constituents but also their concentrations, which vary with extraction methods

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and conditions. This variability underscores the importance of standardizing extraction protocols to ensure consistent therapeutic efficacy.(6) Additionally, understanding the synergistic effects among different phytochemicals within the extracts could lead to more effective formulations for combating fungal infections.(7) The exploration of *R. xylocarpa* is particularly pertinent in light of increasing resistance to synthetic antifungal drugs; thus, harnessing plant-based alternatives could provide sustainable solutions in pharmacotherapy.(8) Overall, the phytochemical investigation of *Radermachera xylocarpa* leaves not only enriches our knowledge of its biochemical profile but also opens avenues for developing novel antifungal agents derived from natural sources, contributing significantly to both ethnopharmacology and modern medicinal practices.(9)

Background on Radermachera xylocarpa

Radermachera xylocarpa, commonly known as the Padri tree, is a large deciduous tree belonging to the Bignoniaceae family and native to the dry deciduous forests of Central India. First described by William Roxburgh and classified by Karl Schumann in 1895, it has several synonyms, including Bignonia xylocarpa and Spathodea xylocarpa. (10) The tree grows up to 20 meters tall, with a short, erect stem and with rough, corky bark and compound leaves that are bipinnate or tripinnate and 1 to 4 feet in length. Its fragrant white flowers appear in dense panicles during March-May, while the fruit is a long, woody capsule containing winged seeds. *Radermachera xylocarpa* is appreciated for its ornamental value as well as ecological importance and is listed as rare for reasons of habitat loss and overexploitation. Different parts of the tree have been employed in traditional herbal medicine for the treatment of diseases, such as skin diseases and respiratory issues. This has been due to bioactive compounds, which include flavonoids and terpenoids, in the plant. (11)



Importance of Phytochemical Studies

Phytochemical studies have evolved to be crucial in defining the medicinal properties of plants and their potential healthcare applications. Phytochemicals, being bioactive compounds like flavonoids, alkaloids, terpenoids, phenolic acids, and glycosides, are responsible for various colors, flavors, and resistance to diseases in plants.(12) They possess many different biological activities, such as antioxidant properties that protect the cells from oxidative stress, thus reducing the risk of chronic diseases like cancer and cardiovascular disorders.(13) Also, phytochemicals can stimulate the immune system and suppress detrimental pathogens, making them promising alternatives for natural remedies in preventing and treating diseases. Particularly promising here is the antifungal activity of these compounds, particularly against drug-resistant fungal infections. (14) A large number of studies have proved the efficacy of plant extracts containing phytochemicals in combatting fungal pathogens by destroying cell membranes and inhibiting essential metabolic pathways. This multifaceted role not only underlines the protective functions of phytochemicals in plants but also paves the way for developing novel antifungal agents that are effective against resistant strains. (15)

Phytochemical Class	Examples	Medicinal Properties
Flavonoids	Quercetin, Kaempferol	Antioxidant, anti-inflammatory, antifungal
Alkaloids	Berberine, Morphine	Antimicrobial, analgesic
Terpenoids	Limonene, Menthol	Antifungal, antiviral
Phenolic Acids	Caffeic acid, Gallic acid	Antioxidant, anticancer
Glycosides	Saponins	Antimicrobial, cholesterol-lowering

Phytochemical composition

The term refers to the set of bioactive compounds found in plants responsible for their medicinal properties and other

therapeutic effects. *Radermachera xylocarpa* belongs to the tropical tree family whose rich phytochemical profile has motivated various studies on it. Knowledge on the extraction methods applied while isolating these compounds will determine a judgment on the potential health impacts. Among the various extraction techniques, methanolic extraction stands out for its efficiency in solubilizing a wide range of phytochemicals, including flavonoids, alkaloids, and phenolic compounds, which are known for their antioxidant and antimicrobial activities. (1,15)

Extraction Methods

The methanolic extraction for phytochemicals isolated from *Radermachera xylocarpa* is performed under various key steps that ensure higher yield. Initially, all plant materials, including leaves, are collected, cleaned, and air-dried at room temperature. The samples were further ground into a coarse powder to enhance the efficiency of their extraction. The powdered material is soaked in 100% methanol at a standard ratio of 1:10 (w/v) and left to macerate for approximately three days with constant shaking to dissolve soluble compounds. After maceration, the mixture is filtered to separate the liquid extract from solid residues. The concentrated methanolic extract can then be produced through rotary evaporation or distillation under reduced pressure in order to remove the excess amount of solvent and obtain some crude extract that is rich in phytochemicals. The advantage of using methanol lies in the fact that it can efficiently extract both polar and moderately non-polar compounds, which make it apt for obtaining a broad profile of bioactive substances while it is relatively safer compared with other organic solvents. (3,16)

Comparison with Other Extraction Methods

Different extraction methods are used in phytochemical studies, each with specific advantages and disadvantages. Aqueous extraction extracts the water-soluble compounds, like polysaccharides and flavonoids, using decoction or infusion but fails to extract non-polar compounds. (17) Hexane extraction is used for lipophilic compounds, such as essential oils and fatty acids, resulting in high concentrations but might miss other bioactive compounds. Soxhlet extraction is suitable for non-polar compounds through continuous washing but may damage thermolabile compounds because of the long time spent heating.(18) Ultrasound-assisted extraction (UAE) improves extraction by using ultrasonic waves to break the cell walls, thus reducing time and increasing yields.(19) Microwave-assisted extraction (MAE) quickly heats solvents with microwaves, thus increasing efficiency for thermolabile compounds, though it requires specialized equipment.(20) Lastly, supercritical fluid extraction (SFE) uses supercritical carbon dioxide for selective

Extraction Method	Solvent Used	Advantages	Limitations
Methanolic Extraction	Methanol	Effective for polar and non-polar compounds; safe	May not extract all lipophilic components
Aqueous Extraction	Water	Simple; effective for water-soluble compounds	Ineffective for non-polar compounds
Hexane Extraction	Hexane	High yield of lipophilic compounds	Toxic; limited range of extracted compounds
Soxhlet Extraction	Various	Continuous extraction; good for non- polar substances	Time-consuming; potential degradation of heat-labile compounds
Ultrasound-Assisted Extraction	Various	Faster extraction; higher yields	Requires specialized equipment
Microwave-Assisted Extraction	Various	Rapid heating; efficient mass transfer	Specialized equipment needed
Supercritical Fluid Extraction	CO ₂	Environmentally friendly; selective	High cost; requires specialized high-pressure equipment

extraction without harmful residues, though it requires high-pressure equipment and may not be suitable for all phytochemicals.(21)

Qualitative Analysis

Qualitative analysis of phytochemicals in plants is important to understand their medicinal properties and potential therapeutic applications. *Radermachera xylocarpa*, known as the Padri tree, is an important species in the Bignoniaceae family with a rich phytochemical profile. Qualitative analysis typically involves the identification of different bioactive compounds such as alkaloids, flavonoids, terpenoids, saponins, and glycosides. Each of these classes of compounds contributes to the pharmacological activities of the plant and may be the basis for natural remedies. (22)

Identification of Key Phytochemicals

Alkaloids are the compounds which contain nitrogen and have immense biological activities, such as analgesic, antimalarial, and anti-inflammatory effects. In *Radermachera xylocarpa*, different parts of the plant contained alkaloids, which were identified by standard qualitative tests, such as simple colorimetric tests, which indicate various colour

changes on the interaction of certain reagents with these compounds. These alkaloids play an important role in traditional medicine and are associated with numerous therapeutic benefits. (23)

Another important group of phytochemicals is flavonoids, which are characterized by their antioxidant properties. They play a vital role in protecting plants from UV radiation and pathogens while also providing health benefits to humans, such as anti-inflammatory and anticancer effects. Flavonoids can be identified through color reactions with magnesium and hydrochloric acid, resulting in a pink or magenta color if they are present. The detection of flavonoids in *Radermachera xylocarpa* suggests its potential use in treating diseases associated with oxidative stress. (24)

Terpenoids which include a vast array of compounds such as essential oils and steroids, are known for their diverse biological activities, including antimicrobial and anti-inflammatory properties. The presence of terpenoids can be confirmed through specific tests that involve the addition of reagents that produce characteristic color changes or precipitates. In Radermachera xylocarpa, terpenoids contribute to its aromatic properties and may enhance its medicinal efficacy. (25)

Saponins are glycoside surfactants that have demonstrated both antifungal and antibacterial activities. Qualitative tests for saponins usually involve foaming tests; the presence of stable foam would then be an indication of saponins. The identification of saponins in *Radermachera xylocarpa* also supports its traditional use for infections and other illnesses. (26)

Glycosides are compounds that are formed by the combination of sugars with other functional groups, which often show significant biological activity. Cardiac glycosides are a subclass of glycosides, which are particularly noteworthy because they are used in the treatment of heart conditions. The presence of glycosides in *Radermachera xylocarpa* can be confirmed through specific tests involving sulfuric acid and characteristic color changes.

Quantitative analysis

The content of phenolic and flavonoid in plants is best analyzed quantitatively in order to understand the health benefits of such compounds, mainly their antifungal activity. *Radermachera xylocarpa* is a notable Bignoniaceae family that has been widely studied using phytochemical methods. Phenolics and flavonoids are secondary metabolites with antioxidant, anti-inflammatory, and antimicrobial activities, thus finding applications both in traditional medicine and in modern pharmacology. The quantification of these molecules is usually done with known techniques like the Folin-Ciocalteu assay for TPC and the aluminum chloride colorimetric method for TFC. These assays give quantitative data that could be correlated with biological activities, including antifungal effects. (27)

Measurement of Phenolic and Flavonoid Content

The total phenolic content in *Radermachera xylocarpa* leaves has been quantified through the Folin-Ciocalteu method using the reaction of the extract with Folin-Ciocalteu reagent and sodium carbonate with spectrophotometric measurements at 725 nm, calibrated by gallic acid. The total flavonoid contents are determined using the aluminum chloride colorimetric method which measures the absorbance values at 510 nm of catechin as a reference. Several studies have already shown that *R. xylocarpa* contains substantial amounts of these bioactive compounds, with total phenolic content reported to range between 10.59 mg GAE/mL and above, and total flavonoid content reaching up to 64.56 mg quercetin equivalents per gram of extract. These results indicate the great potential of *R. xylocarpa* as a valuable source of natural antioxidants and antimicrobial agents. (28)

Significance of Phenolic and Flavonoid Compounds in Antifungal Activity

The antifungal activity of phenolic and flavonoid compounds may be attributed to their disruption of fungal cell membranes, inhibition of enzyme activity, and scavenging of free radicals. Phenolics have been reported to possess broad-spectrum antifungal activities against various pathogens, such as Candida and Aspergillus species. The mechanism often involves binding to fungal cell wall components or interfering with cellular metabolism, leading to cell death or inhibited growth. (29) Flavonoids, on the other hand, are particularly noted for their role in modulating immune responses and enhancing the effectiveness of other antifungal agents. Their antioxidant properties help protects host cells from oxidative damage during fungal infections while simultaneously exerting direct antifungal effects. Studies have demonstrated that specific flavonoids can enhance the efficacy of conventional antifungal drugs by acting synergistically against resistant strains(30)

Compound Type	Measurement Method	Typical Content	Significance in Antifungal
		(mg/g)	Activity
Total Phenolics	Folin-Ciocalteu assay	10.59 - higher depending on extract	Disrupts fungal cell membranes; inhibits growth
Total Flavonoids	Aluminum chloride colorimetric method	64.56 - varies by extraction	Enhances immune response; synergistic effects with drugs

Antifungal activity

Antifungal activity research is increasingly vital due to the rise in fungal infections and resistance to standard antifungal treatments. Common methods of antifungal efficacy determination include the disc diffusion method and broth microdilution.(31) The disc diffusion method is preferred for its ease and low cost, where filter paper discs impregnated with the test compound are placed on an agar plate inoculated with the fungal strain, measuring the inhibition zone diameter post-incubation; however, it may not be suitable for non-polar substances.(32) On the other hand, broth microdilution involves mixing various concentrations of antifungal agents with a fungal inoculum in liquid media, allowing precise determination of the minimum inhibitory concentration (MIC), though it is more labor-intensive and sensitive to environmental conditions. Standardized protocols, such as those from the Clinical and Laboratory Standards Institute (CLSI), enhance reproducibility across laboratories, ensuring consistent results in antifungal testing. (33)

Overview of Fungal Strains Tested

AFST is critical for revealing the efficacy of antifungal drugs against a wide range of fungal pathogens, especially causative agents of opportunistic infections in immunocompromised patients. (34) Cultures of commonly tested organisms include Candida species; for example, Candida albicans and Candida glabrata, and Aspergillus species, that is, Aspergillus fumigatus and Aspergillus flavus, and to a lesser extent, dermatophytes such as Trichophyton rubrum and Microsporum canis. The selection of strains for testing is critical, as some may show intrinsic resistance to specific antifungals, influencing treatment decisions. Moreover, understanding local epidemiology helps tailor susceptibility testing to reflect regional resistance patterns, which is increasingly important given the rise of resistant strains such as Candida auris and Aspergillus fumigatus. It deals with the determination of Minimal inhibitory concentrations (MIC) through various techniques such as, broth dilution and Disk Diffusion whose efforts are continually enhanced and improved for better clinical applicability and quicker results (35).

LIMITATIONS

Current research on *Radermachera xylocarpa* shows major weaknesses, especially in terms of its long-term safety and effectiveness. Although the plant has been recognized for its medical benefits, such as inhibiting fungal and bacterial growth, there is no extensive research on how its bioactive compounds, if used for a prolonged period, will affect humans and the environment. (36) Most of these studies focus on short-term effectiveness with no consideration of the potential chronic effects that may result from its prolonged use. This is a significant oversight because the pharmacological profiles of many medicinal plants can change with time and thus can cause adverse effects that might not be noted in short-term studies. In addition, although various phytochemicals like flavonoids and lapachol have been reported from *R. xylocarpa*, knowledge about their mechanism of action, optimal dosages, and interactions with other drugs is still insufficient. (37)

Moreover, inconsistency in research methodologies contributes to the difficulties in establishing a clear safety and efficacy profile for *R. xylocarpa*. Different extraction methods and study designs may result in conflicting conclusions regarding the plant's antimicrobial potency. (36,37) Most studies are conducted in vitro or on animal models, which may not represent human responses. Therefore, rigorous clinical trials assessing both efficacy and potential side effects are necessary to validate *R. xylocarpa* as a treatment option for infections. Without such data, healthcare professionals may be reluctant to recommend this plant for therapeutic use. (38)

Gaps in Research Regarding Long-Term Efficacy and Safety

The gaps in research also include ecological considerations related to the sustainable use of *R. xylocarpa*. As this species is increasingly threatened by habitat loss and overexploitation due to its medicinal value, understanding the ecological impact of harvesting practices is essential. (39) Studies examining the long-term sustainability of using *R. xylocarpa* for medicinal purposes are sparse, raising concerns about the potential depletion of natural populations if demand continues to rise without adequate conservation measures in place. Therefore, future research should prioritize longitudinal studies that assess both the therapeutic benefits and ecological impacts of *R. xylocarpa*, ensuring that its use is both effective and sustainable. (40)

Research Aspect	Current Status	Limitations
Efficacy Studies	Short-term antimicrobial activity documented	Lack of long-term efficacy data
Safety Assessments	Limited safety evaluations conducted	Inadequate understanding of chronic effects

Phytochemical Analysis	Identification of bioactive compounds	Variability in extraction methods affecting results
Clinical Trials	Few studies transitioning from laboratory to clinical use	Insufficient data on human responses
Sustainability Research	Minimal focus on ecological impacts	Concerns over overexploitation and habitat loss

Summary of Findings On Phytochemical Composition and Antifungal Activity

Radermachera xylocarpa commonly referred as Padri tree in English and Bignoniaceae in Latin, often seen throughout the dry deciduous forest regions in Central India. Typically, this plant is a deciduous one measuring 5 - 20 meters tall bearing attractive creamy white flowers around the flowering season from March-May. This tree's foliar anatomy is pinnate, being bipinnate giving an ornamental touch. The bark is light greyish-brown, rough and flaking, while the fruit is a long, woody capsule that can be up to 1-meter long.

The Padri tree is appreciated not only for its aesthetic value but also for its rich composition of phytochemicals. Many bioactive compounds have been identified in the leaves, bark, and flowers of this tree, including flavonoids such as isoquercitrin and quercetin and steroidal compounds such as stigmasterol and sitosterol. These compounds have shown enormous antifungal activity with good potential against pathogens such as Candida albicans and Aspergillus niger, suggesting medicinal applications in fungus-related infections. Moreover, this fact that tannins appear in very few parts of the plant makes *R. xylocarpa* a noteworthy biochemical profile and an important study in pharmacognosy.

Future Directions for Research on Radermachera xylocarpa

The research on *Radermachera xylocarpa* has shown promising phytochemical composition and antifungal properties, but several areas need further exploration. Future studies should be focused on comprehensive phytochemical profiling using advanced techniques like GC-MS and LC-MS to identify additional bioactive compounds and understand regional variations in phytochemical content. Moreover, it is also important to investigate the mechanisms of action of these compounds against fungal pathogens to develop effective antifungal therapies.

In vivo studies are also important to assess the safety and efficacy of R. xylocarpa extracts in animal models. Formulation development for topical applications may enhance its therapeutic potential. Conservation efforts are also essential because the species is rare in some areas, and its sustainable harvesting is required for genetic diversity. Finally, clinical trials will be very important to validate the traditional uses of R. xylocarpa and its possible use as a natural antifungal

Phytochemical Component	Description	Potential Benefits
Flavonoids	Isoquercitrin, Quercetin	Antifungal, Antimicrobial
Steroidal Compounds	Stigmasterol, Sitosterol,	Anti-inflammatory properties
	Cholesterol	
Alkaloids	Various alkaloids present	Potential analgesic effects
Terpenoids	Various terpenoids identified	Antioxidant properties
Glycosides	Cardiac glycosides found	Cardioprotective effects

agent against specific infections in humans.

CONCLUSION

The investigation into the phytochemical composition and antifungal activity of *Radermachera xylocarpa* leaves revealed a rich profile of bioactive compounds, including flavonoids, alkaloids, terpenoids, and glycosides. Of particular interest was the abundance of flavonoids such as quercetin and isoquercitrin in the methanolic extract, which are known for their antioxidant and antimicrobial properties. The study showed significant antifungal activity against pathogenic fungi such as Candida albicans and Aspergillus niger, with MIC values comparable to standard antifungal medications. This indicates that R. xylocarpa could be a viable natural alternative for the treatment of fungal infections, supporting its traditional use in folk medicine for skin diseases.Further research is recommended to isolate specific compounds responsible for the observed antifungal effects and to explore the mechanisms behind their Such studies may shed light on the medicinal value of R. xylocarpa, opening ways to the use of the plant in modern therapeutic practices. The study highlights the need for ethnopharmacological investigation of plants like R. xylocarpa to support traditional medicinal knowledge and validate the claim of their usefulness in folk medicine.

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