Exploring The Anti-Bacterial Potential of Radermachera Sinca: A Phytochemical Based Invitro Study

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***Abstract*— The plant extracts of Radermachera sinica were prepared using various solvents and subjected to preliminary phytochemical screening to detect the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds. Antibacterial activity was evaluated using the agar well diffusion method against Staphylococcus aureus, with ampicillin employed as the standard drug. The study aimed to investigate the in vitro antibacterial potential of Radermachera sinica in relation to its phytochemical composition. Medicinal plants represent a valuable source for the discovery of novel bioactive compounds with significant therapeutic potential. Their applications extend beyond traditional uses and offer promising alternatives for the treatment of various diseases, supported by longstanding cultural practices and empirical knowledge. The present investigation focused on assessing the antibacterial efficacy of Radermachera sinica, which has been frequently reported for its medicinal properties. The results demonstrated a significant increase in the zones of inhibition produced by the plant extracts, indicating notable antimicrobial activity. This effect may be attributed to the presence of bioactive phytoconstituents such as alkaloids, flavonoids, terpenoids, phenolic compounds, and steroids. Based on these findings, Radermachera sinica may be considered a promising candidate for further exploration as a potential antimicrobial agent.**

***Index Terms*— Radermachera sinica, Phytoconstituents, Antibacterial activity, Ampicillin**

I. INTRODUCTION

Bacteria are microscopic, single-celled prokaryotic organisms that are widely distributed across diverse environments, including soil, water, air, and living organisms [(1)]. They are among the earliest forms of life on Earth and have developed various adaptive mechanisms to survive under extreme conditions such as fluctuations in temperature, pH, and salinity [(2)]. In relation to humans, bacteria may be beneficial, neutral, or pathogenic [(3)]. While some species cause infections, many play essential roles in digestion and nutrient cycling within ecosystems [(4).] Bacteria reproduce rapidly through binary fission, resulting in exponential population growth [(1)]. They exhibit diverse morphological forms such as cocci, bacilli, vibrios, and spirilla, and may occur in arrangements like chains or clusters, which are important for identification and understanding pathogenicity [(5)]. The Gram staining technique is commonly employed to classify bacteria into Gram-positive and Gram-negative groups based on differences in cell wall composition [(6)]. Gram-positive bacteria possess a thick peptidoglycan layer and retain the crystal violet stain, appearing purple, whereas Gram-negative bacteria have a thinner peptidoglycan layer and an outer membrane [(6)]. Some bacteria may exhibit Gram-variable or Gram-indeterminate characteristics [(7)]. Structurally, bacteria contain both external and internal components. External structures include flagella for motility, pili and fimbriae for adhesion, and capsules for protection [(5)]. Internally, they possess ribosomes for protein synthesis, a nucleoid region containing genetic material, plasmids that may confer traits such as antibiotic resistance, and cytoplasm where metabolic activities occur [(1)]. Additional structures such as inclusion bodies, cytoskeletal elements, and gas vacuoles contribute to storage, structural integrity, and buoyancy [(8)].

Antibiotics are chemical agents that act by targeting essential bacterial processes, including cell wall synthesis, protein synthesis, nucleic acid synthesis, and metabolic pathways, thereby inhibiting growth or causing bacterial death [(9)]. They have significantly reduced morbidity and mortality associated with bacterial infections and remain fundamental to modern medicine [10]. However, the widespread and inappropriate use of antibiotics has led to the emergence of antibiotic-resistant bacteria, posing a serious global health challenge [11]. This has intensified the need for the development of novel antibacterial agents, particularly from natural sources [12]. Medicinal plants are rich in bioactive phytochemicals such as alkaloids, flavonoids, tannins, and saponins, which exhibit potential antibacterial properties [13]. In this context, Radermachera sinica has gained attention due to its diverse phytoconstituents [14]. Therefore, the present study aims to evaluate the antibacterial activity of these plant-derived compounds, with the objective of identifying safer and effective alternatives to conventional antibiotics and addressing antimicrobial resistance.

1.1 Aim

To assess the phytochemical constituents and to evaluate the anti-bacterial activity of Radermachera sinica leaf extract using standard laboratory procedure.

1.2 Objectives

- To gather and verify plant material from Radermachera sinica for research purposes.
- To make R. sinica plant extracts with the right solvents.
- To test extracts antibacterial efficacy against particular Gram-positive and Gram-negative microorganisms.
- To assess how well the plant extracts work against germs in comparison to conventional antibacterial medications.
- To use conventional microbiological techniques to identify the zone of inhibition generated by R. sinica extracts.
- To assess the plant extracts' concentration-dependent antibacterial activity.
- To evaluate Radermachera sinica's potential as a natural substitute for artificial antibiotics.
- To provide scientific evidence in favour of the usage of medicinal plants in combating antibiotic resistance.

1.3 Plant Profile

Radermachera sinica:

Radermachera sinica is an ornamental plant popularly known as china doll plant serpent tree or emerald tree. It belongs to the family of Bignoniaceae. It is native to southern china and Taiwan. Its leaves are bipinnate, divided in to small glossy green leaflets Leaves are arranged along the stemas opposite or whorled, non-alternate and growig in pairs. It typically has white or yellow trumpet shaped flowers. R. Sinica seed pods are long, twisted, resembling a serpent shape.

Cultivation:

It is widely cultivated as decorative household plant for its elegant and glossy appearance. It requires optimal environmental conditions like good light availability and consistent moisture. Direct sunlight may lead to leaf scorch; indirect sunlight promotes leaf growth irregular watering results in leaf wiliting. The optimum growth of the plant has been observed at 18 -30. soil must be well drained with high organic matter for the development of healthy roots. Balanced liquid fertilizers appilied during growing phase. It should be protected from strong drying winds and sudden changes in climatic conditions.

Fig-1: Radermacher sinica

1.4 Uses:

- Environment and aesthetic value. It improves the quality of air by reducing dust accumulation and enhancing humidity.
- It has the potential medical uses
- It has vast bioactive compounds that are associated with antioxidant, antidiabetic, antihistaminic activities.
- It is used for research purpose (phyto chemical screening, In-vitro pharmacological studies)

- It is economically important in commercial horticultures.
- It isused as decorative purpose.
- It has hepatoprotective activity.
- It is used in stress tolerance and plant physiological studies.
- It is used in herbal drug discovery.
- It is also used in anticancer research for its antiproliferative and apoptosis inducing effects.

II. METHODOLOGY

2.1 Collection and AUTH entification of plant material: Collect fresh leaves of Radermachera sinica from around of Narayana Pharmacy College,Nellore. Got the Authentification frombotanist G.Prabhkar in Government Degree College, kovur.

2.2 Preparation of powders:
- Washthecollectedleavestoremovedirtandcontaminants.
- Drytheleavesinashaded, wellventilatedareatopreventdegradationofbioactive compounds.
- Grind the dried leaves were coarsely powdered using a mechanical grinder and stored in an air tight container for further use.

Fig-2: Stem and leaves of Radermchera sinica

2.3 Preparation of the extract:
The plant extract of Radermachera sinica was prepared using the Soxhlet extraction method to ensure efficient extraction of phytochemicals. About 20 g of finely powdered plant material was accurately weighed and packed in a muslin cloth, which was then placed into the siphon tube of the Soxhlet apparatus. The apparatus was properly assembled, and the round-bottom flask (RBF) was filled with 100 mL of solvent along with porcelain chips to prevent bumping during heating. Extraction was carried out separately using solvents of varying polarity, namely ethanol, distilled water, chloroform, and diethyl ether. The system was heated on a water bath at a controlled temperature, allowing the solvent to vaporize, condense, and percolate through the plant material. Once the extractor filled to the siphon level, the solvent containing dissolved phytoconstituents returned to the RBF, and the process was continued for multiple cycles until the solvent became colorless, indicating complete extraction.
The extraction conditions were maintained according to the boiling point of each solvent, where ethanol was extracted at 75°C for 5–6 hours, diethyl ether at 35°C for 7 hours, distilled water at 100°C for 10 hours, and chloroform at 45°C for 6–7 hours. The solvents were heated on a water bath under controlled temperature, allowing vaporization, condensation, and repeated percolation through the plant material.

Fig-3: Soxhlet Extraction

2.4 Antimicrobial activity
The antimicrobial activity was evaluated using Staphylococcus aureus, a Gram-positive, spherical bacterium widely employed as a test organism due to its clinical significance and sensitivity to various antimicrobial agents, making it suitable for screening studies. Ampicillin was used as the standard broad-spectrum antibiotic for comparison of antibacterial activity against Staphylococcus aureus. For the preparation of bacterial inoculum, a pure culture of Staphylococcus aureus was obtained from a stock

culture and subcultured on nutrient agar plates, followed by incubation at 37°C for 18–24 hours to obtain fresh colonies. A few well-isolated colonies were selected using a sterile inoculating loop and transferred into sterile nutrient broth or saline solution. The suspension was mixed thoroughly to achieve a uniform bacterial inoculum, and its turbidity was adjusted to match the 0.5 McFarland standard. The prepared inoculum was then used immediately for antibacterial testing, such as the agar well diffusion method.

2.5 Agar well diffusion method:
The antibacterial activity of Radermachera sinica plant extracts was evaluated using the agar well diffusion method, a standard laboratory technique for assessing antimicrobial potential. In this method, sterile agar plates were inoculated with the prepared bacterial suspension of Staphylococcus aureus, and wells were aseptically created in the solidified agar using a sterile corn borer. The test samples were then introduced into the wells, allowing the extracts to diffuse into the surrounding medium. The plates were incubated under suitable conditions, and the antibacterial activity was determined by observing the clear zones formed around the wells. The effectiveness of the test samples was quantified by measuring the zone of inhibition in millimeters (mm) using a scale, where the diameter of the clear area indicates the extent of antibacterial activity.

III. RESULTS

3.1 Preliminary Phyto-Chemical Screening:
The data corresponding to table no.4 describes the screening reports of extraction of the plant Radermacher sinica.

Chemical tests	Ethanol extract	Distilled water	Diethyl ether	ChCl3extract
Alkaloids	□	□	_	□
Flavonoids	□	□	_	□
saponins	□	□	_	_
Terpenoids	□	□	□	□
Glycoside	□	□	_	□
Carbohydrate	□	□	_	_
Amino acids	□	_	_	_
Tannins	□	□	_	_
Phenols	□	□	_	□
Steroids	□	□	□	□

Table no.4: Preliminary Phytochemical Screening


Fig.4: Phytochemical Screening of Ethanol Extract

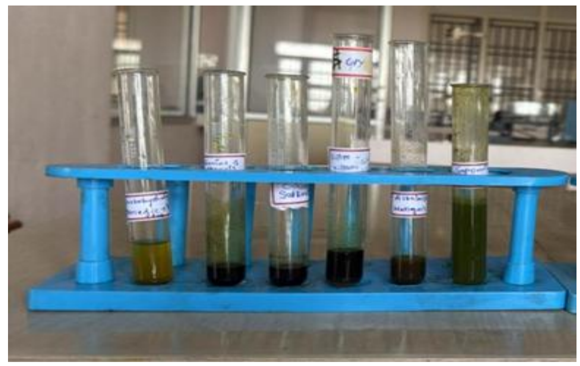


Fig.5: Phytochemical Screening Distilled Water Extract

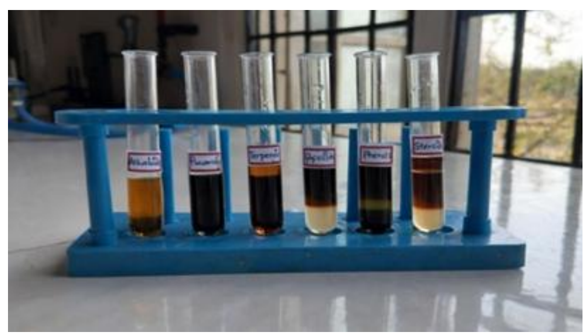


Fig.6: Phytochemical Screening of Chloroform Extract


Figure–7 Phytochemical Screening of Diethyl Ether Extract (Terpinods & steroids)

3.2 Evalution of Anti-Bacterial Activity:

The antibacterial activity of the Radermachera plant extaract was done by using Agar well diffusion method and results of the extract compare with the standard antibiotic drug. The intensity of activity was calculated as mm.

S.No	Samples	Zoneof inhibition			
		25%	50%	75%	100%
1	Ethanol	3mm	4mm	4mm	6mm
2	Distilled water	2mm	2.5mm	4mm	5mm
3	Diehylether	0mm	1mm	1.5mm	2mm
4	Chloroform	1mm	3mm	4mm	5mm
5	Standard (Ampicillin)	1mm	5mm	7mm	9mm

Tab.5: Comparitive zone of Inhibition of different Solvents Extracts of Radermachera sinica

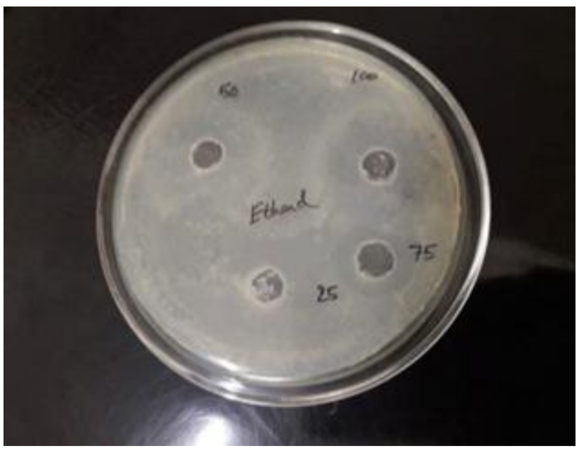


Fig-8: Agar Well Diffusion method of Ethanol Extract

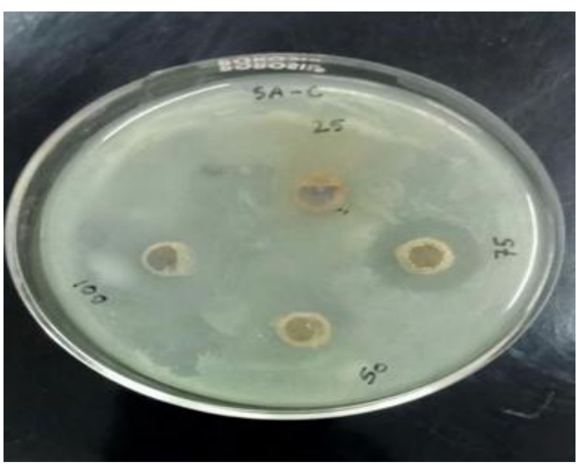


Fig-9: Agar Well Diffusion of Diethyl Ether Extract

Fig-10: Agar Well Diffusion of Distilled Water Extract

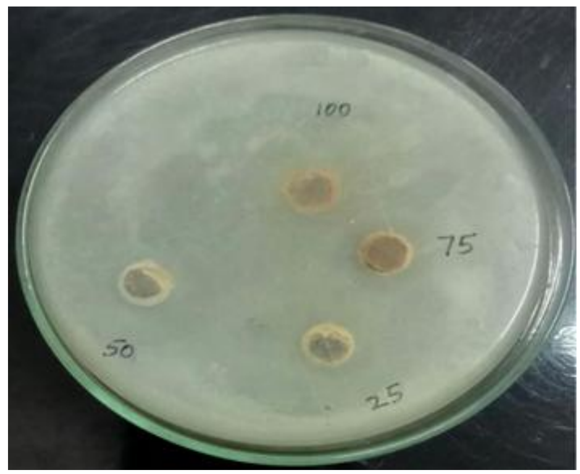


Fig-11: Agar Well Diffusion of Chloroform Extract

3.3 Graphical representation of Anti-Bacterial Activity of different Solvents:

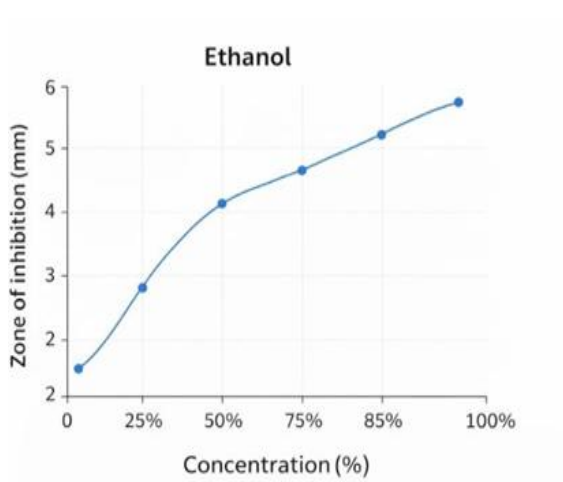


Fig-12: Anti-Bacterial Activity graph of Ethanol

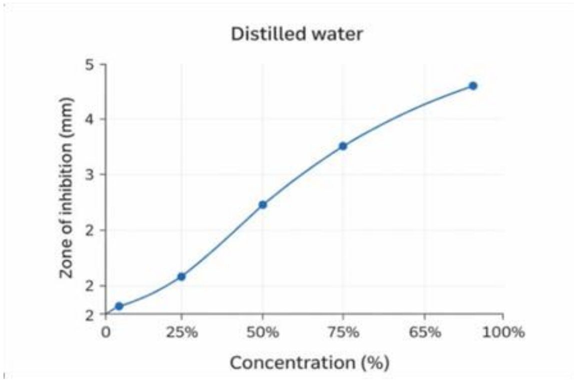


Fig.13: Anti-Bacterial Activity graph of Distilled water

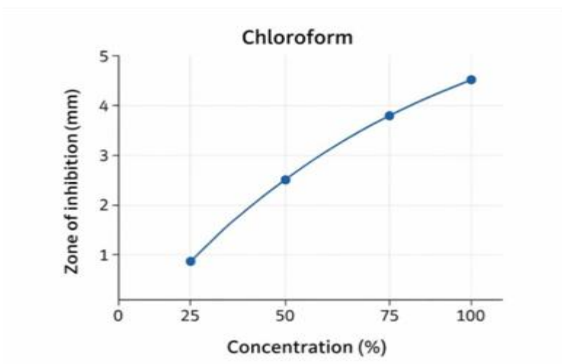


Fig.14: Anti-Bacterial Activity graph of Chloroform

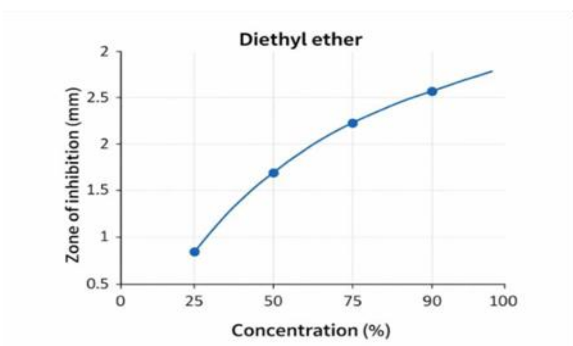
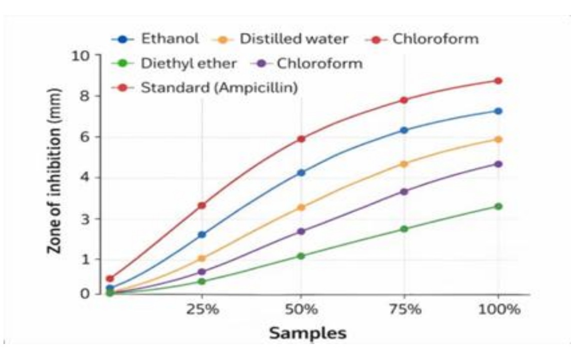


Fig.15: Anti-Bacterial Activity graph of Diethyl ether



Fig–16: Anti-Bacterial Activity graph of Solvents

IV. DISCUSSION

The antibacterial capabilities of Radermachera sinica were assessed in this study using an in vitro approach based on phytochemicals. This study aimed to ascertain if the antibacterial activity of R. sinica is influenced by the bioactive compounds present in medicinal plants, which are widely recognized for their abundance.

Phytochemical screening revealed significant secondary metabolites in the plant extract, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds. It is well known that these phytoconstituents have antimicrobial properties. For instance, although flavonoids and phenols can harm microbial cell membranes, tannins may inhibit microbial proteins and enzymes, preventing the growth of bacteria.

The antibacterial activity against specific test species, such as Staphylococcus aureus, was assessed using the agar well diffusion method. The plant extract showed a measurable zone of inhibition, indicating how effectively it prevented the growth of bacteria. The claimed efficacy may be caused by the synergistic impact of many phytochemicals[15] in the extract. When compared to the widely used antibiotic ampicillin, the plant extract showed noticeable but somewhat diminished antibacterial activity. Although R. sinica may not be as effective as produced drugs, this suggests that it has interesting natural antibacterial properties that can be further studied. The results of the study are consistent with previous research demonstrating that plant-based extracts have antibacterial qualities due to their phytochemical composition. Although the in vitro design of the study provides preliminary evidence, additional research is needed to prove its therapeutic efficacy, including in vivo analysis, toxicity assessment and active component separation potential.

V. CONCLUSION

Radermachera sinica's antibacterial qualities were demonstrated using an in vitro technique based on phytochemicals. Phytochemical screening revealed significant bioactive chemicals with antibacterial properties, such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds. The plant extract's potent antibacterial activity against the test organism

Staphylococcus aureus was shown by the formation of a zone of inhibition in the agar well diffusion method. Even though the activity was somewhat lower than that of the widely used drug ampicillin, the results demonstrate the plant's significant natural antibacterial capacity. This study generally supports the use of medicinal plants as alternative sources of antibacterial chemicals. To fully ascertain Radermachera sinica's medical potential, however, additional study is needed, including toxicity studies, in vivo analyses, and the isolation of the active chemical.

Disclosure statement the authors declare no potential conflicts of interest.

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