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Phytochemical and Antimicrobial Properties of Rothia Indica against Various Bacterial Species

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Rothia indica (L.) Druce is an annual herb that belongs to the family Fabaceae. It is native to India, Sri Lanka, China to peninsular Malaysia and Australia that grows primarily in wet tropical biomes. It is highly used in medicinal applications for its varied chemical compounds that acts as a good source of treating various diseases. The present study evaluates the root, leaf and stem extracts obtained by four different solvents i.e., methanol, chloroform, ethyl acetate and hexane. Phytochemical analysis was performed for the extracts obtained by following standard tests for identification of different compounds. Later the extracts are subjected for the identification of their antimicrobial potential against various bacteria: Streptococcus mutans, Pseudomonas fluroscens, Staphylococcus aureus, and Bacillus subtilis. Root, stem and leaf showed adequate activity, among them root showed the highest activity. Highest activity was observed in ethyl acetate and chloroform followed by methanol, and hexane extracts. The results provide evidence that Rothia indica root, leaf and stem extract possesses vital phytochemicals and antimicrobial properties.

1. Introduction

Infectious diseases remain a significant health issue, representing 41% of the worldwide disease burden assessed in terms of Disability-Adjusted Life Years (DALYS) (1). One significant factor contributing to this issue is the extensive prevalence of acquired bacterial resistance to antibiotics, posing a severe threat to worldwide public health today. This threat manifests not only in the form of epidemics, but also in pandemics of antibiotic resistance as highlighted by Chopra (2000), Chanda et al. (2010), and Osman et al. (2012) (2-4). As

resistance to antibiotics becomes a pressing issue, focus is now turning towards exploring the biologically active compounds derived from plant species traditionally utilized in herbal medicine. These compounds hold promise as a novel and potent reservoir of antibacterial and antifungal properties (5,6). The ability of plants to produce various secondary metabolites with complex structures is closely linked to their antimicrobial properties, as highlighted in studies by Matasyoh et al. (2009) and Souza et al. (2005) (7,8).

The search for novel antimicrobial and antioxidants from natural sources has increased in response to the emergence of drug-resistant microorganisms and negative health effects of synthetic antioxidants [9,10]. Herbal drugs have gained a reputation in recent years because of their safety, efficacy, and cost-effectiveness. In the present day, nearly four billion people living in the developing world depend on plant-derived medicines as their first line of action for combating diseases and maintaining health [11-13]. Phytochemical analysis of medicinal plants plays a crucial role in understanding the various beneficial compounds that nature provides us. These plants, which have been used for centuries in traditional medicine, contain a variety of chemical substances known as phytochemicals. These compounds are the reason many plants have therapeutic properties, helping to prevent and treat ailments. Phytochemicals can include alkaloids, flavonoids, terpenoids, and phenolic compounds, each contributing to the plant's unique benefits. Through phytochemical analysis, researchers can identify these compounds and their potential health benefits, leading to the development of new medicines and therapies. This analysis often involves extracting the active ingredients from the plant materials and testing their effects in the lab. Due to the presence of phytochemicals like alkaloids, flavonoids, and terpenoids exhibit strong antibacterial activity. These compounds can disrupt bacterial cell walls, interfere with their metabolism, or prevent them from reproducing, offering a promising alternative for treating infections.

By exploring the chemical makeup of medicinal plants, scientists can better understand how they work within the body, how they can be combined for enhanced effects, and how they compare to synthetic drugs. The continued study of phytochemicals not only helps preserve traditional herbal knowledge but also encourages sustainable practices in sourcing these plants, ensuring that future generations can benefit from their natural healing properties. Ultimately, phytochemical analysis is a bridge connecting ancient wisdom with modern science, highlighting the importance of biodiversity in health and medicine.

2. Methodology

Collection and Authentication of plant material

Rothia indica plants were collected during the field work in Visakhapatnam district of Andhra Pradesh. The plant material was pressed and made into herbarium and was deposited in the Andhra university herbarium (AUV). After confirming the plant species, the plant material was collected. The leaf, stem and root were separated and dried in shade. Later made into powder and subjected for Soxhlet extraction to obtain extracts by using different solvents.

Taxonomic description

Prostrate annual herbs; ascending, to 25 to 30 cm, Stems adpressed-pilose, velvet-hairy. Stipules lanceolate, 4 × 1.5 mm. Leaves trifoliolate; petioles to 1 mm; leaflets 0.7-1.8 x 0.3-

0.7 cm, obovate to oblanceolate, 1-2.2 × 0.4-0.8 cm, both surfaces adpressed pilose, base cuneate, apex rounded to broadly acute base wedge-shaped, tip blunt or rounded, mucronate, appressed hairy beneath; stipules about 3 mm long, linear-lanceolate shaped; leaf-stalk 1-1.5 cm long. Flowers nearly stalkless, in leaf-axils, solitary or fascicled, very tiny, about 5 mm across; bracts 2-3 mm long, Inflorescence leaf-opposed or extra-axillary, 1-3-flowered, congested; bracts filiform, 1.5 mm; peduncle 0-2.5 mm. Pedicel 1.5 mm. Calyx obconical, 4-6 mm, divided to slightly below middle; lobes triangular, apex acuminate. sparsely fringed with hairs. Corolla yellow, exserted from calyx; standard oblong-orbicular, 5.5-6.5 mm, abaxially pilose along midline; wings oblong, slightly shorter than standard; keel ± as long as standard. Petals are yellow, clawed; standard about 6 mm long, ovate-oblong; wings narrow; keels cohering, sickle shaped. Stamens 10, monoadelphous. Ovary about 3 mm long, linear, hairy; stigma capitate. Fruit, legume narrowly oblong, linear, 3.5-5.5 × 0.15 - 0.2 compressed, mucronate, velvet-hairy. Seeds brown, many, about 1.5 mm long, kidney-shaped.

Phenology: Flowering and fruiting from August to March.

Habit and distribution: Commonly grows in deciduous forests, also in the Plains, in India it is distributed in Andhra Pradesh, Tamil Nadu, Karnataka, Kerala, Odisha.

Fig.1. Rothia indica habit

Fig.2. Plant material collected

Phytochemical analysis of plant extracts is a scientific method used to identify and quantify the various chemical compounds found in plants that may contribute to their medicinal properties. The process typically begins with the collection of plant materials, such as leaves, roots, or flowers, which are then dried and powdered to increase surface area for extraction. Various solvent extraction techniques, such as maceration, soxhlet extraction, or cold percolation, are employed to dissolve the phytochemicals from the plant material. Common solvents include water, ethanol, methanol, and hexane, depending on the compounds of interest. After extraction, the resulting solutions are filtered to remove solid debris. Additionally, colorimetric assays can help determine the concentration of specific phytochemicals, such as flavonoids or tannins. Qualitative analysis is primarily focused on identifying the various categories of phytochemicals present in the extract, such as

Carbohydrate, Tannin, Saponin, Flavonoid, Alkaloid, Quinone, Glycoside, Cardiac Glycoside, Terpenoid, Phenol and Protein. This is typically conducted using specific chemical tests established by Harborne (1973) and Sofowora (1993), where reagents are added to the extract to observe any reactions or colour changes, indicating the presence of certain phytochemical classes (14,15).

Antimicrobial activity of plant extracts is assessed through various methodologies that help researchers understand how these natural substances can inhibit or kill harmful microorganisms like bacteria and fungi. One common approach is the disc diffusion method, where small discs soaked in plant extracts are placed on an agar plate that has been inoculated with the target microorganisms. After incubation, the plates are examined for clear areas around the discs, known as zones of inhibition, which indicate the effectiveness of the extracts against the pathogens. Another popular technique is the broth micro dilution method, which involves diluting the plant extracts in a liquid culture medium with the microorganisms. By determining the lowest concentration of the extract that prevents growth, researchers can establish the minimum inhibitory concentration (MIC). Additionally, some studies employ the well diffusion method, which creates wells in the agar plates filled with the plant extract, and the growth inhibition is measured similarly to the disc diffusion technique. Advanced techniques may also include time-kill studies to observe the antibacterial effect over time. These methodologies not only provide insight into the antimicrobial properties of various plant extracts but also contribute to the development of new antimicrobial agents that are needed due to increasing resistance to conventional antibiotics.

3. Results

The preliminary examination of the phytochemical profile suggests that various extracts of the formulation encompass a varied assortment of crucial phytochemicals, including alkaloids, flavonoids, amino acids, glycosides, cardiac glycosides, tannins, terpenoids, phenols, steroids, quinones, proteins, carbohydrates, and saponins. The outcomes of the different phytochemical screening tests conducted during the experiment can be found in Table 1. The methanol extract displayed the greatest proportion of secondary metabolites, with the chloroform, ethyl acetate, and hexane extracts following in descending order, each exhibiting lower percentages. The alkaloids were found in all methanol and chloroform extracts of the root, stem, and leaf, while being notably absent in the remaining extracts. All the extracts lack amino acids, proteins, and steroids. Carbohydrates can be found exclusively in the root chloroform extract, stem ethyl acetate extract, and leaf extract. All the extracts indicate the lack of saponins, except for the Methanol extracts from the root, stem, and leaf. All extracts have

been verified to contain flavonoids, except for the ethyl acetate extracts from the root, stem, and leaf, as well as the chloroform extracts from the stem and leaf, and the hexane extracts from the root. All extracts confirm the lack of quinones, except for the Methanol root extract and chloroform stem extract. Glycosides and cardiac glycosides can be found in methanol extracts from the stem and leaf, chloroform extracts from the root and leaf, and ethyl acetate extracts from the root and stem. All extracts have been found to lack terpenoids and phenols, except for the Methanol root and chloroform stem extracts. All extracts have been verified to lack tannins, except for the Methanol root and chloroform stem and leaf extracts.

Root, stem and leaf showed adequate activity, among them root showed the highest activity. Highest activity was observed in ethyl acetate and chloroform followed by methanol, and hexane extracts. All the three extracts showed highest activity in Bacillus subtilis followed by Streptococcus mutans, Staphylococcus aureus and less or no inhibition was observed in Pseudomonas fluorescens respectively at 10mg/l concentration given in table 2.

Bacillus subtilis (14 mm) exhibited the largest zone of inhibition in the methanol root extract of Rothia indica, followed by Streptococcus mutans (13 mm), Staphylococus aureus (12 mm) and Pseudomonas fluroscens (11 mm). The stem extract showed maximum zone of inhibition in gram negative bacteria i.e., Bacillus subtilis and Pseudomonas fluroscens (15 mm) followed by gram positive bacteria i.e., Staphylococus aureus and Streptococcus mutans (14 mm). Staphylococus aureus (21 mm) showed maximum zone of inhibition in methanol leaf extract followed by Pseudomonas fluroscens and Bacillus subtilis (16 mm) and Streptococcus mutans (13 mm).

Gram positive bacteria i.e., Streptococcus mutans and Staphylococus aureus (18 mm) exhibited the largest zone of inhibition in the chloroform root extract of Rothia indica, followed by gram negative bacteria i.e., Bacillus subtilis (15 mm) and Pseudomonas fluroscens (13 mm). The stem extract showed maximum zone of inhibition in Bacillus subtilis (20 mm) followed by Staphylococus aureus (16 mm), Streptococcus mutans (15 mm) and less or no inhibition was observed in Pseudomonas fluroscens. Bacillus subtilis (19 mm) showed maximum zone of inhibition in chloroform leaf extract followed by Staphylococus aureus (16 mm), Streptococcus mutans (14 mm) Pseudomonas fluroscens (10 mm).

Streptococcus mutans (21 mm) exhibited the largest zone of inhibition in the ethyl acetate root extract of Rothia indica, followed by Staphylococus aureus and Bacillus subtilis (15 mm) and Pseudomonas fluroscens (14 mm). The stem extract showed maximum zone of inhibition in Streptococcus mutans (20 mm) followed by Bacillus subtilis (19 mm) and Staphylococus aureus and Pseudomonas fluroscens (14 mm). Staphylococus aureus and Bacillus subtilis (18 mm) showed maximum zone of inhibition in ethyl acetate leaf extract followed by Streptococcus mutans (15 mm) and Pseudomonas fluroscens (14 mm). Pseudomonas fluroscens (16 mm) exhibited the largest zone of inhibition in the hexane root extract of Rothia indica, followed by Streptococcus mutans (12 mm), Staphylococus aureus and Bacillus subtilis (10 mm) and less or no inhibition was observed in Bacillus subtilis (8 mm). The stem extract showed maximum zone of inhibition in Bacillus subtilis (14 mm) followed by Streptococcus mutans (13 mm) and Staphylococus aureus (12 mm) and Pseudomonas fluroscens (10 mm). Bacillus subtilis (18 mm) showed maximum zone of inhibition in hexane leaf extract followed by Streptococcus mutans and Pseudomonas fluroscens (15 mm) and Staphylococus aureus (10 mm).

Table.1. Showing preliminary phytochemical results of different solvent extracts of Rothia indica (L.) Druce

SNO	COMPOUND	MRR	MRS	MRL	CRR	CRS	CRL	ERR	ERS	ERL	HRR	HRS	HRL
1	CARBOHYDRTES	-	-	-	-	-	+	-	+	+	-	-	-
2	ALKALOIDS	+++	+++	+++	+++	+++	+++	-	-		-	-	-

3	AMINO ACIDS	-	-	-	-	-	-	-	-	-	-	-	-
4	SAPONINS	+	+	+	-	-	-	-	-	-	-	-	-
5	FLAVONOIDS	+	+	+	+	-	-	-	-	-	-	+	+
6	QUINONES	+++	-	-	-	+++	-	-	-	-	-	-	-
7	GLYCOSIDES	-	+	+	+	-	+	+	+	-	-	-	-
8	TERPENOIDS	+ + +	-	-	-	+++	-	-	-	-	-	-	-
9	PHENOLS	+	-	-	-	+	-	-	-	-	-	-	-
10	PROTEINS	-	-	-	-	-	-	-	-	-	-	-	-
11	STEROIDS	-	-	-	-	-	-	-	-	-	-	-	-
12	CARDIAC GLYCOSIDES	-	+	+	+	-	+	+	+	-	-	-	-
13	TANNINS	+	-	-	-	+	+	-	-	-	-	-	-

MRR-Methanol Rothia root, MRS- Methanol Rothia stem, MML- Methanol Rothia leaf, CRR-Chloroform Rothia root, CRS- Chloroform Rothia stem, CRL-Chloroform Rothia leaf, ERR-Ethyl acetate Rothia root, ERS- Ethyl acetate Rothia stem, ERL- Ethyl acetate Rothia leaf, HRR- Hexane Rothia root, HRS-Hexane Rothia stem, HRL-Hexane Rothia leaf.

Table.2. showing anti-bacterial activity of different solvent extracts obtained from Rothia indica (L.) Druce

Sample	Streptococcus mutans			Staphylococcus aureus			Bacillus subtilis			Pseudomonas fluorescens		
	10 mg/µl	5 mg/µl	2.5 mg/µl	10 mg/µl	5 mg/µl	2.5 mg/µl	10 mg/µl	5 mg/µl	2.5 mg/µl	10 mg/µl	5 mg/µl	2.5 mg/µl
MethnolRoot	13	13	8	12	11	9	14	13	8	11	11	9
MethnolStem	14	11	10	14	11	9	15	13	11	15	13	8
Methnol leaf	13	12	8	21	16	12	16	15	12	16	13	12
Chloroform Root	18	10	8	18	14	9	15	12	10	13	12	9
Chloroform stem	15	9	6	16	15	10	20	19	16	14	--	--
Chloroform leaf	14	10	8	16	13	12	19	17	16	10	8	7
Ethyl acetate root	21	12	10	15	13	11	15	14	13	14	13	--
Ethyl acetate stem	20	16	13	14	14	13	19	16	13	14	12	10
Ethyl acetate leaf	15	12	10	18	15	12	18	18	16	14	10	9
Hexane Root	12	10	--	10	10	8	8	6	--	16	8	7

Hexane stem	13	11	9	12	9	8	14	12	12	10	8	6
Hexane leaf	15	12	10	10	10	7	18	15	13	15	13	11

4. Discussion

Plants have been found to contain various Phytochemicals such as tannin, quinine, terpenoid, flavonoid, steroid, alkaloid, cardiac glycoside, glycoside, and volatile oils, among other compounds. Literature suggests that curcumin, extracted from turmeric, is linked to improved insulin resistance, enhanced glucose uptake, impacts on blood pressure, and decreased inflammation. Flavonoids have been associated with a lower risk of developing cardiovascular diseases and cancer. Certain groups of plant compounds, including flavonoids, alkaloids, and tannins, have shown to possess cytotoxic effects. Flavonoids, renowned for their vibrant hues and delightful scents, have been found to possess anti-cancer attributes as well. Moreover, saponins are known for their ability to lower cholesterol levels and possess cytotoxic, antibacterial, and antiviral characteristics (19). Tannins have demonstrated potential anticancer effects, as they have been observed to impede growth (20). Plants abundant in flavonoids have the potential to act as powerful antibacterial agents (17). As a result, these plants can be used as antibacterial and antiseptic agents. Moreover, plants rich in phenolic compounds are highly prized for their antioxidant characteristics. Quinine is well-known for its ability to lower fever, hinting plants like Ocimum, Nyctanthes, and Mentha, which boast quinine, could offer relief in reducing body temperature. Phenolic compounds such as tannins, terpenoids, and flavonoids have anti-parasitic qualities, making these plants effective for treating gastrointestinal problems (21). Plant polyphenolic compounds, such as flavonoids and terpenoids, offer numerous advantages as antioxidants, anti-inflammatory agents, and antibacterial substances. They also play a crucial part in reducing blood pressure and averting heart diseases.

The understanding and application of traditional medicine can be leveraged effectively when supported by a wealth of in vitro and in vivo studies. Based on scientific literature and insights from national pharmacognosy experts, there are four key plants commonly utilized in India for the management of bacterial infections. Antibiotic therapy is typically effective in treating bacterial infections, yet the rising challenge of antibiotic resistance underscores the ongoing necessity for innovative solutions. The management of bacterial infections has become more complex due to the development of resistance to the majority of primary antimicrobial agents. Numerous individuals favor utilizing herbal remedies over antibiotics, particularly those herbal medicines commonly employed in traditional medicine (23, 24). Numerous plants are utilized globally for addressing bacterial infections, although not all have undergone in vitro investigations and clinical trials. Numerous reports delineate the antibacterial properties of plant crude extracts, hindering the growth of different bacteria. However, there is a scarcity of in vitro antimicrobial investigations to ascertain if they surpass, match, or fall short of antibiotics (25,26).

The efficacy of the organic extracts may also be linked to the existence of complex terpenoids with carboxylic acid groups. Many workers have reported the pain-relieving properties of alkaloids (27,28) and the anti-inflammatory and antibacterial characteristics of tannins (29).

These categories of compounds are known for their healing properties against different types of bacteria. It comes as no surprise that herbalists have traditionally used these botanical extracts for addressing bacterial-related ailments. Tannins, accompanied by their protein composition, can be found in 232 African species. The synergistic impact of rainfall and vasoconstriction has the potential to offer advantages in preventing the development of ulcers (30,31). Numerous researchers have extensively documented the diuretic and antibacterial properties of plant extracts abundant in flavonoids (15, 32-34). The alkaloids present in plants are used in the field of medicine as substances for inducing anaesthesia (35). Saponins discovered in plants were pinpointed as the source of the revitalizing and tonic benefits observed in Chinese and Japanese medicinal herbs, as documented by Alinnor in 2008 (36). The study's discoveries indicate that the phytochemical compounds identified could potentially be the active elements contributing to the effectiveness of the leaves of the plants under investigation (37). The documented existence of specific compounds has been associated with antimicrobial effects. Hence, one could deduce that plant extracts have the potential to be a valuable source for creating pharmaceuticals used to fight different types of microbial infections (38).

5. Conclusion

Ultimately, the harmonious combination of phytochemical variety and antimicrobial properties found in plant extracts provides a valuable reservoir of possible antimicrobial substances that can be applied across different fields. These findings provide a strong foundation for incorporating plant-based remedies into modern medical practices. It is important to scientifically evaluate and properly share local ethno-medical remedies and herbal treatments. Moreover, delving deeper into the understanding of traditional botanical preparations using medicinal plants has the potential to facilitate additional advancements in pharmacology, phytochemistry, ethnobotany, and various other biological mechanisms that are essential for the process of drug discovery. Researchers strive to harness the power of nature's medicinal resources to create new methods for addressing microbial challenges and enhancing worldwide health and prosperity.

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