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PRELIMINARY PHYTOCHEMICAL STUDIES AND ANTIBACTERIAL ACTIVITY OF THE LEAF EXTRACTS OF RHODODENDRON NILAGIRICUM

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ABSTRACT

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. The present study involves the leaves of *Rhododendron nilagiricum* which were collected from in and around Nilgiri district, Tamilnadu. The leaves of the selected medicinal plant was washed, air dried and then powdered. The Methanolic and Acetone extract of leaf sample was used for the phytochemical analysis to find out the phytochemical constituents in the plant. The main objective of the research work was to check the presence or absence of the phytochemical constituents in the selected medicinal plant and screen for the Antibacterial activity. The results of the phytochemical analysis of this medicinal plant showed the presence of phenols, saponins and tannins. The methanol and acetone extracts of the medicinal plant was evaluated for activity against medically important bacteria such as *streptococcus sp* . and *Escherichia coli*. The *in vitro* antimicrobial activity was performed by agar well diffusion method and disc diffusion method. The acetone extracts showed minimum antimicrobial activity when compared to methanolic extracts. The methanolic leaf extract of *Rhododendron nilagiricum* showed the maximum activity against *Steptococcus sp*. The use of plant extracts with known antimicrobial properties, can be of great significance in therapeutic treatments. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases. It is expected that the important

phytochemical properties recognized by our study in the medicinal plant of *Rhododendron nilagiricum* will be very useful in the curing of various diseases.

KEYWORDS: *Rhododendron nilagiricum*, Phenols, saponins, tannins, antibacterial activity, *Streptococcus sp*. and *Escherichia coli*.

INTRODUCTION

Nature has been a source of medicinal agents for thousand of years and an impressive number of modern drugs have been isolated from natural sources and many based on their use as traditional medicine.[1-5] India and China are the leading countries enjoying major global market share in medicinal plants. India is a home to thousands of important medicinal plant species; it is ranked sixth among 12 mega diversity countries of the world having two hotspots of biodiversity.[6-11] The genus Rhododendron, having about 72 species, 20 sub species and 19 varieties in India, is mainly distributed in the Eastern Himalayas, while *Rhododendron nilagiricum* is the only endemic species found in western Ghats. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. The main objective of our research work was to analyze the presence or absence of different phytochemicals in the selected medicinal plant from Nilgiri district used for healing and curing of various diseases.

The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in various countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like, phenolic compounds which are part of the essential oils, as well as in tanning.

The screening of plant products for antimicrobial activity have shown that the higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants.[2]

MATERIALS AND METHODS

Collection and Processing of medicinal plant: The leaves of *Rhododendron nilagiricum* were collected from in and around Nilgiri district,Tamilnadu. The leaves of the plant was used to prepare extracts for the study. The plants collected were washed with water to remove the soil and dust particles. Then they were dried in thoroughly shaded place, and blended to form a fine powder and stored in airtight containers.

Preparation of leaf extract and phytochemical screening: For extraction of crude phytochemical, 5g of powdered leaf material was kept in closed conical flask with 20 ml of the solvents like methanol and acetone in a shaker at room temperature for 24 h. After incubation, the extracts were filtered through Whatman No. 41 filter paper and the extracts were collected and stored in the refrigerator at 4°C. All the extracts were subjected to preliminary phytochemical screening as per the methods given by Harborne.[12]

Preparation of leaf extract for Antibacterial study

Methanol extract: About 10g of air dried powder was taken in 50 ml of methanol. Plugged with cotton wool and then kept on rotary shaker at 220 rpm for 24 hrs. Then the supernatant was collected and the solvent was evaporated to make the final volume one- fourth of the original volume and stored at 4^0C in air tight container.

Acetone extract: About 10g of air dried powder was taken in 50 ml of acetone. Plugged with cotton wool and then kept on rotary shaker at 220 rpm for 24 hrs. Then the supernatant was collected and the solvent was evaporated to make the final volume one- fourth of the original volume and stored at 4^0C in air tight container.

Preparation of sterile disc: Whatman's No.3 filter paper was punched into 5 mm disc form and they sterilized, each sterile disc was incorporated individually with 20 - 60l of extracts using micropipette. Precautions were taken to prevent the flow of the solvent extract from the discs to the outer surface. The condensed extracts were applied in small quantities on discs and they were allowed to dry in air. After sometimes another doses of extracts were applied on discs. Then they were stored at 4^oC.

Assay of Antimicrobial activity using Agar well diffusion method: The 20 ml of sterilized Muller Hinton Agar was poured into sterile petriplate, after solidification, 100 µl of fresh culture of human pathogens were swabbed on the respective plates. The wells were punched

over the agar plates using sterile gel puncher at various concentration (20, 30, 40, 50 and 60) of the plant extract were added to the wells. The plates were incubated for 24 hours at 37C.[14] After incubation the diameter of inhibitory zones formed around each discs were measured in mm and recorded.

Antimicrobial activity of commercially available antibiotics: The antimicrobial activities of some selected plant extracts on human pathogenic bacteria were compared with the commercially available antibiotics. Sterile Muller Hinton Agar plates were prepared and the test organisms were swabbed over the surface of agar plates using sterile cotton swab. The antibiotic disc such as Kanamycin and Streptomycin was placed on the surface of the plates. The plates were incubated at 37^{o}C for 24 hours and after incubation the diameter of the inhibition zones were measured in mm and recorded.[13]

RESULTS

Phytochemical screening: The results of the phytochemical screening of the leaves of *Rhododendron nilagiricum* findings are reported in Table 1.

Table. 1. Results of preliminary phytochemical screening of the leaf extracts of *R. nilagiricum*.

Phytochemical constituents	Methanol	Acetone
Alkaloids	------	------
Phenol	+++	+++
Flavanoids	------	------
Saponins	+++	+++
Proteins	------	------
Quinone	------	------
Steroid	------	------
Tannin	+++	+++
Xanthoprotein	------	------
Carboxylic acid	------	------
Coumarin	------	------
Carbohydrates	+++	+++

(---) – absence, (+++) – presence

Antibacterial activity

Anti-bacterial activity of Methanolic leaf extract of *R.nilagiricum* on *E.Coli:* Antibacterial activity of methanolic leaf extract of *R. nilagiricum* against *E.coli* showed activity at different concentration such as 10mg/ml (80µl,40µl). At concentration the zone size was found to be 21mm at higher concentration 10mg /mL (40 µl) and 10 mg /mL (80µl) there an increase in

zone size by 2.0 mm comparing to 40µl. Thus, it could be confirmed that at 80µl (10mg/ml) dosage shows maximum inhibition of 21mm. The antibiotic kanamycin was used as positive control.

Table. 2. Anti-bacterial activity of Methanolic leaf extract of *R.nilagiricum.*

Concentration of extract (mg/mL)	Zone of inhibition (mm diameter)
Control (Kanamycin)	24.00
10(80µl)	21.00
10(40µl)	19.00

Plate. 1. Anti-E.coli activity of methanolic leaf extracts of *R.nilagiricum.*

Anti-bacterial activity of Acetone leaf extract of *R.nilagiricum:* Antibacterial activity of Acetone leaf extract of *R. nilagiricum* against *E.coli* showed activity at different concentration such as 10mg/ml(80µl,40µl). At 40µl concentration the zone size was found to be 16mm .At higher concentration 10 mg /mL (80µl)there an increase in zone size by 4.0mm comparing to 40µl. Thus, it could be confirmed that at 80µl (10mg/ml) dosage shows maximum inhibition of 20mm. The antibiotic kanamycin was used as positive control.

Table. 3. Anti-bacterial activity of acetone leaf extract of *R.nilagiricum.*

Concentration of extract (mg/mL)	Zone of inhibition (mm diameter)
Control (Kanamycin)	22.00
10(80µl)	20.00
10(40µl)	16.00

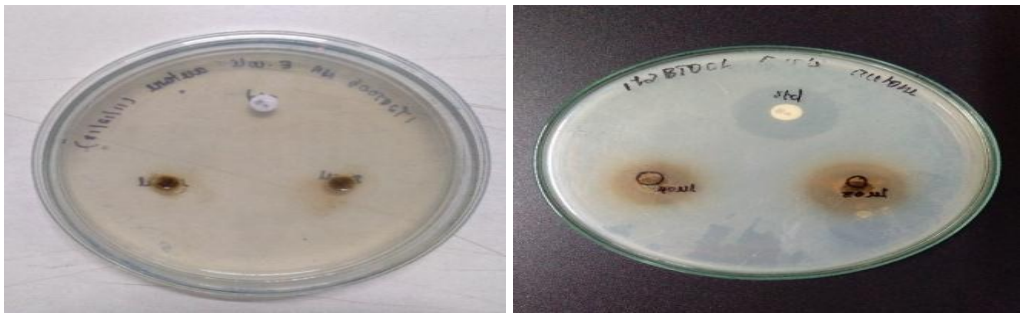

Plate. 2. Anti-E.coli activity of acetone leaf extracts of R. Nilagiricum.

Anti-bacterial activity of Acetone leaf extract of *R.nilagiricum* on *Streptococcus*

Antibacterial activity of Acetone leaf extract of *R. nilagiricum* against *Streptococcus* showed activity at different concentration such as 10mg/ml(80µl,40µl). At 40µl concentration the zone size was found to be 20mm. At higher concentration 10mg /mL (80µl) there an increase in zone size by 7.0mm comparing to 40µl. Thus, it could be confirmed that at 80µl (10mg/ml)dosage shows maximum inhibition of 27mm. The antibiotic streptomycin was used as positive control.

Table 4. Anti-bacterial activity of acetone leaf extract of *R.nilagiricum*.

Concentration of extract (mg/mL)	Zone of inhibition (mm diameter)
Control (streptomycin)	27.00
10(80µl)	27.00
10(40µl)	20.00

Plate. 3. Anti-*Streptococcal* activity of acetone leaf extracts of *R.nilagiricum*.

Anti-bacterial activity of Methanolic leaf extract of *R.nilagiricum* on *E.Coli*

Antibacterial activity of methanolic leaf extract of *R. nilagiricum* against *Streptococcus* showed activity at different concentration such as 10mg/ ml(80µl,40µl). At 40µlconcentration the zone size was found to be 21mm. At higher concentration 10mg /mL (80 µl) there an increase in zone size by 2.0 mm comparing to 40µl. Thus, it could be confirmed that at 80µl (10mg/ml) dosage shows maximum inhibition of 21mm. The antibiotic streptomycin was used as positive control.

Table. 5. Anti-bacterial activity of methanolic leaf extract of *R.nilagiricum*.

Concentration of extract (mg/mL)	Zone of inhibition (mm diameter)
Control (streptomycin)	25.00
10(80µl)	23.00
10(40µl)	21.00

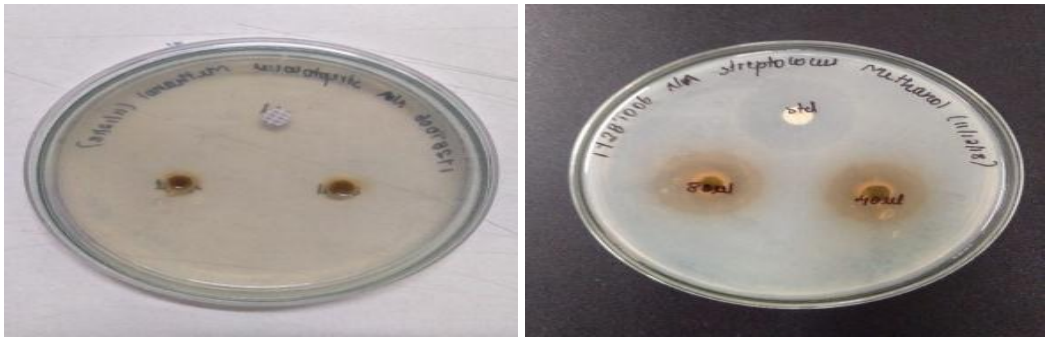

Plate. 4. Anti-*Streptococcus* activity of methanolic leaf extracts of *R.nilagiricum*.

CONCLUSION

The species of *Rhododendron* showed the presence of phenols and tannins. The leaves of the experimental plant could be of considerable attention to the development of novel drugs in the field of biomedicine.In this present study, preliminary screening for antimicrobial activity showed, that the methanolic extract of *R.nilagiricum* exhibited maximum inhibitory zone (30 mm) against *Streptococcus sp.* The antimicrobial assay by agar-well diffusion method revealed that methanol extract of medicinal plants exhibited broad spectrum activity against tested isolates as compared to acetone extracts. Results obtained from this study, indicated that, the plant extracts showed the strongest antimicrobial activity than the commercially available antibiotics.

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