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THE LEAF OF *NILGIRI RHODODENDRON*: A POTENT ANTIMICROBIAL AGENT AGAINST MEDICALLY CRITICAL HUMAN PATHOGENS

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ABSTRACT: Several reports are available for Rhododendron arboreum which is limited to its subspecies nilagiricum. The present study intended to examine the bioactive compounds and the antimicrobial activity of leaf extracts of *R. arboreum* Sm spp. *nilagiricum* (Zenker) Tagg against medically important four bacterial (*Streptococcus pyogene*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*) and two fungal (*Candida albicans* and *Trichoderma viride*) strains. Agar well diffusion method is applied to assess the antimicrobial activity of the aqueous and methanol extracts of the plant sample. Various fractions of aqueous, ethanol, methanol and chloroform extracts confirmed the presence of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, steroids, carbohydrates, glycosides, amino acids, and proteins. The methanol extract of leaf revealed a promising antibacterial and antifungal activity against *S. aureus* and *T. viride* respectively. This study shows a broad and great therapeutic potential of the leaf extract. However further studies are necessary for this potent leaf extracts to evaluate the other parameters of antimicrobial efficacy.

INTRODUCTION: Nature being the major source of traditional medicine provides unbelievable remedies for various ailments for about thousands of years. Knowing the impact of these traditional medicines, an impressive number of modern drugs have been isolated from natural sources. Worldwide, fair numbers of plant species have been used for medicinal practices. Folk medicine has gifted many new plant drugs to modern medicine.

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Presently, there is a vital question flickering in the mind of third world researchers is the choice of which disease condition needs to be focused since typical diseases such as cancer and viral type are concentrated on by large pharmaceutical companies. The focus indeed should be on parasitic diseases.

The Nilgiri Rhododendron (*Rhododendron arboreum* Sm spp. *Nilagiricum* (Zenker) (Tagg)) is an interesting species of the genus Rhododendron which is a member of the plant family Ericaceae. It is endemic to the Southern Western Ghats of peninsular India [1]. It is a tree species up to 10m tall with bark brownish, fissured and pinkish blaze. Crimson red bell-shaped flowers are borne in fascicles or pseudocorymbs at branch ends. It has ecological significance and economical importance

in addition to its graceful flowers. The young leaves are said to be poisonous as well as medicinal when applied on the forehead to alleviate headache [2]. Chemical analysis of the leaves revealed the presence of hyperoside (3-D-galactoside of quercetin), ursolic acid and epifriedelinol (a triterpenoid compound) [3]. The genus has been reported to be effective as astringent, diuretic, choleretic, antispasmodic, chronic eczema, diarrhea, dysentery, anti-irritable bowel syndrome therapy [4, 5].

Presently, the spread of multidrug-resistant microbial pathogens has threatened the current antimicrobial therapy. The most problematic human bacterial pathogens include *Escherichia coli, Klebsiella pneumonia, Enterobacterium, Pseudomonas aeruginosa, Staphylococcus aureus, etc.* and fungal pathogens such as *Candida albicans, Trichoderma viride, etc.*

Thus, effective newer antimicrobials are urgently required to treat or inhibit the growth of these human pathogens. The influence of various plant extracts on several diseases is observed to be a promising remedy since ancient time in Chinese medicine, Ayurveda, Arabic, and Unani medicine [6]. Plants used in traditional medicine contains a wide range of bioactive compounds that can be used to treat infectious diseases. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenols [7].

Considering all the above facts, the present study was conducted to screen the bioactive chemical compounds and to assess the antimicrobial activity of leaf of *R. arboreum* Sm spp. *nilagiricum* – an endemic representative of Western Ghats

MATERIALS AND METHODS:

Collection of Plant Material: Plant material was collected from The Nilgiris, Tamil Nadu, India, in January 2015, authenticated by taxonomist at the Department of Botany, Government Arts College, Ooty. Documentation of the plant specimen is made and deposited in the department herbaria.

Preparation of Plant Extracts: The aerial parts of the plant were air dried under shade for three weeks. The dried plant material (leaf) was pulverized by a mechanical grinder, sieved through 40 mesh. To perform the phytochemical screening, various aqueous, ethanol, methanol and chloroform extracts of the plant sample was prepared. Simultaneously, aqueous and methanol extracts were prepared to assess the antimicrobial activity of the plant sample.

Preparation of Inoculum: The test organisms used were clinical isolates *viz*., *Streptococcus pyogene, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia*. Also, the human fungal pathogens *Candida albicans* and *Trichoderma viride* which were obtained from the Department of Microbiology, Hindustan College of arts and science, Coimbatore. The bacterial and fungal cultures were maintained on nutrient agar medium and potato dextrose agar (PDA) medium respectively. The bacterial strains were pre-cultured in nutrient broth overnight in a rotary shaker at 37ºC, centrifuged at 10,000 rpm for 5 min. Pellets was suspended in double distilled water, and the cell density was standardized spectrophoto-metrically (A_{610} nm).

The fungal inoculums were prepared from 5 to 10 days old culture grown on potato dextrose agar medium. The Petri dishes were flooded with 8 to 10ml of distilled water and conidia were scraped using a sterile spatula. The spore density of each fungus was adjusted with the spectrophotometer (A_{595} nm) to obtain a final concentration of approximately 10^5 spores per ml. The composition of Nutrient Agar medium (g/L) is Beef extract: 3g; Peptone: 5g; Agar: 15g and distilled water: 1000ml; pH 7. Similarly, the composition of PDA medium is potato: 200g; dextrose: 20g; Agar: 15g and distilled water: 1000ml; pH 6.2.

Antimicrobial Testing: Antibacterial activity was determined by agar-well diffusion method [8] with modifications according to the present experimental conditions. The test microorganisms were seeded into the respective medium by spread plate method 10µl (10 cells/ml) with the 24 h cultures of bacterial growth in nutrient broth. After solidification, the filter paper wells (5mm in diameter) impregnated with the extracts were placed on test organism – seeded plates. Chloramphenicol (10µg) used as a standard for the antibacterial test. The antibacterial assay plates were incubated at 37 ºC for 24 h.

Antifungal activity was also determined by agar-well diffusion method [9]. The potato dextrose agar plates were inoculated with each fungal culture by point inoculation. The filter paper wells (5mm in diameter) impregnated with the extracts were placed on test organism - seeded plates. Chloramphenicol (10µg) used as positive control. The activity was determined after 72 h of incubation at 28 ºC.

The diameters of the inhibition zones were measured in mm. The experiments were performed in triplicates and results were presented as mean ± SD (Standard deviation). The significance in the difference of mean was determined according to Duncan's multiple range test.

Phytochemical Analysis: The qualitative phytochemical properties of the dried powdered sample were determined using standard methods [10, 11].

RESULTS AND DISCUSSION: Although most bacteria are harmless or often beneficial, several are pathogenic. Pathogenic bacteria contribute to other globally important diseases such as pneumonia, which can be caused by bacteria such as Streptococcus and *Pseudomonas. Streptococcus* and *Staphylococcus* are part of the normal skin microbiota and typically reside on healthy skin or in nasopharyngeal region. Some bacteria such as *E. coli* can induce host epithelial cells to engulf them in a process resembling phagocytosis [12]. In the present study, it is cleared that all the fractions of *R. arboreum* showed statistically significant antibacterial activity against *E. coli, K. pneumonia, S. aureus* and *S. pyogene* as shown in **Table 1.**

TABLE 1: ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT OF *R. ARBOREUM* SPP. NILAGIRICUM

Bacterial strains	The diameter of the inhibition zone (mm)		
	Aqueous	Methanol	Control
EC	10.00±0.82 [a]	11.00±0.80 [d]	10.00±0.22 [b]
KP	8.00±0.50 [c]	11.50±0.90 [c]	8.00±0.20 [d]
SA	6.50±0.10 [d]	12.50±1.10 [a]	10.50±0.60 [a]
SP	8.50±0.55 [b]	11.50±0.50 [b]	8.50±0.55 [c]

Gram-positive: SA-*Staphylococcus aureus*, SP-*Streptococcus pyogene*. Gram-negative: EC- *E. coli*, KP- *Klebsiella Pneumoniae*; the superscripts (a – d) indicates higher to lower inhibition zone

The mean zone of inhibition of the aqueous and methanol extract of leaf against the bacterial strains *E. coli, K. pneumonia, S. aureus* and *S. pyogene* showed 10.00 ± 0.82^{a} and 11.00 ± 0.80^{d}; 8.00 ± 0.50^{c} and 11.50 ± 0.90^{c}; 6.50 ± 0.10^{d} and 12.50 ± 1.10^{a}; 8.50 ± 0.55^{b} and 11.50 ± 0.50^{b} respectively. Likewise, the aqueous and methanol extract of the leaf against the fungal strains *Candida albicans* and *Trichoderma viride* showed 4.00 ± 0.20^{b} and 8.00 ± 0.40^{b}; 5.00 ± 0.30^{a} and 9.00 ± 0.50^{a} respectively. Fungi are microscopic eukaryotic organisms produce superficial, subcutaneous and systemic infections in animals and human beings [13]. Superficial and subcutaneous mycotic infections include dermatophytosis and candidiasis caused by various *dermatophytes* and *candida albicans* while systemic mycotic infections include aspergillosis, cryptococcosis, histoplasmosis, sporotrichosis *etc*. [14] Antifungal activity of aqueous and methanol extracts was tested against *C.albicans* and *T. viride* while chloramphenicol was used as a standard. In the present study, it is cleared that all the fractions of *R. arboreum* showed statistically significant antifungal activity as shown in **Table 2**.

TABLE 2: ANTIFUNGAL ACTIVITY OF LEAF AND BARK EXTRACT OF *R. ARBOREUM* SPP. *NILAGIRICUM*

Fungal strains	The diameter of the inhibition zone (mm)		
	AE	ME	C
CA	4.00±0.20 [b]	8.00±0.40 [b]	8.00±0.45 [a]
TV	5.00±0.30 [a]	9.00±0.50 [a]	8.00±0.42 [b]

CA- *Candida albicans*, TV-*Trichoderma viride*

Successive extraction and isolation of botanical compounds from plant material are largely dependent on the type of solvent used in the extraction procedure. The traditional healers use water primarily as the solvent, but we found in the present study that the plant extracts by alcohol

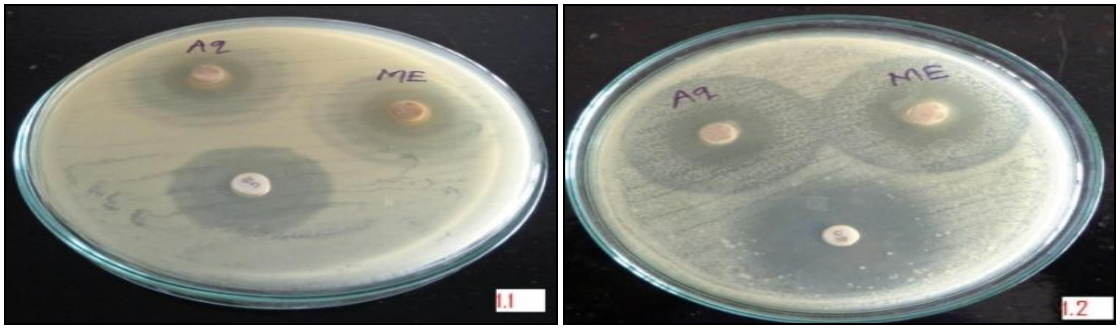
(methanol) provided more consistent antimicrobial activity compared to those extracted by water. The higher antimicrobial activity of methanol extract might be due to its high degree of solubility of active constituents in methanol [15]. The qualitative analyses of phytochemicals present in the leaf extracts are presented in **Table 3**. The result confirmed the presence of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, steroids, carbohydrates, glycosides, amino acids and proteins in both leaf and bark extracts.

TABLE 3: QUALITATIVE ANALYSIS OF PHYTOCONSTITUENTS PRESENT IN DIFFERENT SOLVENT EXTRACTS OF LEAF AND BARK EXTRACT

Phytochemicals	Aqueous	Ethanol	Methanol	Chloroform	Ethyl acetate
Alkaloids	+	+	+	+	+
Phenols	+	+	+	+	+
Flavonoids	+	+	+	-	-
Tannins	-	+	+	+	-
Saponins	-	+	+	+	-
Terpenoids	+	+	+	+	+
Steroids	-	+	+	+	+
Carbohydrates	+	-	-	+	+
Glycosides	-	+	+	-	-
Amino acids	+	+	-	+	-
Proteins	+	+	+	+	-

(+) the sign indicates the presence and (-) sign indicates the absence of the phytoconstituents

The leaf extract showed low to significant antimicrobial activity against the mentioned bacterial and fungal strains. Comparing the aqueous and methanol extract against the microbial strains, the methanol extract showed a promising antimicrobial activity. The methanol extract of the leaf against *Staphylococcus aureus shows* the high antibacterial activity followed by *Streptococcus pyogene*. The aqueous extract of the leaf shows significant activity against *E. coli*. Likewise, the methanol extract of the leaf against the fungal strain *Trichoderma viride* shows the high degree of antifungal activity. Amongst the plant extracts, methanol extract showed the most promising activity against all the bacterial strains. The methanol extracts of leaf showed potent antibacterial activity in the order *S. aureus* > *S. pyogene* > *K. pneumoniae* > *E. coli* and *S. aureus* > *S. pyogene* > *E. coli* > *K. pneumoniae* respectively. Similarly, methanol extracts of leaf showed potent antifungal activity against the studied fungal strains in the order *T. viride* > *C. albicans.*



E. COLI ***K. PNEUMONIAE***

S. AUREUS ***S. PYOGENE***

The high potency of the extracts against bacterial strains shows its scientific basis for its uses in traditional medicine in the treatment of different types of cough, diarrhea, and dysentery. These antibacterial activities are likely due to the presence of the secondary metabolites present in the extract. Flavanoids which recently reported to have antimicrobial activity include quercetin 3'-O-glucoside, rutin [16], coumestrol, genistein and daidzein [17], morin [18], *etc*. It has also been shown that saponins are active antifungal agents. Tannins are also known, antimicrobial agents. Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods. Tannins are water-soluble plant polyphenols that precipitate proteins. Tannins have been reported to prevent the development of microorganisms by accelerating microbial protein and making nutritional proteins unavailable for them [19]. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins [20]. The secondary metabolites identified in *R. arboreum* Sm sp. Nilagiricum could be responsible for antimicrobial activity exhibited by this plant.

C. ALBICANS ***T. VIRIDE***

FIG. 2: THE AQUEOUS AND METHANOL EXTRACT OF LEAF OF *R. ARBOREUM* SPP. NILAGIRICUM SHOWING THE ZONE OF INHIBITION AGAINST 2 FUNGAL STRAINS

CONCLUSION: It may, therefore be concluded that the phytochemical screening of *R. arboreum* Sm spp. nilagiricum confirmed the presence of major bioactive compounds which could be responsible for the significant degree of antimicrobial activity against the studied human pathogenic bacterial and fungal strains. The methanol extracts showed a promising antimicrobial activity compared to aqueous extracts of the plant sample. From the above findings, it could be suggested that further studies are necessary for this potent leaf extracts to evaluate the other parameters of antimicrobial efficacy which could then be utilized to develop a broad spectrum antimicrobial herbal formulation with this plant.

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