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ANTI-BACTERIAL ACTIVITY OF RHODODENDRON ARBOREUM PLANT AGAINST STAPHYLOCOCCUS AUREUS

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ABSTRACT

In the present study, preliminary i*n-vitro* antibacterial screening of the alcoholic extracts of leaves (LE) and flowers (FE) of *Rhododendron arboreum* was performed against bacterial culture, *Staphylococcus aureus* which was isolate from infected throat. Extracts showed prominent inhibition in the development of bacterial growth on agar plate. *Staphylococcus aureus* identified from bacterial culture by the methods of Mannitol salt agar with provide a yellow colony of *Staphylococcus aureus* due to acidic activity. Prepare a mix solution of agar and plant extract, after solidifying the media spread the bacterial culture over the surface of media and leave it for a one day. After a one day see the bacterial growth and his inhibition on agar plate due to the plant extract activity. Antibacterial activity of Plant leaf extract is good as compare to flower extract.

KEYWORDS: Rhododendron Arboreum, Antibacterial Screening, Plant Leaf Extract and Mannitol Salt Agar

INTRODUCTION

The genus *Rhododendron* (Ericaceae) consists of over 850 species, occurring throughout the world. Rhododendron arboreum is one of the most stately and impressive rhododendron species. It is extremely variable in length, hardness, flower color, leaf characteristics and antimicrobial properties. It species name arboreum means tree like [1]. Thought the ages human have keep faith on nature for their general needs, the traditional medicine systems of plant that have been in existence from thousands of years and continue with new remedies. Natural products have, until recently, been the primary source of medicine and drugs. A recent survey revealed that 61% of the 877 drugs introduced worldwide can be traced to inspired by natural products [2].Rhododendron simsii have been used to treat irregular menstruation, traumatic, skin disorders, many immunomodulatory, infectious and inflammatory diseases such as sepsis syndrome, Cerebral malaria, adult respiratory distress syndrome, bacterial meningitis, AIDS and rheumatoid arthritis [3]. It's essential oils present in their twigs, leaves and flowers and can be used as high grade spices and cosmetics. Natural products include: an entire organism, a plant, an animal, or a microorganism. In current status of natural product nature has been a source of therapeutic agents for thousands of years, and an impressive value of modern drugs have been received from natural sources, and lots of drug are being used in modern medicine. Originally discovered in north central India the plant is found in the Himalayas from Kashmir to Bhutan & in the hills of Assam & Manipur at altitudes of 1200-400 m [4]. It grows well at the height of 4500 to 10,500 ft & grows up to 40 to 50 ft high sometimes it attains maximum height up to 100 ft. This is an evergreen much branched tree up to 14 m in height & 2.4 m in girth [5]. The roots, leaves and flowers of *R. arboreum* are important crude source of drugs in traditional system of medicine and are used in dysentery, fever and headaches [4] [5]. Rhododendron arboreum phytoconstituent is rutin, ursolic acid, epifriedelinol, quercetin rhamnoside, friedelin, hyperin, quercetin, taraxerol, betulinic acid, coumaric acid, campanulin, α-amyrin, β-sitosterol, 3, 10-

epoxyglutinane, flavone 5, 2□- dihydroxy-7-methoxy-4□-O- glucoside, dimethyl ester of terephthalic acid [5]. The leaves and flowers of *R. lepidotum* are used as a snuff in headache [6]. The leaves and flowers of *R. ferrugineum* are used for rheumatism in Germany [7]. The poulticed leaves of R. maximum are used to relieve arthritic pain, headaches etc. [8]. *R. ponticum* is a common folk medicine of the Black Sea region, widely used as analgesic for the treatment of rheumatic or dental pain, common colds and edema, both internally and externally [9]. In Tibet and the western part of China, the leaf and flower of R. primulaeflorum are widely used to cure pulmonary disease, indigestion, dropsy, gastroptosis, and gastrectasis. The essential oils from it are used in curing chronic tracheitis. The leaves and flowers of *Rhododendron simsii* have been used in Chinese medicine to treat irregular menstruation, traumatic injuries, rheumatism and subcutaneous swelling [10]. *Rhododendrons* are not only of high value in view but are also of good medicinal importance because of the essential oils present in their twigs, leaves and flowers and can be used as high grade spices and cosmetics, especially due to efficacies such as detoxification, relieving cough and calming asthma, dispelling phlegm, diminishing inflammation and depressing blood pressure [11]. Aavicularin and hyperoside (Figure 2.1) from *R. dauricum* have antinociceptive action [12]. Coumarins i.e., daphnin, daphnetin, daphnetin glucoside, rhodonetin, rhodonin and umbelliferone (Figure 2.2) and their acetyl derivatives isolated from the methanolic extract of Rhododendron lepidotum have shown good antibacterial activity against Staphylococcus aureus, methicillin resistant Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa [13]. The dried leaves of *R. dauricum* are used medicinally as expectorant and in treatment of acute-chronic bronchitis [14]. In Tibet and China, the essential oils from various species of the genus *Rhododendron* are used to cure pulmonary diseases, laryngitis, urethritis, indigestion, gastroptosis, gastrectasis gastric cancer, hepatoma, dropsy and chronic tracheitis.

The flowers and leaves of *R. ferrugineum* are antirheumatic, diaphoretic and diuretic [15]. The methanolic extract with the chemical constituents of *Rhodendron. simsii* showed high antioxidative activity [16]. An unusual oleanane triterpene isolated from the aerial parts of *R. campanulatum* has shown prominent antibacterial and immunomodulatory activities [17]. Courmarins is phytochemical compounds which have a potential of effective inhibitors of tyrosinase enzyme which is isolated fom *R. collettianum* to be used for the treatment of hyper pigmentation associated with the high production of melanocytes [18]. The phagostimulants nature of these compounds Sitosterol and quercetin 3- galactoside are isolated from *R. catawbiense*. Tibetan medicine Dali, consisting of two Tibetan plants, *R. anthopogonoides* and *R. primulaeflorum* containing a mixture of essential oils, flavonoids and triterpenoids have been successfully used in eliminating phlegm, relieving cough, relieving inflammation, relaxing smooth muscle and curing asthma. Various stages of tumor development, tumor initiation and promotion is inhibited by Oleanolic acid and ursolic and is also induce tumor cell differentiation and apoptosis [19]. Oleanolic acid and ursolic acid are well known for their anti-HIV and hepatoprotective effects [20]. Betulinic acid Shows a broad spectrum of activity against various cancer cell types [21] and is also known for its anti-HIV [22], anti-inflammatory [23], antimicrobial [24], antimalarial, spasmogenic [25], antinoceceptive [26], antihelmintic [27], and anti-HSV-1 activities.

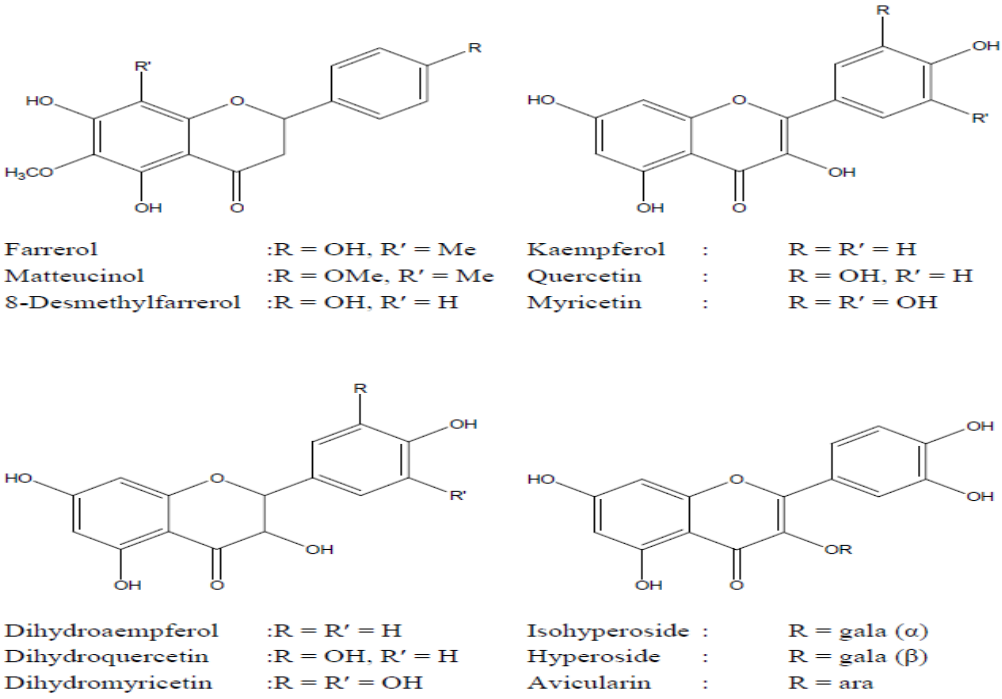


Figure 2.1: Molecular Structures of the Bioactive Flavonoids

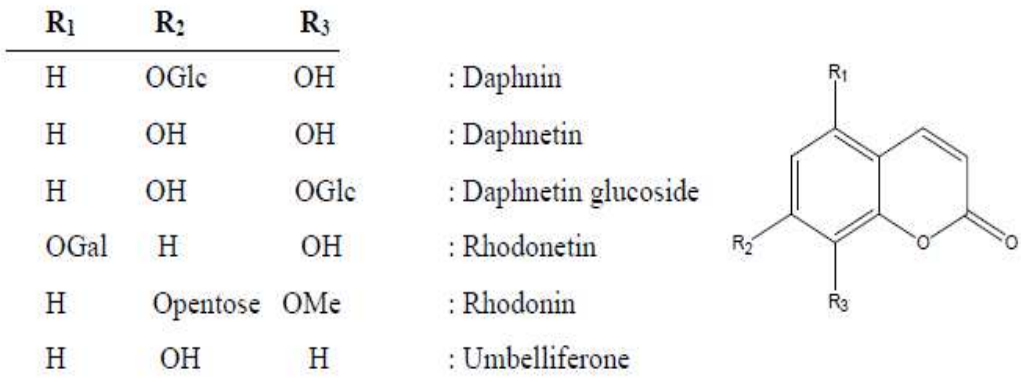


Figure 2.2: Molecular Structures of the Antibacterial Coumarins

MATERIALS AND METHODS

Collection of Plant Materials

Rhododendron arboreum plant leaves and flower collect from Nanital (U.K), India.

Chemicals: - All the chemicals, dyes and media such as algae broth were procured from Himedia, Mumbai, India.

Isolation of Bacteria and Preparation of Pure Bacterial Culture

2.8gm NAM powder was dissolved in 100ml of distilled water, autoclaved at 121^{0c} temperature on 15lbs. Samples from nasal cavity and infected throat area were collected with the help of sterilized cotton swab and spread over the Nutrient agar plate and incubated overnight at 37^{0c} in incubator. After 24hrs of incubation a single colony of growing bacteria from the Nutrient Agar Media plate was collected and streaked over the fresh NAM plate and Nutrient Broth

Medium for cultivation and further conformation and bacterial identification.

Identification of Bacteria

Bacterial Growth was Identified on the Basis of Biochemical Methods and Grams Staining Method

Preparation of Extracts

Alcoholic (Methanol) extracts of flowers and leaves of *Rhododendron arboreum* were prepared by continuous hot percolation method using Soxhlet apparatus.

Mixture of leaf and flower was prepared by drying at room temperature, thereafter crushed mixture was filled in dialysis bag .The ratio of 3:1 metabolic solution was prepared by adding ¾ solution of methanol followed by ¼ double distilled water. The Soxhlet was set at 70 ^{0c} for 8 hrs. Filled bag with dry material were inserted into an extraction tube when methanolic solution boiled, the vapors of methanol were condensed and pure extract was collected.

Antibacterial Activity of Plant Extract

Antibacterial activity of *Rhododendron arboreum* was more effective in leafs as compare to flower. Plant leaf extract reduce or stop the growth of bacteria (*Staphylococcus aureus*). NAM media was prepared with plant leaf extract and flower extract in different petri plate. When media was solidified then spread bacterial culture on it and Left for 24hrs. Bacterial growth was measured on the surface of medium in both plates. Plant leaf extract showed better result as compare to flower extract.

RESULTS AND DISCUSSIONS

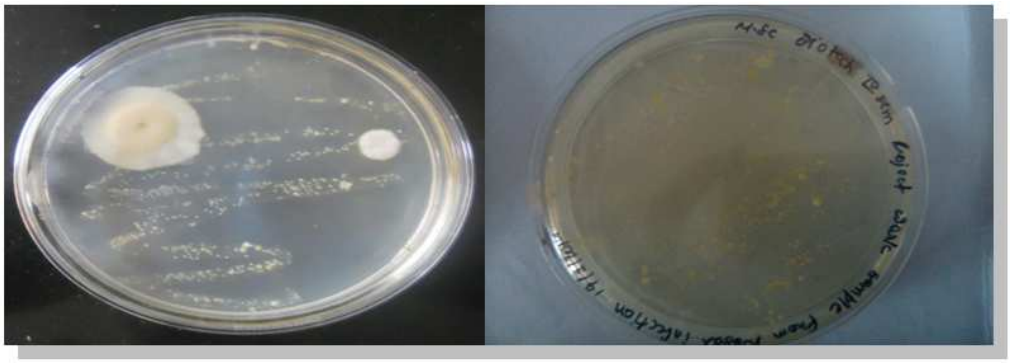


Figure 4.1: Bacterial Culture Collect from the Infected Throat on NAM Medium

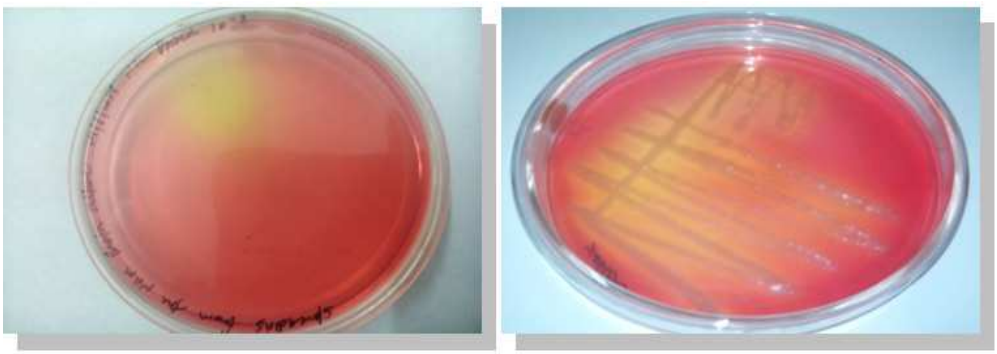


Figure 4.2: Bacteria *Staphylococcous Aureus* Grow on MSA Medium and Yellow Appearance Show Due to the Acidic Activity

Antibacterial Activity of *Rhododendron Arboreum* Leaf and Flower

- Collect a leaf and flower extract of a plant in a different reagent bottle from the soxhlet apparatus.
- Prepare a NAM media in different quantity and add extract of leaf and flower in the media before solidifying media **Figure 4.3.1 and Figure 4.3.2**.

Table 4.3.1: Media Quantity with Leaf Extract

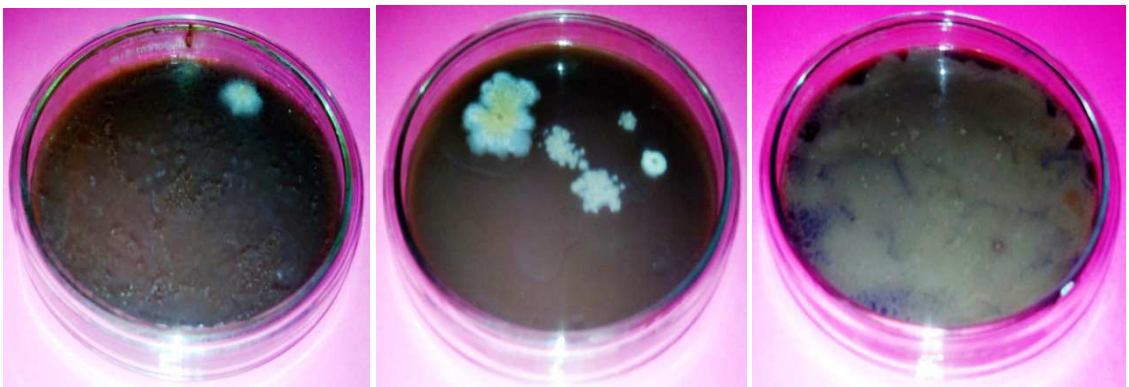
Percentage (%)	50-50	60-40	80-20
NAM	25ml	30ml	40ml
Extract of leaf	25ml	20ml	10ml
Total volume	50ml	50ml	50ml

Table 4.3.1: Media Quantity with Flower Extract

Percentage (%)	50-50	60-40	80-20
NAM	25ml	30ml	40ml
Extract of Flower	25ml	20ml	10ml
Total volume	50ml	50ml	50ml

A **B** **C**

Figure 4.3.1: (A) The Quantity of Media and Leaf Extract is 50-50 Present and no Growth Appear of *Staphylococcous Aureus*, (B) The Quantity of Media and Extract is 60-40 Present and no Growth Appear of *Staphylococcous Aureus*, (C) The Quantity of Media and Extract Is 80-20 Present and Growth Appear of *Staphylococcous Aureus*



A **B** **C**

Figure 4.3.2: (A) The Quantity of Media and Flower Extract is 50-50 Percent and Growth Appear of *Staphylococcous Aureus*, (B) The Quantity of Media and Flower Extract is 60-40 Present and Minimum Growth Appear of *Staphylococcous Aureus*, (C) The Quantity of Media and Flower Extract is 80-20 Present and Growth Appear of *Staphylococcous Aureus*

DISCUSSIONS

Many naturally occurring compounds found in plants, herbs, and spices act as a source of antimicrobial agents against pathogens. Diseases caused by bacterial infection represent an important cause of morbidity and mortality worldwide. Therefore, the development of new antimicrobial agents for the treatment of bacterial infections is of increasing interest. The main objective of the recent study was to define the ability of the plants leaf and flower extract of *Rhododendron arboreum* to inhibit the growth rate of pathogenic bacteria without antibiotics drugs. Antimicrobial activity was recorded when the growth of *Staphylococcus aureus* inhibited by the effect of *Rhododendron arboreum* extract.

4.4.1 Antibacterial Activity of the Plant Extracts

In our test we use leaf and flower extract of *Rhododendron arboreum* with media, before pouring the media add measure quantity of leaf and flower extract in different percentage criteria of media. When media is solidify then spread the liquid culture of *Staphylococcus aureus* bacteria and leave for 24hrs. After 24hrs measure the antibacterial effect of leaf and flower extract on the *Staphylococcus aureus* on the basis of its growth.

In leaf extract the *Staphylococcus aureus* growth is inhibit as shown in figure 4.3.1 A and B, no growth of *Staphylococcus aureus* is observed but in figure 4.3.1C. It indicated that the quantity of leaf extract that inhibit the growth of *Staphylococcus aureus,* at the percentage of 60-40%, but not inhibit the growth on the percentage of 80-20 percent.

In flower extract the *Staphylococcus aureus* growth is inhibit as shown in figure 4.3.2A, minimum growth of *Staphylococcus aureus* is observed in figure 4.3.2B, the maximum growth of *Staphylococcus aureus* appeared in figure 4.3.2C. It indicated that the quantity of flower extract is inhibit the growth rate of *Staphylococcus aureus* at the percentage level of 50-50%, but not inhibit on the percentage of 60-40 and 80-20%.

CONCLUSIONS

On the basis of these results it was observed that the leaf extract of *Rhododendron arboreum* have maximum antibacterial activity against *Staphylococcus aureus* than flower extract. So it means *Rhododendron arboreum* leaves may have desired metabolic compound which play an important role in antibacterial activity against pathogens.

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