ANTIMICROBIAL EXTRACTS OF ROSE ENCAPSULATED INTO POLYMERIC BLENDED MEMBRANE FOR POTENTIAL APPLICATION

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Abstract
The intention of the research was to evaluate the profile of antibacterial activities in Rosa glauca (Rose) petals against some gram-positive and gram-negative bacteria B. subtilis, S. aureus, E. coli and P. florescence. The preliminary phytochemical screening of Rose showed presence of carbohydrates, amino acid, flavonoids, alkaloids, tannins and saponins. Rose petals are expected to synthesize a variety of secondary metabolites capable of providing them protection against the infectious agents. Result data showed that alcoholic extract showed higher antimicrobial activity by dish diffusion method.

Keywords: Antibacterial activity, dish diffusion method, gram-positive and gram-negative bacteria, phytochemical, Rose(Rosa glauca).

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INTRODUCTION

In India, the use of different parts of several medicinal plants to cure specific ailments has been in vague from ancient times. The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries. These systems of medicine cater to the needs of nearly seventy percent of our population residing in the villages. In Homeopathy system, 70% of the medicines are prepared from plants [1]. The plants supply us with large number of excellent “chemicals” which form sources for different types of drugs. The present trend in modern medicine is towards a change from the use of cellulose coated medicinal pills to extracts of plant supplied either in pure forms or in synthetic versions for curing many human ailments. Thus plants have provided the blue prints for the modern medicine. WHO pointed out that more than 80% of world’s population rely on plants based products to meet their primary health care needs. In recent years, multiple drug resistance in both human and plant pathogens has been developed due to indiscriminate use of synthetic drugs. This drives the need to screen medicinal plants for novel bioactive compounds as plant based drugs are biodegradable, safe and have fewer side effects [2,3]. From over 3, 00,000 species of higher plants to occur in nature, only about 2 percent have been screened so far. Extract of plants from 157 families have been reported to be active against microorganisms [4]. In the recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids, etc, which have been found in vitro to have antimicrobial properties [5-7]. Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential [8-10]. The literature showed that Rose having Family-Rosaceae and commonly called as Damask Rose. The Rose is a woody climbing shrub with cylindrical stem, externally yellowish brown and internally yellow and longitudinally fluted. The petals, stems. bark, stem-bark and root of the plant used for several therapeutic purposes. Some of their therapeutic uses are anti-depressant, antioxidant, synergistic, hypnotics, aphrodisiac and skin care. Our present study is aimed to justify the traditional claim of the petals as an
antibacterial activity. The genus Rose contains approximately 100 species that are widely distributed worldwide. The products of Rose are Rose -water, Rose -oil and dried petals that are used in medicine, food and perfume industry as well as in make-up and health products. Rose also widely been used in pharmaceuticals products in cosmetics. People noticed that Rose are well known to use in cosmetics products, spa, as bathing to rejuvenate our bodies. Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Scientific experiments since the late 19th century have documented the antioxidant properties of some spices, herbs, and their components [11]. Many studies reported the activities of spices and herbs on food borne pathogenic microorganisms [12,13]. The purpose of the present study was to investigate the antimicrobial properties of Rose. In this paper we report the results of such studies in order to orient future investigations towards the finding of new, potent and safe antimicrobial compounds.

MATERIALS AND METHODS

The experiment was performed in a Laminar air flow chamber. The apparatus and solution and nutrient media, etc., were sterilized by autoclaving before experiment.

Plant material

Plants used in the study were gathered from the Department of Applied Sciences and Humanities, Jamia Millia Islamia, New Delhi for the purpose of antimicrobial screening test. The petals of Rose were air-dried, coarsely powdered and were then extracted.

Test microorganisms

The organisms used were B.subtilis, S. aureus, E.coli and P. florescence. All the organisms were obtained from microbiology Lab, Department of Biosciences, Jamia Millia Islamia, New Delhi, India.

Preparation of the crude extracts

The samples were prepared in Chemistry Lab, Department of Chemistry, Jamia Millia Islamia. They were washed in sterilized distilled water, followed by washing in mercuric chloride solution (0.1 %) and again washed in sterilized distilled water. Washed plant material was dried at room temperature for 72 h. Plant material was weighed and transferred in to a sterile mortar and pestle for crude crushing of the material, thereafter it was transferred in to a sterile homogenizer and finely crushed to powder. The solvents used for extraction were ethanol, water and acetone. Finely crushed powder deep in different solvents for overnight. Fraction was separated using
sterile muslin cloth and filter through sterile Whatman filter paper (No. 1). The filtered extract was concentrated by a rotary film evaporator.

Antimicrobial sensitivity testing

The paper disc diffusion method was used to determine the antimicrobial activity of the plant extracts, isolated using standard procedure [14,15], studied against different bacteria B.subtilis, E. coli, P. florescence and S. aureus. For antimicrobial activity the microorganisms were cultured in nutrient broth at 370C overnight. First we prepared sterile nutrient agar plates and placed in incubator for 24h. After that with the help of spreader, spread 100 µl inoculum (bacteria) in solid nutrient agar plates until the agar surface in the plates absorbed all inoculums. Subsequently paper discs (Whatman filter paper No.1 of diameter 6 mm), which were sterilized and soaked in different extracts, allowed to dry completely and placed on nutrient agar plate. Filter paper discs (6 mm in diameter) soaked in different solvents and allowed to dry, were used as negative control and tetracycline was used as positive control. The petri-dishes were incubated at 370C for 24 h. The zone of inhibition by the sample was compared with Tetracycline which is used as a standard antibiotic.

Minimal inhibitory concentration (MIC)

MIC is the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism [16-18]. MIC of the extracts were determined by macrodilution broth method [19]. Two ml of extracted solutions at the different concentrations (0.5% w/v to 0.040 % w/v) was mixed with 15 ml of sterile molten agar in conical flask. The mixture was well mixed before being poured into sterile Petri dishes containing 15 mL hard agar. The 2 ml of 40% v/v solvent (ethanol, chloroform, petroleum ether, and acetone) without added test materials was used as the control. The cultures (5 μL) were taken from nutrient broth and added to three places on the medium surface and incubated at 37°C.

Growth curve studies

In case of growth curve studies the effect of increasing concentrations of the Rose flower extract on the growth pattern of different bacterial species have been studied. Increase in concentration leads to significant decrease in growth.

Phytochemical screening

Screening was carried out on extracts to determine the active compounds by using the procedures of Sofowora [19-21]. Extract was measured into a test tube for each of the tests and concentrated.
by evaporating the extractant in a trough. The screening was carried out in the Department of Chemistry of the Jamia Millia Islamia, India. Application study In order to make application of plant extract more effective and sustainable, the plant extract was blended with some polymers. Here in we have proposed blending of the plant extract with PVA and CS. Preparation of PVA-based antimicrobial films PVA (poly vinyl alcohol) powder with different compositions (w/w) of gelatin was dissolved in 25 ml distilled water. Concentrated HCl (0.05ml) was added, and the resulting dispersion was stirred (using an overhead stirrer at 100 rpm) at 60°C for half an hour to carry out esterification reaction between PVA and gelatin (Figure 1) [22-24].

A. Coating with antimicrobial agents
Appropriate coating can sometimes impart antimicrobial effectiveness. Direct surface application of antibacterial substances onto blended film have limited benefits because the active substances are neutralized on contact (Figure 2).

B. Incorporation of antimicrobial additives
The direct incorporation of antimicrobial additives is a convenient means by which antimicrobial activity can be achieved.
RESULT AND DISCUSSION

Different extracts were investigated for their antimicrobial activity by disc diffusion method [15]. Antibacterial activity (in vitro) of each extract was studied against different bacteria *B. subtilis*, *S. aureus*, *E. coli* and *P. florescence* as shown in Table 1. At higher concentration the extract showed significant antimicrobial activity against the tested pathogens. The degree of inhibition varied with the concentration; therefore, higher concentrations were used to get observable results.

Table 1: Antimicrobial activity caused by plant extracts through agar diffusion method

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>Rose Petals extract/presence of antimicrobial activity</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>-</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>-</td>
</tr>
<tr>
<td><em>P. florescence</em></td>
<td>-</td>
</tr>
</tbody>
</table>

(+) susceptibility (inhibition zone > 7 mm)
(-) absence of susceptibility

Paper disc soaked and dried in different solvents used as negative control and terta cycline used as positive control).

The ethanol, acetone and aqueous extracts of *Rose* petals were tested against selected Gram positive and Gram negative bacterial species. These extracts limited the growth of both Gram-positive and Gram-negative bacteria species tested. Among the different extracts, ethanolic extract of *Rose* was found to be more active towards the bacterial species. The highest zone of inhibition was measured in *B. subtilis* 16mm in ethanolic extract and least against *S. aureus* in aqueous extract 8mm (Figure 4).

![Antimicrobial activity of different extracts of Rose petals](image_url)

**Fig. 4:** Antimicrobial activity of different extracts of *Rose petals* (A-Control, B-Ethanol, C-Acetone, D-Aqueous)
Antimicrobial activity of ethanolic extract incorporated in PVA-based film

Antimicrobial activity of the PVA based film incorporated with the ethanolic extract of Rose was expressed in the terms of inhibition zone (Figure 5). The polymeric film (Figure 3) incorporated with ethanol extract had antimicrobial property against all test organisms. The maximum zone of inhibition was 25mm determined against B.subtilis.

Minimum inhibitory concentration (MIC)

The results of the determination of the antimicrobial activity of plant extract and tested bacterial strains are presented as MIC. The values of the MIC are those obtained after 72h incubation at 37°C. The ethanol extract has less MIC as compared to acetone and aqueous extract. It was found that ethanol extract of Rose flower showed inhibitory properties against B.subtilis, E. coli, P. florescence and S. aureus. Such findings are in conformity [18] with the reported literature where Rose leaves, stem and flowers were screened against various pathogenic bacterial strains to study the antimicrobial properties of the plant.

Fig. 3: Polymeric Film (A) with extract of plant and (B) without extract

Ethanol, acetone and aqueous extracts of flower were screened by agar diffusion method [14] against B.subtilis, E. coli, P. florescence and S. aureus respectively. Ethanolic extracts of Rose petals were found to have good antibacterial properties against the entire test microorganisms selected in the study.

Fig. 5: Antimicrobial activity of Rose ethanolic extract-incorporated PVA based film (A- Ethanol, B- Acetone, C- Aqueous, D- Control)
Growth curve studies
In case of growth curve studies the effect of increasing concentrations of the Rose flower extract on the growth pattern of different bacterial species have been studied. Increase in concentration leads to significant decrease in growth. Ethanol extract when treated against bacteria at concentration of 0.12µg/ml the growth pattern has changed, the lag phase was extended by 2 hours, the stationary phase has not reached the same level of cell growth as in case of control and at 0.24µg/ml the lag phase is further extended by 2 hours. At concentration of 0.48µg/ml (MIC level) there is total inhibition of growth showing a flat line (Figure 6 and Figure 7).

Phytochemical analysis of ethanolic extract of Rose petals
Phytochemical analysis of the ethanol extract of the petals of Rose was done using the confirmatory test [25]. The components analyzed are listed in the Table 2. According to the results this study showed that ethanolic extract of Rose used for treatment of resistance against bacteria such as B.subtilis.

Table 2: Phytoconstituents analysis of ethanolic extract of Rose petals

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Rose</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Amino Acids</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
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</tbody>
</table>

Fig. 6: Growth curve of Bacillus subtilis against three different concentration of Rose extract along with control. Series1-Control, Series2-Bacillus+0.12 µg/ml, Series3-Bacillus+0.24 µg/ml, Series4-Bacillus+0.48 µg/ml
Fig. 7: Growth curve of *E. coli* against three different concentration of *Rose* extract along with control. Series1-Control, Series2-Bacillus+0.12 micro µg/ml Series3-Bacillus+0.24 µg/ml Series4-Bacillus+0.48 µg/ml

CONCLUSION

The results obtained showed that the petals of *Rose* have bactericidal effects on pathogenic microorganisms. Figure 3-7 shows that ethanolic extract proved better and improved antibacterial activity in comparison to other solvents. The widest zone of inhibition (16mm) was determined by the ethanolic extract in *B. subtilis* and least against *S. aureus* in aqueous extract 8mm. The study show that the natural antimicrobial compounds of *Rose* can be successfully incorporated into PVA blended film and retain their inhibitory effect against microbial growth in model media based on the inhibitory zone, inhibitory underneath the films, and suspension test. The excellent antimicrobial activity was obtained from PVA blended film incorporated with ethanolic extract. In future this work can be extended for wound dressing.

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REFERENCES


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