

BIOCHEMICAL AND ANTIMICROBIAL ANALYSIS OF ROSE PETALS (*ROSA INDICA*)¹Laxmi Sowmya K., ²Sandhya Deepika D., ³Geetha S. and ⁴Lakshmi Sri M.^{1,2,4}Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India - 530003.²P.G Dept. of Microbiology, Andhra University, Visakhapatnam, Andhra Pradesh, India - 530003.***Corresponding Author: Dr. Sandhya Deepika D.**

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ABSTRACT

The potential presence of naturally occurring antimicrobials in petals of flowers of *Rosa indica* was investigated against human pathogens. Owing to the usage of these flowers in Siddha medicine for common folklore medicine, the extracts of petals were screened for antibacterial activity against pathogenic microbes. *S.aureus* showed high zone of inhibition with the distilled water extract of rose petals. Globalization has contributed not only to a better awareness of consumers but also to the comeback of earlier lifestyles, in which edible flowers played an important role thus the extracts of petals were phytochemically screened. The present study indicates that the extracts of petals of the flowers of *Rosa indica* can be used to discover antibacterial agent for developing new pharmaceuticals.

KEYWORDS: *Rosa indica*, antimicrobials, phytochemical, petals.**INTRODUCTION**

Plants are well known as a major source of modern medicines. From ancient times, humans have utilized plants for the treatment or prevention of diseases, leading to the dawn of traditional medicine. Many plant species produce a wide range of chemical products that are not involved in primary metabolism and called secondary metabolites (Rhodes, 1994). Alkaloid and terpenoids are main secondary metabolites that have many physiological and pharmacological properties to living cells. Secondary metabolites of plants are having good efficacy in controlling various pathological disorders. While plant leaf, stem and root extracts have been widely evaluated for bioactive compounds, screening for plant flower has not been extensive. Medicinal plants are potential sources of antimicrobial and antioxidant compounds. It is therefore essential to thoroughly investigate their composition and activity and thus validate their use. The flower petals which provide physical protection to the reproductive compounds can be expected to synthesize potent bioactive compounds. Interestingly, the symptoms of most plant diseases of bacterial and fungal origin have been reported mostly on the leaves, stems, roots and seldom on petals (Darokar et al., 1998). While the information on antimicrobial activity of plant flowers and especially petals were scanty, the petals provide physical protection to the reproductive organs can be expected to synthesize potent bioactive compounds (Sridevi et al., 2011). The aim of the present study was focused on the Nutritive value and antimicrobial activity of flower petals of Rose with selected water borne pathogens.

MATERIAL AND METHODS

Rose belongs to the family Rosaceae. The medicinal uses and health benefits of a rose (gulab flower) are many. Rose petals are used in making rose oil that is steam distilled by crushing. The byproduct of steam distillation is rose water, which is an excellent relaxing agent, soothes the nerves and adds flavor to a variety of dishes across the world. Rose essence is rich in flavanoids, tannins, antioxidants, and vitamins A, B3, C, D and E, making it beneficial in skin care.

Sample Collection

The flowers were picked in morning and stored in at low temperature for further use. The petals were surface sterilized with 0.1% Mercuric Chloride for 10 seconds and washed with sterile distilled water for three successive times. The petals were dried in a drier and then crushed in mortar and pestle and aqueous extracts were prepared with sterile distilled water and filtered through Millipore filter.

Preparation of Flower Petal Extracts

These flower petals are used to prepare distilled water, methanol and ethanol extracts. 2 gm of powdered samples were dissolved in 20 ml of distilled water, Ethanol and Methanol. Flasks were kept in dark for 2-4 days. The solutions were filtered and left in oven at 50°C till the extract dried. The amounts of evaporated metabolites from methanolic and ethanolic extracts were dissolved in double amount of 100 mM Tris HCl buffer pH 8.

QUALITATIVE PHYTOCHEMICAL SCREENING

The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. Qualitative phytochemical analyses was done using the procedures of Kokate (1994) and Kokate et al (1995). The qualitative phytochemical analysis of flower petal extracts revealed the presence of carbohydrates, proteins, Amino acids, glycosides, alkaloids, tannins, phenols and Flavonoids.

ANTIMICROBIAL STUDIES

The concentrate of all the extracts and isolated compounds were tested for antimicrobial activities against human pathogens.

Test microorganism for antibacterial assay

For the in vitro antibacterial assay the following human bacterial pathogens were studied such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella Spp.*, *Klebsiella pneumonia*, *Vibrio spp.*, for their antimicrobial activity. It was demonstrated by well diffusion assay.

Culture preparation for antibacterial assay

Media preparation

36g of Muller Hinton Media (Hi-Media) was mixed with distilled water and than sterilized in autoclave at 15 lb pressure for 15 minutes. The sterilized media were poured in to petridishes. The solidified plates were pored with 5 mm dia cork pore. The plates with wells were used for antibacterial studies.

Agar well diffusion method

The antimicrobial assay was performed by agar well diffusion method (Perez et al., 1990) for solvent extract. The molten Mueller Hinton Agar (HiMedia) was inoculated with the 100µl of the inoculums (1×10^8 Cfu) and poured into the sterile petri plates. In agar well diffusion method, a well was prepared in the plates with help of cork-borer (8mm). 100µl of test compound was introduced into well. The plates were incubated overnight at 37°C.

RESULTS AND DISCUSSION

Herbal medicines are a valuable and readily available resource for primary health care and complementary health care systems. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value that have yet to be discovered, Thought large numbers of plants are constantly being screened for their antimicrobial effects. The plant may prove to be a rich source of compounds with possible antimicrobial activities but more pharmacological investigations are necessary.

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The medicinal value of plants lies in certain chemical substances that produce a definite physiological action

on the human body. Disc diffusion methods are extensively used to investigate the antibacterial activities of natural antimicrobial substances and plant extracts. These assays are based on the use of discs as reservoirs containing the solution of substances to be examined (Bartner et al. 1994).

Phytochemical Screening

The qualitative phytochemical analysis of flower petal extracts revealed the presence of carbohydrates, proteins, Amino acids, glycosides, alkaloids, tannins, phenols and Flavonoids (Table 1). The rose petals contain all the constituents such as carbohydrates, proteins, Amino acids, glycosides, alkaloids, tannins, phenols and flavonoids. Whereas glycosides and phenols are rich when compare to the other two flower petals.

Table 1: Qualitative analysis of extracts of three types of flower petals

S.No	Phytochemicals	Rose
1.	Carbohydrates	+
2.	Proteins	+
3.	Amino acids	+
4.	Glycosides	++
5.	Alkaloids	+
6.	Phenols	++
7.	Flavonoids	+

(+) = indicates presence, (-) = indicates absence

Phenolic compounds such as flavonoids, phenolic acids and tannins are considered the major contributors to the antioxidant activity of vegetables, fruits or medicinal plants.

Antimicrobial Activity

Methanol extract of the *Rosa indica* shown zone of inhibition against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella Spp.*, *Klebsiella pneumonia*, *Vibrio spp.* were given in the table 2. *Staphylococcus aureus*, and *Klebsiella pneumonia* showed maximum inhibition (22 mm) followed by *Escherichia coli* (12 mm) and *Salmonella Spp* and *Vibrio spp* (10 mm).

Ethanol extract of the *Rosa indica* shown zone of inhibition against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella Spp.*, *Klebsiella pneumonia*, *Vibrio spp.* were given in the table 2. *Staphylococcus aureus*, and *Klebsiella pneumonia* showed maximum inhibition (20 mm) followed by *Escherichia coli* (17 mm) and *Salmonella Spp* and *Vibrio spp* (15 mm).

Distilled water extract of the *Rosa indica* shown zone of inhibition against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella Spp.*, *Klebsiella pneumonia*, *Vibrio spp.* were given in the table 2. *Vibrio spp* showed maximum inhibition (32 mm) followed by *Staphylococcus aureus* (28 mm), *Escherichia coli* (19 mm), *Salmonella Spp* (16 mm) and *Klebsiella pneumonia* (15 mm).

Methanolic extracts of Red rose petals gave the zone of inhibition of 25 mm, 26 mm, 27 mm against staphylococcus aureus, pseudomonas aeruginosa and Escherichia coli respectively. The ethanolic extract of red rose petals shows the zone of inhibition of 26mm, 25mm, 27mm against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli respectively. Earlier also zone of inhibitions of 30mm, 32mm, 21mm against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli have been reported by [Hirulkar NB, et al., 2010].

The fresh powder of Rosa indica shown zone of inhibition against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella Spp.*, *Klebsiella pneumonia*, *Vibrio spp.* were given in the table 2. *Vibrio spp* showed maximum inhibition (28 mm) followed by *Klebsiella pneumonia* (25 mm), *Escherichia coli* (15 mm), *Salmonella Spp* (12 mm) and *Staphylococcus aureus* (10 mm).

The Minimum Inhibitory concentration (MIC) was also performed in order to know the minimum inhibitory concentration of the extract by broth dilution method and the MIC was found to be ranging between 0.12-.65µg/ml earlier also MIC of rose extract has been reported to between 5.0-18.0 mg/ml by [Vieira GDV, 2009].

Table 2: Antimicrobial activity of Rose

Test Organisms	Methanol (mm)	Ethanol (mm)	Distilled water (mm)	Fresh (mm)
<i>Escherichia coli</i>	12	17	19	15
<i>Staphylococcus aureus</i>	22	20	28	10
<i>Salmonella Spp.</i>	10	15	16	12
<i>Klebsiella pneumonia</i>	22	20	15	25
<i>Vibrio spp</i>	10	15	32	28

CONCLUSION

Globalization has contributed not only to a better awareness of consumers but also to the comeback of earlier lifestyles, in which edible flowers played an important role. This new information concerning the composition and nutritional value of edible flower is also important and represents a sufficient reason for their consumption. Even if you are not keen on experimenting with salads or sauces, edible flowers make excellent garnishes which, unlike some decorations which appear in the guise of nouvelle cuisine, are actually nice to eat. Some flowers are safe to eat only in small amounts. Flowers commonly carry traces of pesticides and harbour organisms such as insects. Flowers cultivated as ornamental plants for garden use are not intended for use as food. Common garden flowers which are ALL POISONOUS to a greater or lesser degree should be especially avoided. It is always best to grow your own edible flowers, and then you can be sure that they are clean, fresh and free from pests and disease. The majority of edible flowers are always best picked fresh from the garden the day you want to use them.

N. B. Hirulkar and Mona Agrawal (2010) showed that low dilution of alcoholic extract showed higher antimicrobial activity as compare to high dilution. Petroleum ether extract showed maximum 29 mm zone of inhibition for Pseudomonas aeruginosa as compare to other bacterial strains. Alcoholic extract showed higher inhibitory effect on Streptococcus pneumoniae (30mm), Enterobacter aerogens 28 mm, Staphylococcus epidermidis 25 mm, Bacillus subtilis 30 mm, Pseudomonas Aeruginosa 32 mm. aqueous extract showed higher inhibition against E.coli (21 mm), Enterobacter aerogens (25mm) and Bacillus subtilis (28 mm) as compare to other bacterial strains. Analysis showed that when comparing the antimicrobial activity of Rose petals with control antibiotic, the zone of inhibition was higher to antibiotic at the highest concentration, in the case of Streptococcus pneumoniae and Pseudomonas aeruginosa. Study showed that the average relative antimicrobial activity was found higher with alcoholic extract (25mm) as compare to aqueous (19mm) and petroleum ether (18mm). Statistical data analysis showed that, petroleum ether showed low average relative antimicrobial activity among all solvents.

Amit Pandey (2012) the ethanolic extract of Rosa indica of red and orange color showed positive result against 3 bacterial pathoges- E. coli, S. aureus and P. aeruginosa.

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