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RESEARCH ARTICLE

**STUDIES ON THE ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF
*ROSA INDICA L.***

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Abstract

Medicinal plants are extensively used in traditional medicine to cure various infectious diseases in human. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. Medicinal plants are commonly used in the cure of diseases since times immemorial. In the present study methanol, chloroform and aqueous extracts of *Rosa indica L.* leaf were subjected for antimicrobial activity by well-diffusion method in the case of evaluate the antibacterial activity of Petroleum ether extract of *Rosa indica L.* extract against gram-positive cultures include *B. cereus*, *B. subtilis*. Gram-negative cultures include *Vibrio cholera*, *Klebsiella pneumonia*. The maximum antibacterial activity against *Bacillus cereus* (Zone of inhibition in mm 14.2), *B. subtilis* (zone of inhibition 13.1) and athanolic the lowest activity against *Vibrio cholera* (Zone of inhibition 12.6), *Klebsiella pneumonia* (Zone of inhibition 12.3). The gram positive bacteria more susceptible than the gram negative bacteria. The phytochemical characteristics of *Rosa indica L.* plants tested were summarized in the, it could be seen that, carbohydrates, cardiac glycosids, Flavonoids, Saponins (Foam test) Amino acids , Terpenoids, Phenols and Tannins were present in *Rosa indica* plant extract.

Key words: *Rosa indica L.*, bacterial strains, Antibacterial activity, phyto chemical analysis

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Introduction

Universally, investigation into organically dynamic regular items from plants has drawn in numerous normal item scientists. Different plants have been inspected for their organic exercises and at times dynamic substances have been disengaged and

recognized. Plants have always been an important source of medicines since ancient times and seventy percent of the worldwide population still relies on one or other forms of traditional plant based medicine (Shanmugam et al., 2012). Plant based regular constituents can be gotten from any piece of the plant like bark, leaves, blossoms, roots, natural products, seeds, etc. Medicinal plants are commonly used in the cure of diseases since times immemorial. The concentrates from these plants give limitless freedoms to the turn of events and creation of new medications as they have gigantic remedial potential to treat numerous illnesses (Anthonoy and Dweck, 2008). The fundamental basic rule is the presence of phytochemical constituents (Holme, 2007). Phytochemicals are categorized into primary and secondary compounds. Essential constituents incorporate chlorophyll, proteins and normal sugars, while optional mixtures involve terpenoid, alkaloids and phenolic compounds (Fouda et al., 2009) Phytochemicals constituents in plants have protection component and show restorative job in illness fix. Antimicrobial agents inhibit the growth or kill microbes to control their negative effect e.g. odour, staining and deterioration. Antibacterial agents, commonly known as antibiotics, are used against bacteria, whereas, antifungal agents are used against fungi. They can likewise be characterized by their capacities (Cowan, 1999). Tannins, alkaloids, flavonoids, triterpenoids, sesquiterpenes, phenolic compounds, carotenoids, steroids and ketones present in the medicinal herbs have been suggested to be involved in antibacterial activities (Sathianarayanan et al., 2010).

Rose belongs to the family *Rosaceae*. Hundred (n=100) types of roses are locally accessible. Roses are local basically to the calm areas of the Northern Hemisphere. There is a wide variety of colours available in a rose which enhances the beauty of the rose. Most roses are locally available in Asia, North America and few are available in Europe. There are so many important components of rose which plays an important role in the antibacterial activity of rose. Among them, the components which possess antibacterial activity are flavonoids, terpenes, anthocyanins. Flavonoids are classified as natural plant compounds usually they are secondary metabolites of plants with various phenolic structures

(Saric A . et al., 2009). They have many properties that help to treat cancer Alzheimer's disease like they pose biochemical and antioxidant effects, antimutagenic effect, anticarcinogenic effects, etc. (A.N Panche et al., 2016). They are extracted from plants and are classified as a low molecular weight compound. Rose, onion is the major source of flavonoids. Apart from antibacterial and antifungal properties, flavonoids are also responsible for color and aroma production in flowers.. They are helpful for plants in a way that they act as an UV channel which shields the DNA of plant from UV radiations. Classification is done based on C ring which contains carbon (Calhoun *et al.*, 1993) Terpene is a natural compound present in the rose. Terpenes have extraordinary modern and drug utilization. In food ventures, it is utilized as an enhancing specialist or fragrancng specialist. Pharmaceutical industries have medicinal uses of terpene (Jiang z et al. 2017). Packs in continuous many years have shown that terpenes apply mitigating impacts by blocking distinctive proinflammatory pathways. Terpenes have been displayed to apply movement against malignant growth and tumors.

Individuals from the class *Rosa* (Family *Rosaceae*) are considered as one of the world's most mainstream decorative plants due to their excellence and aroma. Despite the fact that, there are more than 100 types of roses, *Rosa damascena* Mill. is considered one of the most important *Rosa* species for its beauty, flavour and fragrance industry. The name of *R. damascena* species depends on Damascus, Syria, where it initially existed as a wild plant., it is now cultivated in different countries around the world (Das, A., 2012). Although, Taif is known for the production of a high quality rose essential oil, from *R. damascena*, Bulgaria, Turkey, France and India are the largest producers of rose essential oil (Harkness, P., 2003).

Anthocyanins have a place with flavonoids class which incorporates a subset of the polyphenol. Red, blue, and purple shades of natural items, vegetables, grains, blooms, and spices are expected to anthocyanin. Anthocyanin is a Greek word, anthos intends to sprout and kyanos implies blue. Anthocyanins are fantastically found in nature as glycosides of polyhydroxy and polymethoxy

subordinates of 2-phenyl-benzopyrylium or flavylum salts. They are isolated by the amount of hydroxyl and methoxyl kinds of the B-ring, by the quantity of sugars associated with the aglycon and the circumstance of association, and by the nature and number of aliphatic or fragrant acids attached to the sugar stores (Saul Ruiz-Cruz et al., 2017). In a previous report, quiniccorrosiv, 5-hydroxymethylfurfural, pyrogallol, levoglucosan, and 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl were the major recognized portions in methanolic concentrate of *R. indica* petals. In like way, another assessment exhibited that temperamental oils of new sprouts of *R. Damascena*, generally, have citronellol, geraniol, nonadecane, and heneicosane in the basic oil, while they have alcoholic fragments, citronellol and geraniol in rose water parcel.

The significance of plants is known to us well. The plant realm is a treasury of expected medications and in the new years there has been an expanding mindfulness about the significance of restorative plants. Medications from the plants are effectively accessible, more affordable, safe, and proficient and seldom have incidental effects. The plants which have been chosen for restorative use more than millennia establish the most clear decision of looking at the flow look for remedially compelling new medications like anticancer medications (Dewick, P.M. 1996) antimicrobial medications antihepatotoxic compounds. As indicated by World Health Organization (WHO), restorative plants would be the best source to get assortment of medications. About 80% of people from created nations utilize customary medications, which has compounds gotten from restorative plants. Be that as it may, such plants ought to be researched to all the more likely comprehend their properties, wellbeing, and proficiency (Arunkumar, S and Muthuselvam . 2009). Therapeutic plants contain some natural mixtures which give distinct physiological activity on the human body and these bioactive substances incorporate tannins, alkaloids, sugars, terpenoids, steroids and flavonoids. These mixtures are blended by essential or rather auxiliary digestion of living beings. Optional metabolites are artificially and systematically amazingly different mixtures with dark capacity. They are broadly utilized in the human treatment, veterinary, horticulture, logical exploration and

endless different regions .An enormous number of phytochemicals having a place with a few compound classes have been displayed to effectsly affect a wide range of microorganisms in vitro. Plant items have been important for phytomedicines since days of yore. This can be gotten from barks, leaves, blossoms, roots, natural products, seeds. (Vasu, K., et al., 2009).

(Sahira banu K and L Cathrine. 2015) Phytochemicals are the synthetic compounds that present normally in plants. Presently a-days these phytochemicals become more mainstream because of their innumerable therapeutic employments. Phytochemicals assume a crucial part against number of sicknesses like asthma, joint inflammation, malignant growth and so forth dissimilar to drug synthetics these phytochemicals don't have any incidental effects. Since the phytochemicals fix sicknesses without making any mischief people these can likewise be considered as "man-accommodating prescriptions". This paper primarily manages assortment, extraction, subjective and quantitative examination of phytochemicals. The current investigation was started with following destinations are, To contemplate the phytochemical examination of *Rosa indica* L. concentrate like sugars, cardiovascular glycosids, Flavonoids, Saponins (Foam test) Amino acids , Terpenoids, Phenols and Tannins. To break down antibacterial movement of *Rosa indica* L. against Gram positive microscopic organisms like *B. cereus*, *B. subtili* and Gram negative microorganisms like *Vibrio cholera*, *Klebsiella pneumonia*.

Materials and method

Sample collection

Fresh leaves and free from infection of *Rosa indica* L were collected from the beautiful premises of Annamalai University Horticulture Department, Annamalai nagar, Tamilnadu, India in January 2021.. This flower plant was identified by Dr. M. Subramanian, Specialization of Plant biology, working in Sri vinayaga College of Arts and Science, Ulundurpet, Tamil Nadu.

The samples of flower petals were collected from the plants being maintained in the gardens of Horticulture Department Annamalai University, Annamalai Nagar, India. The rose flowers were sampled during January to February in 2021. The

blossoms were picked in morning and put away in at low temperature for additional utilization. Rose flowers of same number petals five petals were randomly selected and drawn the petals. Drawn petals were surface sterilized by dipping in 0.1% mercuric chloride for about 2 min and washing 6 times. By using Soxhlet extraction method, rose petals extract were obtained with various solvents. Here petroleum ether, alcohol and water used as a solvent. All extracted material applied in 1:1, 1:2 and 1:3 dilutions with distilled water.

Extraction of plant materials

The plant materials leaves of *Rosa indica* L. were air-dried at room temperature (26°C) for about fourteen days, after which it was crushed to a uniform powder Fig. 2. The ethanol removes were ready by dousing 100 g every one of the dry powdered plant materials in 1 L of ethanol at room temperature for 48 h. The concentrates were sifted after 48 h, first through a Whatmann channel paper No. 42 (125mm) and afterward through cotton fleece. The concentrates were concentrated utilizing a rotational evaporator with the water shower set at 40°C. The rate yield of concentrates went from 7–19% w/w.

Preparation of Aqueous extract

For aqueous extraction, 10g of air dried powder was placed in 100 ml of distilled water (1:0 ratios) and boiled for 6 hours and then filtrate was condensed in boiling water bath and used for further analysis.

Preparation of Ethanol extract

For aqueous extraction, 10g of air dried powder was placed in 100 ml of ethanol and boiled for 6 hours and then filtered, the filtrate was condensed in boiling water bath and used for further analysis.

Preparation of petroleum ether extract

For aqueous extraction, 10g of air dried powder was placed in 100 ml of petroleum ether and boiled for 6 hours and then filtered, the filtrate was condensed in boiling water bath and used for further analysis.

Antibacterial activity/susceptibility testing

To assess the antibacterial activity of the rose extract, Four cultures were collected from the known clinical laboratory Pondicherry. Gram-positive cultures include *B. cereus*, *B. subtilis*. Gram-negative cultures include *Vibrio cholera*, *Klebsiella pneumonia*. They were all gram stained and affirmed by biochemical testing and afterward they were refined on supplement agar plates and saved for additional utilization.

Antibacterial activity by disc diffusion methods

The bacteriostatic of the compounds was tested by disc diffusion method as described by Bauer Kirby,s method .

Qualitative of phytochemical analysis

Following standard protocols were used for qualitative analysis of samples to check for the presence of carbohydrates, cardiac glycosids, Flavonoids, Saponins (Foam test) Amino acids , Terpenoids, Phenols and Tannins were carried out on both extracts. The technique utilized is momentarily depicted beneath.

Carbohydrates

To 1 ml of concentrate, a couple of drops of Molisch's reagent were added and afterward 1 ml of concentrated sulphuric corrosive was poured along the edge of the cylinders. The blend was then permitted to represent 2 to 3 minutes. Formation of red or dull violet colour indicated the presence of carbohydrates in the sample extract.

Cardiac glycosides

Test for cardiac glycosides 5 ml of every unrefined crude concentrate was added with 2 ml of glacial acidic acids and a few drops of 5% iron chloride, and 1 ml of concentrated sulphuric acid was added. A more obscure ring creation at the interface exhibited the presence of heart glycosides.

Flavonoids

To 2 ml of each concentrate, a couple of drops of 20% sodium hydroxide were added till development of extreme yellow tone was noticed. To this, a few drops of 70% dilute hydrochloric acid were

added and the yellow colour disappeared. Formation and disappearance of yellow colour indicated the presence of flavonoids in the sample extract.

Saponins(Foam test)

To 2 ml of each extract, 6 ml of distilled water was added and shaken vigorously; formation of bubbles or persistent foam indicated the presence of saponins.

Amino acids

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for Amino acids.

a. Ninhydrin test

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to 2 ml of aqueous filtrate. Appearance of purple colour indicates the presence of amino acids.

Terpenoids

To 1 ml of extract of each solvent, 0.5 ml of chloroform was added followed by a few drops of concentrated sulphuric acid. Formation of reddish brown precipitate indicated the presence of terpenoids in the extract.

Phenols

To 2 ml of each extract, 2 ml of 5% aqueous ferric chloride was added; formation of blue colour indicated the presence of phenols in the sample extract.

Tannins

To 2 ml of each extract, 10% of alcoholic ferric chloride was added; formation of brownish blue or black colour indicated the presence of tannins.

RESULT

Antibacterial activity of *Rosa indica* L.

The antibacterial activity is checked against all four test organisms. The results of the zone of inhibition showed in Table 1 and Figure 3, 4, 5,6

In this study aimed to evaluate the bacterial antibacterial activity of Petroleum ether extract of *Rosa indica* L. extract against gram-positive cultures include *B. cereus*, *B. subtilis*. Gram-negative cultures include *Vibrio cholera*, *Klebsiella pneumonia*. The maximum antibacterial activity against *Bacillus cereus*, *B. subtilis* and *athanolica* the lowest activity against *Vibrio cholera*, *Klebsiella pneumonia*. The gram positive bacteria more susceptible than the gram negative bacteria.

The results of antimicrobial activity of petroleum ether leaf extract of *Rosa indica* L. are summarized in Table 1. Fig 3,4,5,6 Petroleum ether leaf extract shows strongest antibacterial activity on *B.cereus* (Gram positive) with 14.2 mm zone of inhibition (Fig. 3). However, the petroleum ether leaf extract shows the least antibacterial activity on *Klebsiella pneumonia* (Gram negative) with 12.3 mm zone of inhibition. (Fig. 6)

Table 1 : Antibacterial activity of *Rosa indica*

S.No	Name of the organisms	Petroleum ether extract
		Zone of inhibition in mm
1	<i>B. cereus</i>	14.2
2	<i>B. subtilis</i>	13.1
3	<i>Vibrio cholera</i>	12.6
4	<i>Klebsiella pneumonia</i>	12.3

Fig. 1. Antibacterial activity of *B. cereus*



Fig.2. Antibacterial activity of *B.subtilis*

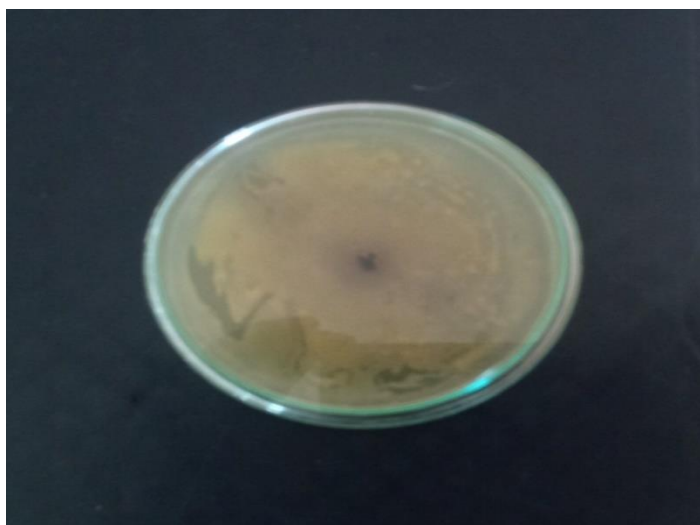


Fig.3. Antibacterial activity of *Vibrio cholera*

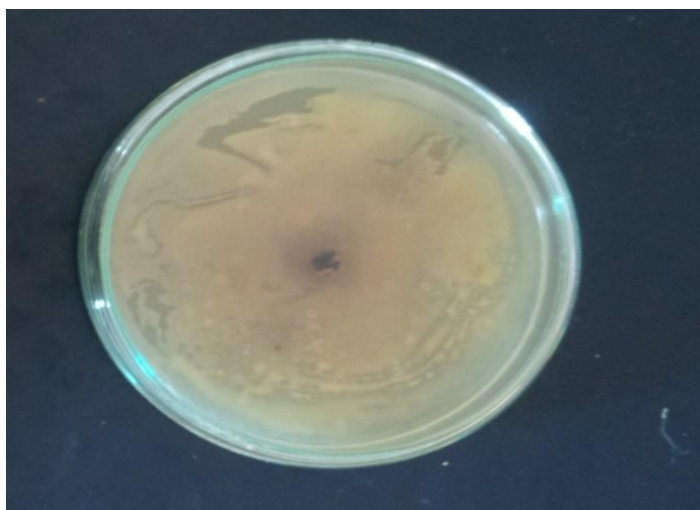
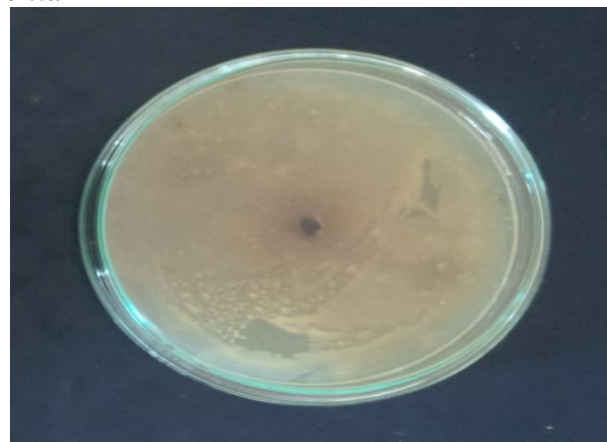


Fig.4. Antibacterial activity of *Klebsiella pneumonia*



The phytochemical characteristics of Rose indica plants tested were summarized in the table-2 and Fig. 5. From the table, it could be seen that, carbohydrates, cardiac glycosids, Flavonoids, Saponins (Foam test) Amino acids , Terpenoids, Phenols and Tannins were present in Rose indica plant extract.

Table 2: Qualitative phytochemical analysis of the Rose indica extract

S.No	Phytochemical	Reaction
1	carbohydrates	+
2	cardiac glycosids	+
3	Flavonoids	+
4	Saponins (Foam test)	+
5	Amino acids	+
6	Terpenoids	+
7	Phenols	+
8	Tannins	+

Fig. 5. Phytochemical analysis test for petroleum ether extract



Discussion

In this study *Rosa indica* L. petroleum ether extracts of petals were evaluated for their antimicrobial activities against Gram positive bacteria and gram negative bacteria and phytochemical analysis of extracts.

Present investigate to evaluate the bacterial antibacterial activity of ethanolic extract of rose indica extract against. Gram-positive cultures include *B. cereus*, *B. subtilis*. Gram-negative cultures include *Vibrio cholera*, *Klebsiella pneumonia*. The maximum antibacterial activity against *Bacillus cereus*, *B. subtilis* and athanolic the lowest activity against *Vibrio cholera*, *Klebsiella pneumonia*.

Antimicrobial movement of Rosa was performed against Bacilli caryophylloid dicot class, staphylococci aureus, and Salmonella typhi. The pathogenicity of *B. cereus*, regardless of whether enteric or nonintestinal, is personally identified with tissue-damaging/responsive exoenzyme creation.

Present study the results of antimicrobial activity of petroleum ether leaf extract of *Roae indica* L. petroleum ether leaf extract shows strongest antibacterial activity on *B.cereus* (Gram

positive) with 14.2 mm zone of inhibition, *B. subtilis* shows the zone is 13.1. Gram negative organisms like *Vibrio cholera* zone formed 12.6, *Klebsiella pneumonia* zone formed 12.3. However, the petroleum ether leaf extract shows the least antibacterial activity on *Klebsiella pneumonia* (Gram negative) with 12.3 mm zone of inhibition. Former researcher Yusra Safdar and Taqdees Malik (2020) investigated antibacterial activity for Rose extract against the organism are *E.coli*, *Pseudomonas*, *Klebsiella*, *Vibrio*, *Entero bacter* respectively zone formation in mm viz., 15mm, 21mm, 19mm, 17mm, 20mm.

Similarly, Sathiya S. 2008, Mishra. R.P. 2011 researched the outcomes acquired showed that the leaves, stem and blossom of *Rosa indica* L (red) effectsly affect pathogenic microorganisms. Shows that methanolic extract proved better and improved antibacterial activity in comparison to ethyl acetate. The broadest zone of restraint (18mm) was controlled by the methanolic concentrate of *Rosa indica* petals, trailed by (14mm) leaves and (17mm) stem remove. A remarkable 13mm zone of inhibition was recorded by ethyl acetate extract of *Rosa indica* stem against *P. aeruginosa*, while *E. coli* and *S. aureus* found insensitive. Similar type of

investigations was reported earlier with ethanolic extracts.

The present study phytochemical characteristics of *Rose indica* plants tested were summarized in the Phytochemical characteristics it could be seen that, carbohydrates, cardiac glycosids, Flavonoids, Saponins (Foam test) Amino acids , Terpenoids, Phenols and Tannins were present in *Rosa indica* L. plant extract. Similarly, Chemical tests were performed on crude extracts of seven Phytochemical analysis of Tannins of ethanolic extracts were positive in all tests plants which were previously supported by Akrayi and Abdulrehman (2013), Okere et al. (2014) and Adham (2015). The phytochemical screening of the plants studied showed the presence of flavonoids terpenoids, saponins and tannins, *M. indica*, *V. amygdalina* and *P. guajava* showed the absence of anthraquinones. *M. indica* and *P. guajava* tested negative for the presence of alkaloids and only *M. indica* tested negative for the presence of cardiac glycosides (GA Ayoola et al., 2008)

Phytochemical screening of the plants uncovered a few contrasts in the constituents of the four plants tried. *C. papaya* tried positive for every one of the phytochemicals tried; *M. indica* showed the shortfall of anthraquinones, alkaloids and heart glycosides; *V. amygdalina* tried positive for all with the exception of anthraquinones while *P. guajava* tried positive for all aside from Anthraquinones and alkaloids. Every one of the plants showed powerful cell reinforcement movement. The presence of flavonoids and tannins in all the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (Ayoola GA 2008)

The present study concluded that when comparing the antimicrobial activity of *Rose petals* with control antibiotic activity it was observed that, in the case of evaluate the antibacterial activity of Petroleum ether extract of *Rosa indica* L.extract against gram-positive cultures include *B. cereus*, *B. subtilis*. Gram-negative cultures include *Vibrio cholera*, *Klebsiella pneumonia*. The maximum antibacterial activity against *Bacillus cereus*, *B.*

subtilis and athanolic the lowest activity against *Vibrio cholera*, *Klebsiella pneumonia*. The gram positive bacteria more susceptible than the gram negative bacteria. The phytochemical characteristics of *Rosa indica* L. plants tested were summarized in the, it could be seen that, carbohydrates, cardiac glycosids, Flavonoids, Saponins (Foam test) Amino acids , Terpenoids, Phenols and Tannins were present in *Rose indica* plant extract.

Conclusion

The present study concluded that when comparing the antimicrobial activity of *Rose petals* with control antibiotic activity it was observed that, in the case of evaluate the antibacterial activity of Petroleum ether extract of *Rosa indica* L.extract against gram-positive cultures include *B. cereus*, *B. subtilis*. Gram-negative cultures include *Vibrio cholera*, *Klebsiella pneumonia*. The maximum antibacterial activity against *Bacillus cereus*, *B. subtilis* and athanolic the lowest activity against *Vibrio cholera*, *Klebsiella pneumonia*. The gram positive bacteria more susceptible than the gram negative bacteria. The phytochemical characteristics of *Rosa indica* L. plants tested were summarized in the, it could be seen that, carbohydrates, cardiac glycosids, Flavonoids, Saponins (Foam test) Amino acids , Terpenoids, Phenols and Tannins were present in *Rose indica* plant extract.

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