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IN VITRO ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *ROSA INDICA* EXTRACTS AGAINST HUMAN PATHOGENS

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ABSTRACT

In the present study of *in vitro* antibacterial activity and phytochemical analysis of *Rosa indica* extracts against human pathogen explored against different pathogens. Antimicrobial component were extracted by solvent extraction where in methanol, ethanol, benzene, ethyl-acetate, hot distilled water and distilled water extracts were prepared. The antibacterial nature of extracts was assessed by agar well diffusion methods. Then the methanol, ethanol, ethyl-acetate and benzene extract of red rose petal were found to be most effective against all pathogen used, they gave a zone of inhibition. Benzene extract show the highest zone of 13.5mm against *Staphylococcus aureus* and methanol gave positive results 10.5 mm against *Escherichia coli* and ethanol extract gave zone of 15.5mm against *Escherichia coli*. . Ethyl acetate leaves extract show the positive result against alkaloids, protein, phenol and amino acid. Benzene petal extract show the positive result against flavonoids and carbohydrate. Ethyl acetate petal extract show the positive result against flavonoids, protein and phenol.

Key words: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, Flavonoids

INTRODUCTION

A Rose is a woody perennial of genus ***Rosa,*** within the family Roseaceae. There are over 100 species and thousand cultivars. Flowers of rose vary in size and shape and are usually large and showy. In colour ranging from white through yellow and red. Different species of rose can be hybridizing easily, and this has been used in the development of the wide range of Garden roses.

The majority of ornamental roses are hybrids that were bred from their flowers generally they are harvested and cut when in bud, and kept in refrigerated conditions until ready for display at the time of sale.

The rose may be used as a perfume. Rose perfumes are made from attar of roses or rose oil, which is a mixture of volatile essential oils obtained by steam distilling the crushed petal of roses.

Rose may also be used as rose water which is used for ooking, cosmetics, medicine and in religious practices. Rose hips are occasionally made into jam, jelly, marmalade and soup or are brewed from tea, primarily for their high content of vitamin C. They are also pressed and filtered to make rose hip syrup. Rose petals or buds are sometimes used to flavour ordinary tea, or combined with other herbs to make herbal teas (**Kumar, U., *et al* 2014).**

Rosa indica are a biological name of roses. Rose has influenced culture aesthetically, economically, medically, religiously and spiritually since human kind could smell and appreciate its fragrance. The high concentration of anthocyanins in the petals give credence to these indications because anthocyanin are known for their ability to strengthen the vascular system, prevent blood platelets stickiness and also have a powerful antioxidants, antibacterial and inflammatory activity. Rose petal water can also be use as an eyewash and mouthwash. Rose petal tea is also recommended for treating breast pain or mastitis. Plants are rich in a wide variety of secondary plant metabolized such as alkaloids and Flavonoids.

It is rich sources of antimicrobial agents. Many plants have been used on traditional treatment of numerous human diseases for thousand years ago in many parts of the world (**Hirulkar N.B., *et al.,* 2010).**

Medicinal plants represent a rich source of antimicrobial agent. Plants are used medicinally in different countries and are a good source of many potent and powerful drugs. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different part used include roots, stem, flower, fruits, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries. Although hundred of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated (**Nayak. S., *et al .,* 2011).**

Antimicrobials: -

Antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to microorganism they act primarily against. For example, antimicrobials are used against bacteria and antifungals are used against fungi. They can also be classified according to their function agents that kill the microorganisms. The Antimicrobial activity of rose petal is that the high concentration anthocyanine in the petal give credence to this indication. They are expected to synthesize the variety of secondary metabolized capable of providing them protection against the infection agents (**Yilmaz. S. O., *et al* ., 2011).**

Antimicrobial activity of *Rosa indica* leaves:-

The history of antimicrobial begins with the observation of Pasteur and Joubert. They had discovered the one type of bacteria could prevent the growth of another. Leaves are used in protection against infectious agent also use in injury portion for treatment of them.

The use of herbal antimicrobials is because of fewer side effects. Natural antimicrobial compound in plants have been found to posses antimicrobial activity. Plants have been a valuable resource of natural products for maintains human health. India has rich tradition in use of medicinal plant to develop drugs. According to world health organization any plant which contains substance that can be used for therapeutic purposes or which are precursor of chemo pharmaceutical semi synthetic new drug is referred as medicinal plants.

Nature is a source of medicinal agent and an impressive number of modern drug have been isolated from natural sources, many based on their use in traditional medicine. Antimicrobial and antioxidant properties of secondary metabolites from red rose flower. Antimicrobial activity of red rose petals extract against some pathogenic bacteria. Rose petal tea as an antioxidant rich beverage: extract against pathogen (**Mishra, R.P., *et al.,* 2010).**

Rosa indica is used to treat the diseases which are related to the skin irritation and allergy. But now today scientists will discover the medicine of cancer. Disinfectant is antimicrobial substance used on non-living objects or outside the body. The history of antimicrobial begins with the observations of Pasteur and Joubert, discovered that one type of bacteria could prevent the growth of another (**Kumar, M.A., *et al.,* 2012).**

METHODOLOGY

Sample collection:

The samples *Rosa indica* were collected from the local area of Dehradun, Uttrakhand.

Preparation of extracts:

The flowers, leaves and stems were separated manually, and washed with distilled water to remove the dirt.

Further the samples were sun dried and converted to powder by grinding.

The samples were dissolved in methanol (1:10) and then incubated for 48 hours at room temperature.

Samples were then filtered in the weighed bowl and the solvents were evaporated. The remaining residues dissolved in the dimethyl sulphoxide.

Antibiogram analysis of extracts:

The extracts were allowed to screened by using agar well diffusion method and the zone of inhibition was calculated for analyzing the best activity of extracts.

Phytochemical tests of extracts:

Phytochemical screening of methanol extracts of *Rosa indica* were carried out to check Saponins, carbohydrates, phenols, alkaloids, Flavonoids, proteins and amino acid.

RESULTS

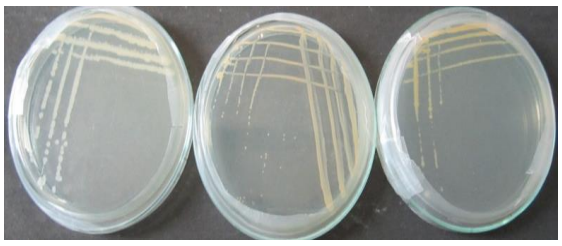
Test materials:

The fresh plant materials of *Rosa indica* plants were collected from Dehradun, Uttrakhand.

Figure 1: (a) Powdered form of leaves
(b) Powdered form of petals

Test micro-organism:

The bacterial strains used in the study were *Pseudomonas aeruginosa*; *Staphylococcus aureus* and *Escherichia coli.* Bacterial cultures were maintained on nutrient agar plate.



E .coli *S. aureus* *P. aeruginosa*

Figure 2: Pathogens

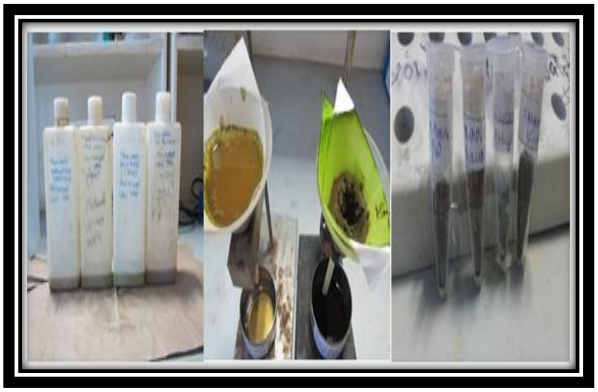


Figure 3: Preparation of solvent extraction

Antibiogram activity:

Petals of *Rosa indica*

A. Antibiogram activity of petal methanol and ethanol against different pathogens:

Antibiogram analysis of petals was carried out against all three available pathogens. It can be seen in result below in **Table 4** and **Figure 4**, in which the maximum zone of inhibition was obtained against *Escherichia coli* (13mm), *Staphylococcus aureus* (11.2mm) followed by *Pseudomonas aeruginosa* (11mm).

Table 4: Antibiogram activity of petals of *Rosa indica* methanol and ethanol extracts: Antibiotic Sensitivity Test /Multiple Drug Resistance Test (MDR):

Pathogens	ZOI by tetracycline in (mm)	ZOI by DMSO in (mm)	ZOI by Benzene in (mm)	ZOI by Ethyl aetate in (mm)
P .aeruginosa	20mm	0	11mm	0
S. aureus	20mm	0	11.2mm	0
E. coli	20mm	0	13mm	0

B. Antibiogram activity of petal benzene and ethyl acetate extracts against different pathogens:

Antibiogram analysis of petals was carried out against all three available pathogens. It can be seen in result below in table 5 and figure 5, in which the maximum zone of inhibition was obtained *Escherichia coli* (13mm), *Staphylococcus aureus* (11mm) followed by *Pseudomonas aeruginosa* (10mm)

Table 5: Antibiogram activity of petals of *Rosa indica* benzene and ethyl acetate extracts:

Pathogens	ZOI by tetracycline in (mm)	ZOI by DMSO in (mm)	ZOI by Methanol in (mm)	ZOI by Ethanol in (mm)
P .aeruginosa	20mm	0	0	10mm
S. aureus	20mm	0	0	11mm
E. coli	20mm	0	13mm	0

C. Antibiogram activity of petal distilled water and hot distilled water extracts against different pathogens:

Antibiogram analysis of petals was carried out against all three available pathogens. It can be seen in result below in table 6 and figure 6, in which the maximum zone of inhibition was obtained *Pseudomonas aeruginosa* (10.8mm),*Escherichia coli* (10.7mm) followed by *Staphylococcus aureus*(10mm).

Table 6: Antibiogram activity of petals of *Rosa indica* Distilled water and Hot distilled water extracts:

Pathogens	ZOI by tetracycline in (mm)	ZOI by DMSO in (mm)	ZOI by Distilled water in (mm)	ZOI by Hot distilled water in (mm)
P .aeruginosa	20mm	0	0	10.8mm
S. aureus	20mm	0	10mm	0
E. coli	20mm	0	0	10.7mm

Leaves of *Rosa indica*

A. Antibiogram activity of leaves methanol and ethanol against different pathogens:

Antibiogram analysis of leaves was carried out against all three available pathogens. It can be seen in result below in table 7 and figure 7, in which the maximum zone of inhibition was obtained *Escherichia coli* (12.5mm),(10.5mm) followed by *Pseudomonas aeruginosa* (1.0mm)

Table 7: Antibiogram activity of leaves of *Rosa indica* Distilled water and Hot distilled water extracts:

Pathogens	ZOI by tetracycline in (mm)	ZOI by DMSO in (mm)	ZOI by Methanol in (mm)	ZOI by Ethanol in (mm)
P .aeruginosa	20mm	0	10.0mm	0
S. aureus	20mm	0	0	0
E. coli	20mm	0	10.5mm	12.5mm

B. Antibiogram activity of leaves benzene and ethyl acetate against different pathogens:

Antibiogram analysis of leaves was carried out against all three available pathogens. It can be seen in result below in table 8 and figure 8, in which the maximum zone of inhibition was obtained *Pseudomonas aeruginosa* (13.5mm), (14mm), *Staphylococcus aureus* (7.5mm), (13.5mm) followed by *Escherichia coli* (8.0mm),(8.0mm)

Table 8: Antibiogram activity of leaves of *Rosa indica* benzene and ethyl acetate extracts:

Pathogens	ZOI by tetracycline in (mm)	ZOI by DMSO in (mm)	ZOI by Benzene in (mm)	ZOI by Ethyl acetate in (mm)
P .aeruginosa	20mm	0	13.5mm	14mm
S. aureus	20mm	0	7.5mm	13.5mm
E. coli	20mm	0	8mm	8.0mm

C. Antibiogram activity of leaves distilled water and hot distilled water against different pathogens:

Antibiogram analysis of leaves was carried out against all three available pathogens. It can be seen in result below in table 9 and figure 8, in which the maximum zone of inhibition was obtained *Pseudomonas aeruginosa* (13.5mm), (14mm), *Staphylococcus aureus* (7.5mm), (13.5mm) followed by *Escherichia coli* (8.0mm),(8.0mm)

Table 9: Antibiogram activity of leaves of *Rosa indica* Distilled water and Hot distilled water extracts**:**

Pathogens	ZOI by tetracycline in (mm)	ZOI by DMSO in (mm)	ZOI by Distilled water in (mm)	ZOI by Hot distilled water in (mm)
P .aeruginosa	20mm	0	0	0
S. aureus	20mm	0	0	0
E. coli	20mm	0	0	0

Phytochemical screening:

Table 10: Phytochemical screening of leaves extract with chemical Saponins, alkaloids, flavonoids.

LEAVES EXTRACT	SAPONINS	ALKALOIDS	FLAVONOID
Benzene	-ve	+ve	+ve
Ethyl acetate	-ve	+ve	-ve
D.W	+ve	-ve	-ve

In it benzene leaves extract shows the positive result in Alkaloids and Flavonoids. Ethyl –acetate show the positive result in Alkaloids. D/W leaves extracts show positive result in Saponins.

Table 11: Phytochemical screening of leaves extract with chemical protein, carbohydrate, phenol and amino acid

LEAVES EXTRACT	PROTEIN	CARBOHYDRATE	PHENOL	AMINO ACID
Benzene	+ve	+ve	+ve	+ve
Ethyl acetate	+ve	-ve	+ve	+ve
D.W	-ve	+ve	+ve	-ve

Benzene leave extracts show the positive results in Protein, Carbohydrates, Phenol and amino-acid .ethyl – acetate leaves extracts show the positive results in protein , phenols, amino-acid. D /W leaves extract show positive results in test carbohydrates and phenol.

Table 12: Phytochemical screening of petal extract with chemical Saponins, alkaloids and flavonoids

LEAVES EXTRACT	SAPONINS	ALKALOIDS	FLAVONOID
Benzene	-ve	-ve	+ve
Ethyl acetate	-ve	-ve	+ve
D.W	+ve	-ve	-ve

In above table benzene petal extracts show the positive result in flavonoid test. Ethyl – acetate petal extracts show positive results in flavonoids test. D/W petal extract show the positive result in saponins.

Table 13: Phytochemical screening of petal extract with chemical protein, carbohydrate, phenol, and amino acid.

LEAVES EXTRACT	PROTEIN	CARBOHYDRATE	PHENOL	AMINO ACID
Benzene	-ve	+ve	-ve	-ve
Ethyl acetate	+ve	-ve	+ve	-ve
D.W	-ve	-ve	+ve	+ve

In above table benzene petal extract show the positive result in carbohydrates. Ethyl acetate petal extracts show the positive results in proteins, phenol. D/W petal show the positive result in phenol and amino-acids.

DISCUSSION

Herbal products are best alternative to antimicrobial agent. Natural products are safe, easily; biodegradable, minimum environment hazards have no adverse effects, easily available and more dependable. Plants are the best source of drugs which can be used to treat the disease. Herbal medicines have less or no side effects, allergic problems and very safe to use. Medicinal plants would be the important source of obtaining a variety of drugs as Phytochemical are more specific, biodegradable and are supposed to have fewer side effects, phytochemical offers unique plate form for structural diversity and biological functionality which is indispensible for drug discovery **(Verporte, 2002)**.

In Antibiogram analysis of petal extract in Methanol gave zone of inhibition of 11mm, 11.2mm, 13mm, against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Eschrechia coli*, respectively. Ethanol showed no zone of inhibition against any pathogen used. Benzene gave a zone of inhibition of 13mm against *Eschrechia coli.* Ethyl acetate gave a zone of n of 10mm, 11mm against *Pseudomonas aeruginosa,* and *Staphylococcus aureus,* respectively. Hot distilled water gave a zone of inhibition 10.7mm against *Eschrechia coli* and 10.8 against *Pseudomonas aeruginosa.* Distilled water gave a zone of inhibition of 10mm against *Staphylococcus aureus*.

In Antibiogram analysis of leaves extract Methanol and Ethanol extracts gave a zone of inhibition of 10.0 mm against *Pseudomonas aeruginosa* and 10.5 against *Eschrechia coli*. Benzene gave maximum zone of inhibition of 13.5mm against *Pseudomonas*. Ethyl acetate extract gave maximum zone of inhibition of 14mm against *Pseudomonas aeruginosa.*

Leaves extract in d/w and hot d/w showed negative results against all pathogens used.

The maximum antibacterial activity was found in methanol, ethanol, benzene and ethyl-acetate extracts of *Rosa indica* petal and leaves. However the zone formed by standard antibiotics tetracycline was comparable in some case but also more than the zone formed by crude extracts.

In Phytochemical analysis, Benzene showed presence of flavonoids, proteins. Ethyl acetate showed presence of Flavonoids. Distilled water extract showed the presence of proteins, amino-acids, Saponins.

CONCLUSION

Based on the above research work it can be concluded that *Rosa indica* petals and leaves can be very good source for herbal drugs and specially the solvent like methanol, ethanol, benzene and ethyl acetate. It can be explored further for the extraction of antimicrobials by more sophisticated procedures for extraction in order to increase the yield. Petal extract also give the response in phytochemical screening test of Saponins, carbohydrates, phenol, protein, amino-acids, alkaloids and flavonoids.

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