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CORRELATION BETWEEN PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIBACTERIAL ACTIVITY STUDY OF *ROSA INDICA* LINN. LEAVES

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ABSTRACT
To know the role of different phytochemicals in relation to the *in vitro* antibacterial activity of both the ethanolic and methanolic extracts of Pink Rose and Maroon Rose varieties of *Rosa indica* leaves (Family- Rosaceae), present study was conducted. Following standard methods, phytochemical screening of each extract was performed. For antibacterial activity study, both the extracts were used at 1 mg /ml, 5 mg/ml and 20 mg /ml concentrations. From our study it seems that the Pink Rose variety was effective against both *Staphylococcus aureus* and *Escherichia coli.* Moreover, the zone of inhibition produced by this variety of rose plant and tetracycline (positive control) was found to be more or less similar against *Escherichia coli.* On the other hand, both the extracts of the Maroon Rose variety displayed antibacterial activity against *Staphylococcus aureus* only. From our antibacterial activity study, it is clear that the Pink Rose variety possesses more antibacterial activity than the Maroon Rose variety. Our phytochemical screening and antibacterial activity study indicated that alkaloids, flavonoids, saponins, tannins and phenols were present in both the extracts of the Pink Rose variety. Probably those phytochemicals were responsible for its antibacterial activity. On the other hand, the both the extracts of the Maroon Rose variety seemed to be antibacterially effective due to the presence of flavonoids, saponins, tannins, steroids and phenols.
KEYWORDS: Pink Rose, Marron Rose, Ethanolic extract, Methanolic extract, Phytochemical screening, Antibacterial activity

INTRODUCTION

Plant materials have been used for the treatment of serious diseases throughout the world before the advent of modern clinical drugs[1]. The use of medicinal plants still plays an important role to cover the basic health needs in the developing countries[2]. Several top selling drugs of modern times such as Quinine, Artemisinin, Shikonin, etc. are obtained from plants[1]. Most of the phytochemicals, secondary metabolites of plants, are physiologically active[2]. Majority of phytochemicals are known to produce therapeutic activities like antibacterial, antifungal, antioxidant, etc. Alkaloids, tannins, flavonoids and phenolic compounds are the most important of bioactive constituents of plants. In addition to their use for therapeutic purposes, natural phytochemicals are effective as precursors for the synthesis of novel useful drugs. About 50% of modern drugs are natural products, which play an important role in drug development in Pharmaceutical Industry[1].

Recently, several infections have increased enormously and antibiotic resistance has become an ever-increasing therapeutic problem. Natural products of some plants may possess a potential source of antimicrobial agents with possibly novel mechanisms of action. They are effective in the treatment of infectious diseases while decreasing many of the side effects that are often found in synthetic antimicrobials. Therefore, in order to validate their use in folk medicine, it is very important to perform a screening of these plants. This evaluation is also useful to show their active principle by isolation and characterization of their constituents. Systematic screening of them may lead to the discovery of novel active compounds [2]. The selection of crude plant extract for the determination of the antibacterial activity has the potential of being more successful in the initial steps than screening of pure compounds[1].

Considering the above mentioned importance of phytochemical screening along with the antibacterial activity of its extracts, we used extracts of pink rose and maroon rose varieties of *Rosa indica* leaves. Rose is a perennial plant of the genus *Rosa*, within the family Rosaceae. There are over hundred species of roses. They form a group of erect shrubs and climbing plants, with stems armed with sharp pickles. Flowers are large and showy and come out in many colours. Most species are native to Asia, Europe, North America and North West Africa. They are cultivated for their beauty and fragrance[3].

Rose Tea (petals and leaves brewed as a tea) can bring down fever. It works as a diuretic to flush the toxins from the body. It can also relieve bronchial and chest congestion, provide relief from a sore throat and stop runny nose. Rose water has antiseptic properties and is used as an eye wash to treat eye irritation. Rose hips are used in cooking where they add flavour as well as nutrition. Rose oil is used for skin treatment to smooth and moisturize the skin and to relieve skin irritation[4].

Till now reports on the antibacterial activity study using leaves of rose plants are scanty. However, Koday et. al. 2010 mentioned the antibacterial activity of rose petals[5]. Considering this information, we tried to explore the antibacterial activity of the leaves of Pink Rose and Maroon Rose varieties of Rose plant (*Rosa indica*).

It is already known that the beneficial effects of medicinal plant materials typically result from the combinations of secondary products present in the plant. Due to the presence of several phytochemical constituents like alkaloids, flavonoids, glycosides, saponins, tannins, steroids, anthraquinones, phenols, resins, fatty acids and gums, the plant extract generally show antibacterial property[6,7, 8].

Since several phytochemicals possess antibacterial activity, we decided to correlate phytochemical screening with antibacterial activity of both ethanolic and methanolic extracts of Pink Rose and Maroon Rose varieties of *Rosa indica* leaves.

MATERIAL AND METHODS

Plant material- The authenticated leaves of the plant *Rosa indica* (Pink Rose and Maroon Rose varieties) were collected from Chhend, Rourkela, during November 2010. The shade dried leaves were powdered and stored in a dessicator until evaporation.

Preparation of extract- The powdered leaves were passed through a sieve (No.40) and stored in a dessicator. For the extraction of the leaves, maceration method was performed.

Maceration method:

The powdered leaves (4 gm) were macerated in 30 ml of 95% ethanol for 3 days at room temperature. The resulting extract was filtered through a filter paper (Whatman No.1). The residue was further extracted using the same procedure. The filtrates obtained were combined and then evaporated to dryness. The same method of

extraction was followed using methanol instead of ethanol[9]. After drying, both the extracts were dissolved in dimethylsulfoxide (DMSO)[10].

a) Phytochemical screening

Following chemical tests were performed for testing different chemical groups present in both the extracts:

Alkaloids

Mayer's test:-To 2-3 ml of the extract, few drops of the Mayer's reagent (1.36 gm of Mercuric chloride and 5 gm of Potassium iodide in 100 ml distilled water) were added. Formation of a cream colour precipitate indicated the presence of alkaloids.

Amino acids

Millon's test:-To 2 ml of the test extract about 2 ml of Millon's reagent (Mercury nitrate) was added. White precipitate indicated the presence of amino acids.

Carbohydrates

Molish test:-To 2 ml of the test extract, at first, few drops of alcohoholic α-napthol were added. Then through sides of test tube, few drops of concentrated sulphuric acid were mixed with it. Purple to violet colour ring appeared at the junction indicated the presence of carbohydrates.

Flavonoids

Alkaline reagent test:-To 2 ml of the test extract, few drops of sodium hydroxide solution were added. At first, intense yellow colour was formed, which was subsequently turned to colourless on addition of few drops of dilute acid indicated the presence of flavonoids.

Glycosides

Borntrager's test :- The test extract was boiled with 1 ml of sulphuric acid in a test tube for 5 minutes. While hot it was filtered, then it was cooled. Shaking of the mixture was done with equal volume of chloroform. Two layers of solution were formed. The lower layer of chloroform was separated. Then the lower layer was shaken with half of its volume of dilute ammonia. Production of a rose pink to red colour suggested the presence of glycosides.

Saponins

Froth formation test :-Two millilitre of the extract was shaken vigorously with water in a test tube. Formation of persistent foam indicated the presence of saponins.

Tannins

Gelatin test :- To 2 ml of the extract, 1% gelatin solution containing 10% sodium chloride was added. Formation of a precipitate suggested the presence of tannins.

Proteins

Warming test :- Two millilitre of the extract was heated in a boiling water bath. Proteins get coagulated due to heating.

Steroids and Triterpenoids

Salkowski test :- The test extract was treated with few drops of concentrated sulphuric acid. Red colour at lower layer indicated the presence of steroids, and formation of yellow colour at the lower layer suggested the presence of triterpenoids.

Phenols

Ferric chloride test:-The test extract was treated with few drops of ferric chloride solution. Appearance of intense colour, suggested the presence of phenol.

Agar well diffusion method- In order to determine the *in vitro* antibacterial activity of the ethanolic and methanolic extracts of the leaves of Maroon Rose and Pink Rose varieties of *Rosa indica*, the nutrient agar well diffusion method as described by Schillenger and Luke (1989) was performed. Sterile nutrient agar medium was inoculated with 0.1ml of fresh overnight nutrient broth culture of each bacterium (approx.10^7CFU/ml) and poured into sterile petriplates[12]. For our study we used bacterial suspensions of *Staphyloccus aureus, Bacillus subtilis* and *Escherichia coli*. In each plate, wells of 6mm in diameter were punched using a sterile borer and the plates were allowed to dry for 5min[12,13]. For the study both ethanolic and methanolic extracts of each variety of the plant were used at 1mg/ml, 5mg/ml and 20mg/ml concentrations[12]. Each concentration of the ethanolic and methanolic extracts of Pink Rose variety was, at first, dispensed separately into different wells using sterile micropipettes. Then both the extracts of Maroon Rose variety were used in a similar manner. In addition, DMSO (negative control) and tetracycline at a concentration of 25µg/ml (positive control) were also dispensed separately into different wells[14]. The volume of different solutions used in each well was 50 µl. After holding the plates at room temperature for 2 hours to allow diffusion of the extracts and controls into the nutrient agar medium, the plates were incubated at 37 ^{0}C for 24 hrs. After the incubation period, the plates were examined for inhibition of the bacterial growth around the wells. The diameters of the zones of inhibition in each case were measured[13].

RESULTS

We performed phytochemical screening of Pink Rose and Maroon Rose varieties. For that both the ethanolic and methanolic extracts of both the varieties were tested for different phytoconstituents .Using standard methods, we have tested for alkaloids aminoacids, carbohydrates, flavonoids, glycosides, saponins, proteins, steroids, tannins, triterpinoids and phenol. In case of the ethanolic extract of Pink Rose variety, alkaloids, amino acids, carbohydrates, flavonoids, saponins, tannins, triterpinoids, phenols were present. On the other hand, the methanolic extract of the same variety gave positive results in case of alkaloids, flavonoids, saponins, tannins, triterpenoids and phenols (Table 1).

In case of both the ethanolic and methanolic extracts of Maroon Rose variety, carbohydrates, flavonoids, saponins, tannins, steroids and phenols were detected (Table 2).

Both the ethanolic and methanolic extracts of Pink Rose variety did not produce any antibacterial activity at 1mg/ml. While those extracts at 5mg /ml showed moderate zones of inhibition against *Staphylococcus aureus* and *Escherichia coli*, maximum zones of inhibition were produced against them at 20mg/ml. *Bacillus subtilis* was totally resistant to both the ethanolic and methanolic extracts at different concentrations used in our study (Table 3).

The ethanolic and methanolic extracts of Maroon Rose variety were only effective against *Staphylococcus aureus* at 20mg/ml. *Bacillus subtilis* and *Escherichia coli* were totally resistant to both the extracts of this variety (Table 4).

Tetracycline produced maximum zone of inhibition against *Staphylococcus aureus*, and it was minimum in case of *Escherichia coli*. DMSO did not produce any zone of inhibition against the bacteria used in the study (Tables 3 and 4).

DISCUSSION

Till now reports on the antibacterial activity study using leaves of rose plants are scanty. However, Koday et. al. 2010 mentioned the antibacterial activity of rose petals[5]. From our study it seems that the Pink Rose variety was effective against both *Staphylococcus aureus* and *Escherichia coli.* Moreover, the zone of inhibition produced by this variety of rose and tetracycline was found to be more or less similar against *Escherichia coli* (Table 3). On the other hand, both the extracts of the Maroon Rose variety displayed antibacterial activity against *Staphylococcus aureus* only (Table 4).

From our antibacterial activity study, it is clear that the Pink Rose variety possesses more antibacterial activity than the Maroon Rose variety. In addition, it may be mentioned that the Pink Rose variety and the positive control are more or less equally effective against *Escherichia coli*.

It is known that due to the presence of several phytochemical constituents like alkaloids, flavonoids, glycosides, saponins, tannins, steroids, anthraquinones, phenols, resins, fatty acids and gums, the plant extract generally show antibacterial property[6,7, 8]. From our phytochemical screening and antibacterial activity study, it may be mentioned that alkaloids, flavonoids, saponins, tannins and phenols are present in both the extracts of the Pink Rose variety. Probably those phytochemicals are responsible for its antibacterial activity [6,7, 8]. On the other hand, the both the extracts of the Maroon Rose variety seems to be antibacterially effective due to the presence of flavonoids, saponins, tannins, steroids and phenols [6, 7, 8].

In conclusion, phytochemicals present in the two varieties of the plant may be responsible for displaying different antibacterial activities. Systematic screening of plants (using crude plant extract of different varieties instead of taking pure compounds) may lead to the discovery of novel active compounds.

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Table 1- Qualitative analysis of various extracts of *Rosa indica* leaves (Pink Rose)

Phytoconstituents	Ethanolic extract	Methanolic extract
Alkaloids	+	+
Amino acids	+	_
Carbohydrates	+	_
Flavonoids	+	+
Glycosides	_	_
Saponins	+	+
Protiens	_	_
Steroids	_	_
Tannins	+	+
Triterpenoids	+	+
Phenol	+	+

'+' = Present; '-' = Absent

Table 2- Qualitative analysis of various extracts of *Rosa indica* leaves (Maroon Rose)

Phytoconstituents	Ethanolic extract	Methanolic extract
Alkaloids	_	_
Amino acids	_	_
Carbohydrates	+	+
Flavonoids	+	+
Glycosides	_	_
Saponins	+	+
Protiens	_	_
Steroids	+	+
Tannins	+	+
Triterpenoids	–	–
Phenol	+	+

'+' = Present; '-' = Absent

Table 3: Antibacterial activity pattern of the extracts and controls of the Pink Rose variety

Microorganisms	Zone of inhibition after 24 hrs(in mm)							
	Ethanolic Extract			Methanolic Extract			Tetracycline 25µg/ml	DMSO
	1mg/ml	5mg/ml	20mg/ml	1mg/ml	5mg/ml	20mg/ml		
Staphylococcus aureus	0	19.5	23	0	18	23	31	0
Bacillus subtilis	0	0	0	0	0	0	26	0
Escherichia coli	0	12	15.3	0	14	14.5	15	0

Table 4: Antibacterial activity pattern of the extracts and controls of the Maroon Rose variety

Microorganisms	Zone of inhibition after 24 hrs(in mm)							
	Ethanolic Extract			Methanolic Extract			Tetracycline 25µg/ml	DMSO
	1mg/ml	5mg/ml	20mg/ml	1mg/ml	5mg/ml	20mg/ml		
Staphylococcus aureus	0	0	22	0	0	21	32	0
Bacillus subtilis	0	0	0	0	0	0	26	0
Escherichia coli	0	0	0	0	0	0	19	0

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