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Phytochemical Analysis and Antibacterial Activity of Rose Water Samples (*Rosa damascena*)

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Abstract: Nature has so many treasures of medicines in the form of herbal plants. These plants were used as traditional medicines in olden days. Till today this herbal plant are in use for their phytochemical components which plays an important role in discovery and designing of drugs. One among them is Rosaceae family. Roses are beautiful flowers which has enormous variety of colours. They are considered as the King of Flowers. They are cultivated worldwide. The Rose flower influence culture, economically, medically, religiously and spiritually. They contain many properties like anticonvulsant, anti-HIV, anti-diabetic, antimicrobial, antioxidant, anti-inflammatory, anti-aging, anti-obesity, antispasmodiac etc. *Rosa damascena* is a small aromatic pink flower which are grown in gardens, around houses and for medicinal properties. The present study aimed to compare rosewater samples and to study their phytochemical and antibacterial effect. The methodology followed was steam distillation method, Qualitative phytochemical analysis and agar well diffusion method. A rose water sample was prepared using steam distillation method. The other sample was bought from a local market and was used for the study. Two Gram-positive bacteria and one Gram-negative bacteria were used for the study. Results showed the presence and absence of phytochemical components.

Keywords: Paneer rose, Rose water, Distillation, Inhibition, Phytochemical, Antibacterial

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Introduction

Rosa damascena Mill (Rosacea) commonly known as Gole Mohammadi, Gul-e-Surk (in Persian), Ward-e-Ahmar (in Arabic), Damascus rose or otto rose (in English) and gulab (in Hindi) (Anonymous, 1981; Loghmani *et al*., 2007), is a well-known ornamental plant, referred as the king of flowers having more than 200 species throughout the world (Gudin, 2000). The name of *Rosa damascena* is derived from the city name Damascus, Syria, where it exists as a wild plant. It is also called as Damask rose or as Rose of Castile. The flower petals are also edible. Apart from the

use of *Rosa damascena* as ornamental plant in parks, gardens, and houses, they are principally cultivated for using in perfume, medicine and food industry (Jabbarzadeh and Khosh-Khuri, 2005). An extensive range of phytochemicals exists within Rosacea (Olsson *et al.*, 2005; Uggla *et al.*, 2005). Plant tannins and flavonoids have been reported to have antimicrobial effects and many biological activities (Sami *et al.*, 2013). The flowers of the plant *Rosa damascena* are also rich sources of flavonoids (Velioglu and Mazza, 1991). Natural products derived from plants represent antimicrobial agents since they are natural and affordable, especially for rural societies in poor developing countries (Ghosh *et al.*, 2008). It has been reported that the rose water has antimicrobial effects and can inhibit *Candida albicans* mycelia growth and has bactericidal activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (Maruyama *et al.*, 2017). Since rose petal extracts are known foe antimicrobial activity, the rose petal extract was used in this study against some Gram-positive and Gram-negative bacteria to confirm the antimicrobial activity.

Materials and Methods

The fresh flowers of *Rosa damascena* were collected from Walajapet, Vellore district, Tamil Nadu, India. The species was identified and authenticated by Mrs. P. Jabeena Begum, Department of Plant Biology and Plant Biotechnology, Justice Basheer Ahmed Sayeed College for Women. The other sample was purchased from a market in Chennai city. The sample was stored in air tight container.

Preparation of the flower extract:

The petals were separated from the flower discarding the green parts. They were washed thoroughly with distilled water to remove any contaminants. Rose water was extracted using Steam distillation method. 150 g of petals were used along with 300 ml of distilled water (Milja *et al.*, 2013).

Workstages:

- A large steel pot was taken in which a kadappa stone was placed in the mid-base of the pot.
- Filled the base of the pot with rose petals (measured) followed by 100 ml of distilled water.
- Placed the small receiving cup at the centre of the pot (on top of the kadappa stone), on the configuration so that the small receiving cup was not in water.
- The level of water should not touch the small receiving cup.
- Covered the steel pot with a lid. If the lid has a handle in the middle, it acted as a good collector for the liquid from which the distillate drops directly into the receiving cup.
- Boiled water, when the water starts to boil filled the lid of the pot with ice. This keeps the lid cool and the vaporizing liquid was effectively condensed on the lid's inner surface, from where it drips into the receiving cup.
- Let the liquid simmer at a mild temperature.
- The distillate was collected in the receiving cup.
- The process takes place within 15 min. The first 5 min for boiling and the remaining 10 min for simmering. After 15 min, the gas-stove was turned off.
- It was again allowed to cool down for 10 min for the distillate to settle down. The set-up was undisturbed during this process.
- The distillate was the rose water which was collected in the small receiving cup. The distillate was stored in an air tight container.
- The distillate received is around 45 ml. The process was repeated to gather more rose water.

Phytochemical analysis (*Harborne, 1973; Sofowora, 1993*):

Test for carbohydrates:

To 200 µl of plant extract, 100 µl of Molisch

reagent and few drops of concentrated sulphuric acid were added. Presence of purple colour or reddish colour indicated the presence of carbohydrates.

Test for tannins:

To 100 µl of extract, 200 µl of 5% ferric chloride was added. Formation of dark blue as greenish black indicated the presence of tannins.

Test for saponins:

To 200 µl of plant extract, 200 µl of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise. Formation of 1 cm layer of foam indicated the presence of saponins.

Test for flavanoids:

To 200 µl of plant extract, 100 µl of 2N sodium hydroxide was added. Presence of yellow colour indicated presence of flavonoids.

Test for alkaloids:

To 200 µl of plant extract, 200 µl of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green colour or white precipitate indicated the presence of alkaloids.

Test for quinines:

To 100 µl of extract, 100 µl of concentrated sulphuric acid was added. Formation of red colour indicated the presence of quinones.

Test for glycosides:

To 200 µl of plant extract, 300 µl of chloroform and 10% ammonia solution was added. Formation of pink colour indicated the presence of glycosides.

Test for cardiac glycosides:

To 50 µl of extract, 200 µl of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 100 µl of concentrated sulphuric acid. Formation of brown ring at the interface indicated presence of cardiac glycosides.

Test for terpenoids:

To 50 µl of extract, 200 µl of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown colour at the interface indicated presence of terpenoids.

Test for triterpenoids:

To 150 µl of extract, 100 µl of chloroform was added and shaken with concentrated sulphuric acid. Lower layer turned yellow on standing which indicated the presence of triterpenoids.

Test for phenols:

To 100 µl of extract, 200 µl of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green colour indicated presence of phenols.

Test for coumarins:

To 100 µl of extract, 100 µl of 10% NaOH was added. Formation of yellow colour indicated presence of coumarins.

Test for steroids and phytosteroids:

To 100 µl of plant extract, equal volume of chloroform and few drops of concentrated sulphuric acid was added. Appearance of brown ring indicated the presence of steroids and appearance of bluish brown ring indicated the presence of phytosteroids.

Test for phlobatannins:

To 100 µl of plant extract, few drops of 2% HCl was added. Appearance of red colour precipitate indicated the presence of phlobatannins.

Test for anthraquinones:

To 100 µl of plant extract, few drops of 10% ammonia solution was added. Appearance of pink colour precipitate indicated the presence of anthraquinones.

Antibacterial activity test:

Bacterial sub-culture:

Two Gram-positive and one Gram-negative bacteria were used for the antibacterial activity. The ATCC bacterial strains of the test bacteria *Staphylococcus aureus* (ATCC 27661), *Streptococcus pneumonia* (ATCC 6305),

Enterobacter cloacae (13048), were labelled as S1, S2, S3 and were aseptically inoculated into sterile nutrient broth tubes and incubated at 37°C for 24 h for agar diffusion experiments.

Antibacterial activity:

The experiment was performed in a laminar air flow chamber. The apparatus, solutions, and nutrient media, etc., were sterilized by autoclaving before experiments. The antibacterial activity was determined by agar well diffusion method. It was measured using the standard method of diffusion disc plates on agar (Erturk *et al*., 2003). The prepared sterile media was poured into the sterilized petri plates and allowed to solidify. The broth cultures were swabbed and inoculated on sterile Mueller-Hinton agar plates. A cork-borer was used to drill wells in each agar plate for different concentrations of the test samples. Simultaneously different concentrations (5, 2.5, 1.25, 0.62, 0.31 mg/ml) of the samples extracted through serial dilution at a concentration ratio of 1:2 were prepared and used for screening the efficacy of extracts by agar well diffusion assay. 20 µl of each different concentrations (5, 2.5, 1.25, 0.62, 0.31 mg/ml) of the extract was added to different wells of each petri plate. 20 µl of distilled water was added as control in each petri plate. The petri plates were left undisturbed for 30 min and then incubated in upright position for 24 h at 37°C. Using a ruler, zone of inhibition formed around wells was measured. The presence of zone of inhibition around the wells was the indication of antibacterial activity of extracts.

Test Samples:

The commercially bought sample was labelled as T1 and the homemade rosewater (distillate) sample as T2. Both were used for the phytochemical analysis and antibacterial activity.

Results and Discussion

The qualitative phytochemical analysis of commercial sample and distilled extract sample showed the presence of carbohydrates, alkaloids and tannins (Table 1). This is in accordance with findings of Safia *et al.* (2018) as they also noticed the presence of tannin in all samples. In this study, 12 samples of rose water were used among them 6 samples were collected from market, 4 samples were provided by a lab and the last sample were prepared in the lab using hydro-distillation method. Aqueous extract of *Rosa damascena* showed the presence of carbohydrates (Table 1) which is in conformity with reports of Tatke *et al.* (2015). The method of preparation is different in this study. The presence of phytochemical components may be the reason for its good antimicrobial- activity. The *Staphylococcus aureus* was the most sensitive strain (Eman, 2014). This report is in accordance with the present study because only the homemade rose water sample inhibited this species and the commercial rose water sample did not showed any inhibition. Maruyama *et al*. (2017) evaluated the effects of rose water against methicillin resistant *Staphylococcus aureus* (MRSA) and showed rose water decreased MRSA 99.99% within 1 h. This strengthens the findings of the present study where *S. aureus* is also inhibited by the rose water (Table 2). This antibacterial property could be due to the presence of several phytochemical constituents like alkaloids, flavonoids, glycosides, saponins, tannins, steroids, anthraquinones, phenols, resins, fatty acids and gums (Rumana Saeed *et al*., 2014). The present study is in accordance with the above statement with the presence of tannins and alkaloids which showed good antibacterial activity. Aqueous extract of rose petals exhibited antibacterial activity, suggesting a possible utilization of rose petal boiling water after rose oil distillation. Aqueous extract of dilution 1:1 showed inhibitory activity against *Staphylococcus aureus* 13 mm (Table 2).

There is no reports for third bacteria *Enterobacter cloacae* regarding the effects of rose aqueous extract. The finding of antibacterial activity in rose petals in this study indicates its ethno botanical evidences on the use of petals of rose flowers to cure diarrhoeal diseases, opportunistic infection and skin infection.

Table 1: Phytochemical analysis of test samples

PHYTOCHEMICAL TEST	SAMPLES	
	T1	T2
Carbohydrates	+	+
Tannins	-	+
Saponnis	-	-
Flavonoids	-	-
Alkaloids	+	-
Quinones	-	-
glycosides	-	-
Cardiac glycosides	-	-
Terpenoids	-	-
Triterpenoids	-	-
Phenols	-	-
Courmarins	-	-
Steroids and phytosteroids	-	-
Phlobatannins	-	-
Anthraquinones	-	-

Table 2: Antibacterial activity of test samples

DETAILS		ZONES OF INHHIBITION (in mm)				
		5 mg	2.5 mg	1.25 mg	0.62 mg	0.31 mg
SAMPLE 1- *Staphylococcus aureus*	T 1	-	-	-	-	-
	T 2	15	12	8	8	7
SAMPLE 2- *Streptococcus pneumonia*	T 1	12	10	-	-	-
	T 2	17	13	13	8	-
SAMPLE 3- *Enterobacter cloacae*	T 1	-	-	-	-	-
	T 2	-	-	-	-	-

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