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ANTI MICROBIAL AND ANTI OXIDANT ACTIVITY EVALUATION OF *ROSA DAMASCENA MILL* FLOWERS

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ABSTRACT

Rosa damascena mill L, commonly known as Damask rose, is known as Gole Mohammadi in Iran. It is one of the most important species of Rosaceae family. Rosaceae The alcoholic extract of the *Rosa damascene* flowers shows the significance anti microbial and anti oxidant activity in a concentration of 500µg/ml and 30µg/ml respectively. The results were compared with standards like ofloxacin and ascorbic acid respectively.

KEYWORDS: *Rosa damascene,* anti microbial activity, anti oxidant activity, DPPH activity.

INTRODUCTION

Rosa damascena mill L, commonly known as Damask rose.[1], is known as Gole Mohammadi in Iran.[2] It is one of the most important species of Rosaceae family. Rosaceae are well- known ornamental plants and have been referred to as the king of flowers.[3,4] At present time, over 200 rose species and more than 18000 cultivars form of the plant have been identified.[5] Apart from the use of *R. damascena* as ornamental plants in parks, gardens, and houses, they are principally cultivated for using in perfume, medicine and food industry.[6] However, *R. damascena* is mainly known for its perfuming effects.[7] The rose water were scattered at weddings to ensure a happy marriage and are symbol of love and purity and are also used to aid meditation and prayer. There is a strong bond between Iranians and this plant. Its popularity is not only because of the medicinal effects but also is due to holy beliefs about it. People call this plant Flower of Prophet Mohammed (Gole mohammadi), because they believe its nice aroma reminds them of prophet Mohammad.[8]

At the present time, this plant is cultivated in Iran (especially in Kashan) for preparing rose water and essential oil.[9, 10] Because of the low oil content in *R. damascena* and the lack of natural and synthetic substitutes, essential rose oil of this plant is one of the most expensive ones in the world markets.[11] The *R. damascena* has also been used for medicinal purposes.[12] Various products and isolated constituents from flowers, petals and hips (seed-pot) of this plant have been studied in a variety of *in vivo*and *in vitro* studies. However, there are not any reviews to collect pharmacological effects of *R. damascena*in the present time. Therefore, in this review we collect and discuss important pharmacological effects of *R. damascena* that recently have been published in numerous studies.

MATERIALS AND METHODS

Preparation of plant extract

The fresh petals of flower *Rosa damascene* were shade dried. The dried petals were grinded to get coarse powder. 250 gm of coarse powder was subjected to cold maceration process using ethanol (70:30) as solvent. The extraction was continued for 7days at room temperature with occasional shaking. Then the extract was filtered, collected and concentrated at 70^{o}C on a heating mantle until a softy mass obtained. It was then thoroughly air dried to remove all the traces of solvent and then was subjected to freeze drying. The obtained plant extract was preserved in cold condition i.e. below 0^{o}C till the end of treatment period.

4.3. Preliminary Phytochemical Screening.[13,14,15,16]

Standard qualitative screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites using standard procedures (Khandelwal, 2005).

Test for Tannins

1. A small portion of extract was treated with 5%ferric chloride solution. Appearance of green to blue color was taken as a positive test for tannins.
2. Small portion of extract was treated with lead acetate. Appearance of creamy precipitate was considered as a positive test for tannins.

Test for Alkaloids

1. **Mayer's Test:** The Extract to be tested is treated with few drops of dilute 2N HCL and 0.5 ml

Mayer's reagent. White precipitate was obtained which confirm the presence of alkaloids.

2. **Wagner's Test:** The extract is treated with few drops of 2N HCL and 0.5 ml Wagner's reagent. Brown flocculent precipitate was obtained which confirm the presence of alkaloids.
3. **Hager's Test**: The extract is treated with few drops of dilute 2N HCL and 0.5 ml Hager's reagent. Yellow colored precipitate was obtained which confirms the presence of alkaloids.

Test for Steroids

1. **Salkowski reaction:** To 2ml.of extract, add 2ml of chloroform and 2ml.conc.H_2SO_4. Shake well, Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
2. **Liebermann-Burchard reaction:** Mix 2 ml extract with chloroform. Add 1-2ml.aceteic anhydride and 2 drops of conc.H_2SO_4 from the side of test tube.
3. **Libermann's reaction:** mix 3ml.extract with 3ml. acetic anhydride. Heat and cool. Add few drops conc. H_2SO_4. Blue color appears.

Tests for Glycosides

1. **Borntrager's test:** About 50mg of extract was hydrolysed with 2ml of concentrated HCl for 2hrs on water bath and filtered. To 2ml of filtrate hydrolysate, 3ml of $CHCl_3$ was added and shaken. $CHCl_3$ layer was separated and 10% NH_3 solution was added. Formation of pink colour indicates the presence of anthraquinone glycosides.
2. **Baljet's test:** The alcoholic or aqueous extract test solution is treated with sodium picrate. Appearance of yellow to orange colour indicates the presence of glycosides.
3. **Keller-Kiliani test:** About 2ml of test solution is treated with few drops of ferric chloride solution and mixed and then sulphuric acid containing ferric chloride solution is added, it forms two layers. Appearance of lower layer in reddish brown and upper layer in bluish green indicates the presence of glycosides**.**

Test for Saponins

Foam's test: A small amount of dry extract was boiled with water and allowed to cool. It was then shaken vigorously for a minute. The formation of persistent honey comb like froth was considered as a positive test for saponins.

Test for Sugars

1. **Molisch's test:** It was performed for the presence of carbohydrates. 1 ml of 10%alcoholic solution of α-napthol was added to the extract and mixed. Then 1ml of concentrated sulphuric acid was carefully poured along the sides of the test tube violet ring formed at the junction which is considered positive test for carbohydrates.
2. **Fehling's test:** 5ml of solution of extract was heated with equal volumes of Fehling's solution A & B. Transition of color from blue through green to reddish orange confirms the presence of reducing sugars.
3. **Benedict's test:** 5 ml of solution of the extract was heated with 5 ml of Benedict's reagent. A green, yellow or orange red precipitate was considered as a positive test for reducing sugars.

Test for Proteins

1. **Biuret test:** A small portion of extract was treated with Biuret reagent.
2. **Xanthoprotein test:** Mix 3ml. T.S. with 1ml.conc. H_2SO_4. White precipitate is formed. Boil. Solution turns black or brownish due to Lead sulphide formation.

Pharmacological evolution

Antioxidant activity by DPPH method[20]

Antioxidant behaviour of the extracted compound was measured *in vitro* by the inhibition of generated stable 2,2-diphenyl- 1-picrylhydrazyl (DPPH) free radical. Methods vary greatly as to the generated radical, the reproducibility of the generation process, and the end point that is used for the determination. The DPPH solution was prepared by dissolving accurately weighed 22 mg of DPPH in 100 ml of ethanol. From this stock solution, 18 ml was diluted to 100 ml with ethanol to obtain 100 µM DPPH solutions. The sample solution was prepared by accurately weighed 2.1 mg of each of the compounds and dissolved in 1 ml of freshly distilled DMSO separately to obtain solutions of 2.1 mg/ml concentration and the standard solution of was prepared by accurately weighed 10.5 mg of α-Tocopherol and dissolved in 1 ml of freshly distilled DMSO to get 10.5 mg/ml concentration.

A different concentration of extract was prepared by the addition of ethanolic solution of DPPH radical. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm against the corresponding blank solution. The final concentration of the samples and standard α-Tocopherol solutions used is 100 µg/ml. The percentage scavenging DPPH radical inhibitions were calculated by using the following formula.

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Where, Abs control was the absorbance of DPPH radical and ethanol, Abs sample was the absorbance of DPPH radical and sample/standard.

The scavenging activity was expressed in terms of IC50, the concentration of the samples required to give a 50% reduction in the intensity of the signal of the DPPH radical. The results were done at least in triplicate.

RESULTS

Table 1: Results of Preliminary Phytochemical Screening of ERD.

S. No	Name of the Test	Result
1.	Flavonoids	++
2.	Phenols	++
3.	Alkaloids	++
4.	Saponins	++
5.	Carbohydrates	++
6.	Proteins & amino acids	++
7.	Tannins	++
8.	Cardiac glycosides	++

Anti microbial activity

Table 2 Anti Microbial Evolution of Compounds

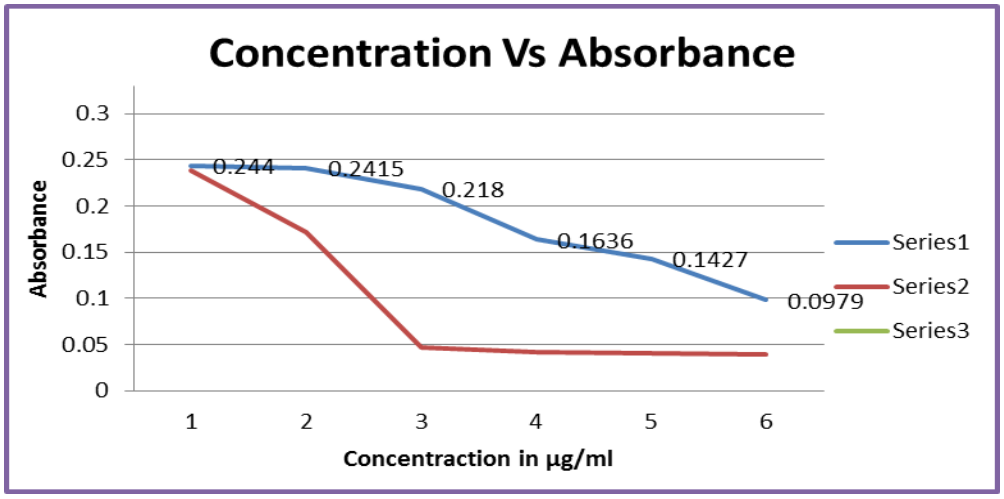
Table 2 Anti oxidant activity of alcoholic extract of *Rosa damascene.*

Concentration (µg/ml))	Ascorbic acid (Abs)	Alcoholic extract of *Rosa damascene* (Abs)
5	0.2380	0.244
10	0.1719	0.2415
15	0.0469	0.218
20	0.0415	0.1636
25	0.0410	0.1427
30	0.0390	0.0979
Control	0.2444	

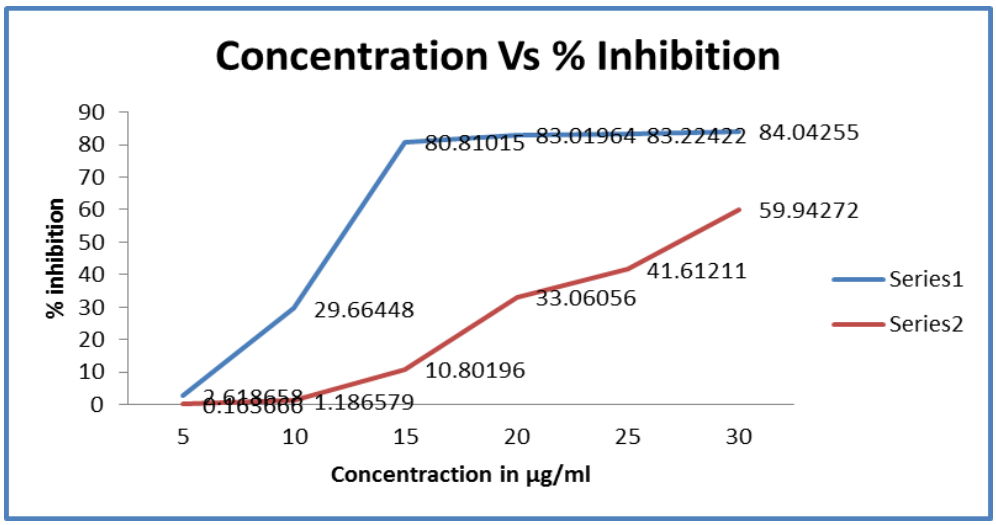
Table 3: % inhibition of alcoholic extract of *Bougainvillea glabra* with ascorbic acid.

Concentration (µg/ml))	Ascorbic acid (% Inhibition)	Alcoholic extract of *Rosa damascene* (% Inhibition)
5	2.618658	0.163666
10	29.66448	1.186579
15	80.81015	10.80196
20	83.01964	33.06056
25	83.22422	41.61211
30	84.04255	59.94272

		Alcoholic extract of *Rosa damascene*			Ofloxacin		
		Zone of inhibition in mm					
		100µg/ml	250mg/ml	500µg/ml	100µg/ml	250mg/ml	500µg/ml
Name of the organisms	*Staphylococcus aureus*	10	14	18	12	20	26
	Bacillus subtilis	8	16	20	14	18	24
	Escherichia coli	6	18	22	12	16	25
	Proteus vulgaris	12	14	20	12	14	22
Control	DMSO	-	-	-	-	-	-



Graph-1: Concentration Vs Absorbance



Graph-2: concentrations Vs % Inhibition.

DISCUSSION

The present results reveals that the alcoholic extract shows the activity less than the standard. The extract was diluted with concentration of 100 µg/ml, 250µg/ml, 500µg/ml. In that the extract with concentration of 500µg/ml shows the significance activity than the remaining concentrations. The Alcoholic extract of *Rosa damascene* tested for antioxidant activity by using DPPH Assay method. Here the results were compared with the standard Ascorbic acid. The result reveals that the extract shows results less than the standard. The concentration of the extract was taken in to 5-30 µg/ml. The % of inhibition shows that the up to 30µg/ml. The % inhibition is therefore it shows more activity than compare with other concentrations.

CONCLUSION

The outcomes of the present study indicated that the alcoholic extract of the *Rosa damascene* flowers shows the significance anti microbial and anti oxidant activity in a concentration of 500µg/ml and 30 µg/ml respectively. The results were compared with standards like ofloxacin and ascorbic acid respectively.

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