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Evaluation of antimicrobial activities of *Rosa damascena* cv. Taifi extract

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Antimicrobial activities were evaluated from the known varied weights of rose petals (50 to 400 mg) and pollens extracts of *Rosa damascena* cv. Taifi in different aqueous solutions and solvents such as sterilized *zamzam* water, distilled water, ethanol, acetone, isoamyl alcohol and 100 millimolar (mM) Tris-HCl at pH 8.7 by using liquid nitrogen. The highest activity was recorded against ethyl alcohol and acetone extracts of *R. damascena* cv. Taifi. The roses were obtained from Al-Kamal factory from *Al-Hada* and Mohammed Bin Othman Farm in *Abha*. The research was conducted at the Department of Biotechnology, Taif University from February to May 2013. The bacterial strains from ATCC and the nutrient agar were utilized for the study. Experiment results shows that the ethyl alcohol and acetone extract of rose's petals and pollen have shown an activity against *Pseudomonas aeruginosa* (ATCC 27853). The acetone extract of rose's petals showed activity against *Candida albicans* (ATCC 14053) and *Pseudomanas aeruginosa* (ATCC 27853). Also, the ethyl alcohol extract of rose's petals had shown activity against *Escherichia coli* (ATCC 25922). The water extract of rose petals showed an activity against *C. albicans (*ATCC 14053) 200 mg/ml. The acetone extract of rose's petals appeared to have a moderate activity against *C. albicans* (ATCC 14053) and showed the highest antimicrobial activities against *P. aeruginosa* (ATCC 27853). Encouraging results were due to the fact that the plants are rich antibacterial sources and further study should be carried out to confirm the purification of the antibacterial compounds.

Key words: Agar well diffusion method, antimicrobial activities, medicinal plant extract.

INTRODUCTION

Rosa damascena cv. Taifi belongs to Rosaceae family, mainly known for its perfuming characteristic and its major products are rose water and essential oil (Lavid et al., 2002). The chemical constituents such as terpenes, glycosides, flavonoids and anthocyanin of this plant have beneficial effects on human health. Its pharmacological

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effects are widespread and most of the central nervous system (CNS) effects are hypnotic, analgesic and anticonvulsant. The respiratory, cardio-vascular, laxative, anti-diabetic, antimicrobial, anti-HIV, anti-inflammatory and antioxidant and other effects of this plant has been proven (Boskabady et al., 2011; Ginova et al., 2013).

R. damascena originated from the Lyzangan Valley near Faris in Iran (Kiani et al., 2008). Farmers shifted it from Iran to Bulgaria in the 17th century and to Turkey in the 1880s. The largest producers of rose oil today are Bulgaria and Turkey (Baydar et al., 2004).

More than 200 roses species and over 18000 cultivar forms of the rose plant have been well known (Gudin, 2000) and used in perfume, medicine and food industry (Jabbarzadeh and Khosh-Khui, 2005). It is mainly known for its perfuming effects (Widrlechner, 1981).

The medicinal functions of Rosaceae are partly due to the presence of phenolics compounds which possess a wide range of pharmacological activities such as free-radical scavengers, antioxidants, anti-inflammatory, anticancer, antimutagenic and antidepressant (Ng et al., 2000; Ren et al., 2003; Hongratanaworakit, 2009). Fresh flower (FF) and spent flower (SF) extracts of *R. damascena* flower were studied against 15 species of bacteria such as *Aeromonas hydrophila, Bacillus cereus, Enterobacter aerogenes, Enterococcus feacalis, Escherichia coli, Klebsiella pneumoniae, Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella enteritidis, Salmonella typhimurium, Staphylococcus aureus* and *Yersinia enterocolitica* for antibacterial activity and both extracts were effective against all the bacteria except *E. coli.* The FF extract was more effective than the SF extract (Özkan et al., 2004).

The essential oils of *R. damascena* and several plants at their low concentrations exhibited inhibitory and bactericidal activities against Gram-positive *S. aureus* (ATCC 25923); Gram-negative *E. coli* (ATCC 25922); *P. aeruginosa* (ATCC 27853) and yeast *C. albicans* (ATCC 14053) as reported by Lisin et al. (1999).

In this work, the rose petals and pollen from *R. damascene* cv. Taifi were extracted with six different solvents against five micro-organisms.

MATERIALS AND METHODS

The delicate and intense fragrance *R. damascena cv. Taifi was* collected from Al Hada region in Taif and Mohamed Bin Othaman Farm in Abha in early morning hours. The flowers were kept in fridge initially for 2 h. The rose petals and pollen were weighed individually and cleaned initially with sterilized distilled water and then ethyl alcohol.

Liquid nitrogen was poured into the cleaned rose petals and pollen which were taken in already cleaned mortar and pestle, homogenized into a fine powder in order to increase the surface area and facilitate the extraction procedure. The microbial strains *B. subtilis* (ATCC 6633); *C. albicans (*ATCC 14053); *E. col*i (ATCC 25922); *M. luteus* (ATCC 9341); *P. aeruginosa (*ATCC 27853) were obtained from American Type Culture Collection (ATCC). The different solvents used were *zamzam* water; sterilized distilled water; ethyl alcohol; isoamyl alcohol; acetone and 100 millimolar Tris-HCl at pH 8.7. The solvents, sterilized water and z*amzam* water were prepared by autoclaving at 121°C at 15 psig for 20 min. The ethyl alcohol, isoamyl alcohol and acetone were purchased from Loba Chemie Pvt. Ltd, Mumbai, India and Tris-HCl at pH 8.7 was prepared in the laboratory. The chemicals used were liquid nitrogen; NaOH; nutrient agar and nutrient broth were obtained from Himedia Lab Pvt. Ltd., Mumbai, India. NaOH was also purchased from Loba Chemie Pvt. Ltd, Mumbai, India. The *zamzam* water was obtained from the bottling factory under the Saudi Ministry of "King Abdullah Bin Abdul-Aziz *zamzam* project" distribution station in Kuday, Mekkah. Oxytetracycline was obtained from Sigma and stored at 4°C.

The rose petals and pollens with bioactive components were subjected to extraction procedures. Varying amounts (50 to 400 mg) of the powdered material was dissolved in 1.5 ml of each solvent and the extract collected was kept in a shaking incubator at 30°C for 2 h. The organic solvents and water extract was filtered and evaporated until dryness. The extract was stored at 4°C until further use. The individual fractions were then centrifuged at 10000 rpm for 10 min. After centrifugation, the supernatant was kept at -20°C. Rose petal from 50 to 400 mg/ml and pollen of equal weight (100 mg) were used for the study of "Evaluation of Antimicrobial Activities of *R. damascena* cv. Taifi Extract". After extraction, the tubes were kept in the shaker at 150 rpm at 30°C for 2 h and then kept in freezer at -80°C. The bacterial strains were maintained on nutrient agar and freshly prepared sub-cultures in nutrient broth were used during this project. The standard agar-well diffusion was employed to determine the antimicrobial activities for both rose petals and pollen extracts method (Collins et al., 1995). Agar was cooled to 50-60°C before adding any thermo-labile substance (Sambrook and Russell, 2001). Suspension of the bacterial cultures were covered wholly on the agar plates and allowed to dry. Then, in the nutrient agar, 30 wells (6 mm in diameter) were made on each plate using sterile yellow tip. Following this, 50 µl of the test solution, that is, the supernatant of rose extract and pollen were added inside the laminar flow cabinet for 15-20 min to allow the solutions in the wells to diffuse. The agar plates were then inverted and incubated for 24 h at 37°C. After incubation, clear areas in the region of the wells containing antibacterial compounds appeared. This diameter of the clear area (called the inhibition zones) around the wells were measured and recorded. Antibacterial activities of each solvent extract were expressed in terms of average diameter of the inhibition zone (evaluated in milliliter). Each rose extract was tested in the same manner. The concentration and solvents that give the optimum result were identified.

The inhibition zone values of the rose petal and pollen extracts was compared with known standard antibacterial agent oxytetracycline. An accurately weighed 10 mg of oxytetracycline base was put in 96% ethanol in 100 ml standard volumetric flask and the flask was swirled to dissolve oxytetracycline base. Accurately measured portion was diluted with sterilized distilled water to get a known concentration of 0.01 mg of oxytetracycline per millilitre. The values obtained for the five tested microorganisms with oxytetracycline at 1.0 µl/ml are *B. subtilis* (ATCC 6633) 3.5 mm/ml; *E. coli* (ATCC 25922) 6 mm/ml; *P. aeruginosa* (ATCC 27853) 2.9 mm/ml; *M. luteus* (ATCC 9341) 4 mm/ml and *C. albicans* (ATCC 14053) 6.5 mm/ml.

RESULTS

The antimicrobial activity of rose petal extracts of *R. damascena* with the six solvents against different types of microbial strains showed diverse inhibition zones. When compared with all the solvent extractions, the *zamzam*

Table 1. Antimicrobial activities (inhibition zones mm/ml) of Taif rose petals extracted with 6 different solvents against 5 microorganisms.

Solvents of extraction	Bacteria	Rose petals weights in milligrams/ml (50 - 400) Inhibition zones (mm)							
		50	100	150	200	250	300	350	400
zamzam	*Bacillus subtilis*	6±1	6±1	8±1	6±1	-	-	10±2	10±2
Distilled water		6±1	8±1	-	-	-	4±2	-	6±1
Ethyl alcohol		4±2	4±2	4±2	8±1	4±2	4±2	-	-
Acetone		-	-	-	-	-	-	-	12±1
Tris-HCl		-	-	4±2	12±1	-	-	6±1	-
zamzam	*Escherichia coli*	4±2	-	-	-	-	4±2	-	4±2
Distilled water		-	4±2	4±2	6±1	-	-	4±2	4±2
Ethyl alcohol		-	-	-	8±1	6±1	10±2	10±2	10±2
Acetone		-	-	-	-	-	4±2	-	-
Tris-HCl		4±2	4±2	-	4±2	4±2	-	4±2	6±1
zamzam	*Candida albicans*	-	4±2	-	-	-	-	-	4±2
Distilled water		-	-	-	10±2	-	-	12±1	-
Ethyl alcohol		8±1	-	-	8±1	4±2	4±2	8±1	6±1
Isoamyl alcohol		-	-	-	4±2	4±2	4±2	-	-
Acetone		4±2	6±1	6±1	-	10±2	12±1	10±2	12±1
Tris-HCl		-	-	-	-	4±2	-	-	-
zamzam	*Micrococcus leutus*	4±2	-	-	-	6±1	-	-	4±2
Distilled water		4±2	4±2	6±1	4±2	4±2	4±2	4±2	4±2
Ethyl alcohol		4±2	4±2	4±2	4±2	4±2	8±1	6±1	4±2
Tris-HCl		6±1	4±2		6±1	-	10±2	6±1	-
zamzam	*Psedomonas aeroginosa*	4±2	6±1	4±2	4±2	-	-	4±2	-
Distilled water		-	-	4±2	6±1	4±2	4±2	4±2	4±2
Ethyl alcohol		8±1	6±1	10±2	12±1	4±2	6±1	6±1	6±1
Acetone		14±2	10±2	14±2	10±2	12±1	14±2	14±2	8±1
Tris-HCl		10±2	-	-	4±2	-	-	-	-

water extracts of rose's petals showed antibacterial activity against *B. subtilis (*ATCC6633) and water extracts of rose petals showed antibacterial activity in *C. albicans* (ATCC 10231), *M. luteus* (ATCC 9341) and *P. aeruginosa* (ATCC 27853). The ethyl alcohol extracts of rose petals showed highest antibacterial activity against *P. aeruginosa* (ATCC 27853) and moderate antibacterial activity against *E. coli* (ATCC8739).

The most effective inhibition value was measured as 13 mm as diameter for *P. aeruginosa* (ATCC 27853) against ethyl alcohol extracts of rose petals. The acetone extracts of rose petals showed clearing zone having a diameter of 6 to 16 mm against *C. albicans* (ATCC 10231) and *P. aeruginosa* (ATCC 27853). The isoamyl alcohol extracts of rose petals showed very little antibacterial activity against *C. albicans (*ATCC 10231). The Tris-HCl at pH 8.9 (100 mM) extracts of rose petals showed less inhibition zone (6 mm) in four microorganisms except, *C. albicans* (ATCC 10231) (Table 1).

Comparing the concentration of the extracts with different solvents in which the inhibition values differ against the tested microorganisms, a concentration of 350 and 400 mg/ml z*amzam* water and 200 and 350 mg/ml water extracts of rose's petals showed anti-bacterial activity against *B. subtilis (*ATCC 6633), 150 and 200 mg/ml ethyl alcohol extracts of rose petals showed highest antibacterial activity against *P. aeruginosa* (ATCC 27853) whereas 200 and 400 mg/ml ethyl alcohol extracts of rose petals showed moderate antibacterial activity against *E. coli* (ATCC8739) and *C. albicans* (ATCC 10231) (Table 1).

The antimicrobial activity of rose pollen extracts of *R. damascena* with the six solvents against different types of microbial strains also showed diverse inhibition zones. When compared with all the solvent extractions, the ethyl alcohol extracts showed highest antibacterial activity

Table 2. Antimicrobial activities (inhibition zones mm/ml) of Taif rose pollen grains extracted with 6 different solvents against 5 different microorganisms.

Pollen/solvent (mg/ml)	*Bacillus subtilis* (mm)	*Escherichia coli* (mm)	*Candida albicans* (mm)	*Micrococcus leutus* ((mm)	*Pseudomonas aeroginosa* (mm)
Pollen/zamzam	-	4±2	10±2	6±1	-
Pollen/distilled water	-	-	8±1	4±2	4±2
Pollen/ethyl alcohol	-	14±2	-	4±2	12±1
Pollen/isoamyl alcohol	-	4±2	4±2	-	-
Pollen/acetone	-	4±2	-	-	8±1
Pollen/Tris-HCl	-	4±2	-	4±2	4±2
Pollen/Tris-HCl	-	4±2	-	4±2	4±2
Oxytetracycline at 10 µl/ml	3.5±0.2	6±0.1	6.5±0.2	4±0.2	2.9±0.1

(among all microorganisms) in *E. coli* ATCC 25922 and *P. aeruginosa* (ATCC 27853). The *zamzam* water extracts of rose pollen extracts showed antibacterial activity against *C. albicans* (ATCC 10231) and *M. luteus (*ATCC 9341*)*. The acetone extracts of pollen showed lesser antibacterial activity in *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) as compared to ethyl alcohol extracts of rose pollen. Nil antibacterial activity was found in *B. subtilis* (ATCC 6633) irrespective of the solvents (Table 2).

The most effective inhibition value was measured as 14 mm as diameter for *E. coli* (ATCC 25922) and 13 mm as diameter for *P. aeruginosa* (ATCC 27853) against ethyl alcohol extracts of rose pollen. The *zamzam* extracts of rose pollen showed clearing zone having a diameter of 12 and 7 mm against *C. albicans* (ATCC 10231) and *M. luteus* (ATCC 9341) respectively. The acetone extracts of rose pollen showed inhibition value of 6 and 9 mm as diameter against *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853), respectively. The Tris-HCl at pH 8.9 extracts of pollen showed 6 mm, a less inhibition value against *E. coli* (ATCC 25922), *M. luteus (*ATCC 9341*)* and *P. aeruginosa* (ATCC 27853) (Table 2).

DISCUSSION

The results obtained by the standard agar well diffusion clearing zone method showed that concentrated ethyl alcohol and acetonic extracts had inhibitory effects (upto 16 mm) on most of the tested microorganisms as represented in Table 1. While the concentrated aqueous extract showed variable inhibitory effects on the tested microorganisms (Tables 1 and 2). The ethyl alcohol extracts of rose petals showed highest antibacterial activity against *P. aeruginosa* (ATCC 27853). The earlier antimicrobial study with native isolated bacteria from sea cucumber recorded moderate antimicrobial activity against *P. aeruginosa* as reported by Farouk et al. (2007). The bacteria isolated from flowers of *R. damascena* cv. Taifi also showed antibacterial activity and enzymatic activity was reported by Farouk et al. (2014). At a concentration of 150 and 200 mg/ml ethyl alcohol extracts of rose petals showed highest antibacterial activity against *P. aeruginosa* (ATCC 27853) and *E. coli* (ATCC 8739). This result complies with other reported research outcomes that the low dilution of alcoholic extract showed a higher antimicrobial activity as compared to high dilution (Hirulkar and Mona, 2010; Farouk and Benafri, 2007). Halawani (2014) also reported that the ethanolic extract of *R. damascena* showed a positive significant bacterial activity. *R. damascena* cv. Taifi should be investigated to better understand its antimicrobial properties, safety and efficiency. In the last two decades, various studies were conducted in many countries to verify such efficiency (Ikram and Inamul, 1984; Izzo et al., 1995; Kubo et al., 1995; Shapoval et al., 1994). Lesser research reports are available for the antimicrobial activity of *R. damascena* pollen. The ethanol extracts of pollen showed higher antimicrobial activity against *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) than the acetone extracts of pollen.

Conclusion and recommendations

However, in this study, the ethyl alcohol and acetone extracts of rose petals and pollen of *R. damascena cv. Taifi* had showed moderate result against *P. aeruginosa* and the acetone extract (200 mg/ml) of rose petals showed good antimicrobial activity against *C. albicans* and highest antimicrobial activity against *P. aeruginosa*. In addition, the ethyl alcohol extract (200 to 400 mg/ml) of rose petals showed good result against *E. coli.* The water extract of rose petals showed good result against *C. albicans*. These results are encouraging and promising and further study should be carried out to confirm the purification of the antimicrobial compounds. In this study, with different concentrations of rose petals and pollen extracts, it is shown that *R. damascena cv. Taifi* has medicinal values.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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