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Antibacterial and Antifungal Activity of *Rosa damascena* MILL. Essential Oil, Different Extracts of Rose Petals

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Abstract: *Rosa damascena* petals were extracted by water, hexane and ethanol. The latter was further fractionated with chloroform, ethyl acetate and butanol. Rose oil and different petal extracts were evaluated against three fungi and eleven Gram-positive, Gram-negative and acid-fast bacteria. Rose oil and all extracts exerted broad spectrum antimicrobial activities against the tested organisms. The descending order of antifungal activity of rose oil and different extracts was, *Penicillium notatum, Aspergillus niger* and *Candida albicans.* Ethyl acetate extracted fraction was relatively more active against the tested bacteria than the other tested extracts. Gram-positive bacteria, *Staphylococcus aureus, Bacillus subtilis* and *Streptococcus pyogenes* were more sensitive than Gram-negative bacteria and had MICs and MBCs in the range of 0.125 to 2 mg/ml and 0.5 to 4 mg/ml respectively. *Acinetobacter baumannii,* which is intrinsically resistant to most antibiotics, was relatively more sensitive than other Gram-negative bacteria. On the contrary, *Klebsiella pneumoniae* was the least sensitive Gram-negative bacterium. The MICs of Gram-positive bacteria to different extracts were significantly ($p \leq 0.05$) less than those of *K. pneumoniae.* The acid-fast bacterium, *Mycobacterium phlei*, was intermediate in its sensitivity to the extracted fractions compared to Gram-positive and Gram-negative bacteria. The antibacterial activity of aqueous extracts of petals suggests a possible utilization of rose petal boiling water after rose oil distillation. Further studies are required to separate and identify the active antimicrobial phytoconstituents of petals to utilize them pharmaceutically.

Key words: Antibacterial · Antifungal · Rosa damascena · Rose oil · Extracts

INTRODUCTION

Medicinal plants have been used for treatment of diseases since the early civilizations of the Middle East, India, China and the New World [1]. Although there is a domination of the synthetic chemistry as a method to discover new and novel products for disease prevention and treatment, eighty percent of the world's inhabitants still rely mainly on traditional folk medicine [2]. In recent years, there has been a revival in the use of traditional medicinal plants [3-5] and therefore, pharmaceutical companies are investigating a lot of money in developing natural products extracted from plants [6].

Members of the genus Rosa (Family Rosaceae) are considered as one of the world's most popular ornamental plants because of their beauty and fragrance [7]. Although, there are over 100 species of roses, *Rosa damascena* Mill. is considered one of the most important *Rosa* species for its beauty, flavor and fragrance industry. The name of *R. damascena* species is based on Damascus, Syria, where it originally existed as a wild plant. However, it is now cultivated in different countries around the world [7]. Although, Taif is known for the production of a high quality rose essential oil, from *R. damascena*, Bulgaria, Turkey, France and India are the largest producers of rose essential oil [8].

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In addition, to fragrance industry of *R. damascena* there has been an interest in the medicinal applications of roses. In ancient medicine, *R. damascena* was used for treatment of abdominal and chest pain, menstrual bleeding, digestive problems, depression, grief, nervous stress, tension, skin problems and headaches [9]. Some new medical applications for R.damascena have been reported. Flavonoids of petals were found to possess strong resistance to UV radiation (254 nm) and they potentially could be used in antisolar creams [10]. Flavonoids have also antioxidant activity [11] and were found to be protective to DNA from oxidative damage [12]. The essential oil of R. damascena was found to reduce systolic blood pressure and breathing rate [13] and was found to be cytotoxic against human prostate carcinoma cells (PC-3) [14]. In addition aqueous extracts of rose petals showed anticonvulsant effect and therefore could be used as an adjunctive therapy for pediatric refractory seizer [15].

One of the major problems that concerns public health, is bacterial resistance against antibiotics [16, 17]. Therefore, researchers have been screening natural sources for as yet undiscovered antimicrobial agents [18-20]. Extracts of *R. damascena* were evaluated qualitatively by disk diffusion method and were found to have antibacterial activity [6,14, 21-23]. In this study, we quantitatively evaluate the antimicrobial activity of *R. damascene* essential oil and petal extracts by different solvents against both bacteria and fungi.

MATERIALS AND METHODS

Collection and Preparation of Plant Extracts: Rose flowers were collected early morning from Shafaa, Taif.

Extraction with Ethanol: Rose petals were extracted with alcohol. The extract was dried under reduced pressure using a rotary vacuum evaporator at 60°C. The dried extract was then fractionated consequently with chloroform, ethyl acetate and butanol. All solvent fractions were dried under reduced pressure at 60°C

Extraction with Hexane: Rose petals were extracted with hexane. The hexane was evaporated under reduced pressure at 40°C to get the concrete.

Extraction with Water: Petals were boiled with water for 30 min and the water extract was dried under reduced pressure at 60°C.

Bacterial Strains and Growth Conditions: Bacteria used in this study were obtained from the microbial collection of the Department of Microbiology, College of Pharmacy, Taif University (Table 1). Bacteria were subcultered from stocks maintained in nutrient broth containing 20% glycerol at -80°C.

Determination of Antifungal Activity: This was done as previously described by Halawani and Shohayeb [24]. Wells of 5mm diameter were punched in Sabouraud's dextrose agar plates with sterile cork borer and filled with 50μl of 40 mg/ml plant extracts dissolved in dimethylsulfoxide (DMSO). The plates were incubated at 25°C for 48 hrs. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition of the respective plant extract.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): MIC and MBC were performed as previously described [25]. Briefly, the reconstituted extracts in DMSO were serially diluted two-folds in nutrient broth medium in microtiter plates. Each dilution was inoculated with 5 x 10^4 CFU/ml of the test bacterial strain. The plates were incubated at 37°C for 18 hours. MIC was taken as the highest dilution (Least concentration) of extract showing no detectable growth. Subinhibitory concentrations were sub-cultured onto nutrient agar plates to determine the minimum bactericidal concentrations.

Statistical Analysis: All determinations were carried out in triplicates and the statistical analyses were carried out using SPSS 13.0.

RESULTS

Antibacterial Activity of Rose Extracts Against Different Types of Pathogenic Bacteria: *R. damascena* petals were extracted with water, hexane and ethanol. The latter extract was further fractionated with solvents of different polarities (Choloroform, ethyl acetate and n-butanol). All extracts and rose oil were evaluated for their antimicrobial activities against seven Gram-negative bacteria, an acid fast bacterium (*M. phlei*) and three Gram-positive bacteria (Table 1).

The MICs of rose oil and different extracts against the tested bacteria ranged between 0.125 and 8 mg/ml. On the other hand the MBCs of the tested preparations ranged between 0.5 and >8 mg/ml (Tables 2 and 3). Usually the MBCs of a tested extracts were 2 to 4 the values of MICs (Tables 2 and 3).

Table 1: Microorganisma used in this study and their sources

Bacteria	*Characteristic*	*Source*
Bacillus subtilis	Standard 168 strain	Faculty of Pharmacy, Tanta University, Egypt
Staphylococcus aureus	Ap, Cf, Sm, Gn	Clinical isolate, Taif University, KSA
Streptococcus pyogenes	Clinical isolate	Clinical isolate
Mycobacterium phlei	Standard strain (ATCC 6841)	Tanta University, Egypt
Pseudomonas aeruginosa	PAO1 standard strain	Clinical isolate, Taif University, KSA
Escherichia coli	Ap, Tc, Cm, Sx, Sm	Clinical isolate, Tanta University, Egypt
Klebsiella pneumoniae	Ap, Pp, Cp, Tc, Cm, Sx	Clinical isolate, Tanta University, Egypt
Salmonella typhimurium	Ap, Tc, Sx, Sm	Clinical isolate, Taif University, KSA
Shigella flexneri	Ap, Tc, Sx, Sm	Clinical isolate, Tanta University, Egypt
Proteus vulgaris	Ap, Pp, Cp, Tc, Cm, Sx, Ctx	Clinical isolate, Taif University, KSA
Acinetobacter baumannii	Ap, Pp, Cp, Tc, Cm, Sx, Ctx, Cip	Clinical isolate, Taif University, KSA
Aspergillus niger	Standard strain (ATCC-13794)	Faculty of Pharmacy, Tanta University, Egypt
Penicillium chrysogenum	Standard strain (ATCC-18226)	Faculty of Pharmacy, Tanta University, Egypt
Saccharomyces cerevisiae	Standard strain (ATCC-9080)	Faculty of Pharmacy, Tanta University, Egypt

* Clinical isolate resistance to: Ap, ampicillin; Cf, cephalexin; Cp, cephoperazone; Pp, piperacillin; Tc, tetracycline; Sm, streptomycin; Cm, chloramphenicol; Sx, sulfamethoxazole; Gn, gentamicin; Ctx, Cefotaxime; Cip; ciprofloxacin.

Table 2: Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of rose oil and different extracts of petals against Gram-positive, acid-fast and two none-*Enterobacteriaceae* Gram-negative bacteria

	Streptococcus pyogenes		*Bacillus subtilis*		*Staphylococcus aureus*		*Mycobacterium phlei*		*Pseudomonas aeruginosa*		*Acinetobacter baumannii*	
Extract	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
						mg/ml						
Rose oil	0.25	0.5	0.25	2	0.25	4	2	4	4	8	2	4
Aqueous extract	2	4	0.25	4	0.25	4	2	4	8	8	4	4
Concrete	4	4	1	4	2	4	4	4	4	>8	2	4
Ethanol extract	0.125	0.5	0.5	1	0.5	2	2	2	4	8	2	2
Chloroform fraction	1	2	0.125	2	1	2	1	2	4	>8	4	8
Ethyl acetate fraction	0.125	0.5	0.125	0.5	0.125	0.5	0.25	0.5	2	4	2	4
Butanol fraction	2	4	0.25	2	0.5	4	2	4	4	>8	2	4
Residue fraction	2	4	0.25	2	0.25	4	2	4	4	>8	2	4

Table 3: Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of rose oil and extracts of petals against five Gram-negative *Enterobacteriaceae* bacteria

	Proteus vulgaris		*Escherichia coli*		*Salmonella typhimurium*		*Shigella flexneri*		*Klebsiella pneumoniae*	
Extract	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Rose oil	4	8	4	>8	4	>8	4	>8	4	>8
Aqueous extract	4	8	8	8	4	4	4	8	8	>8
Concrete	4	8	4	>8	4	>8	4	>8	4	>8
Ethanol extract	4	4	4	4	2	4	4	8	4	8
Chloroform fraction	2	4	4	8	2	4	4	8	4	>8
Ethyl acetate fraction	4	>8	4	>8	4	>8	4	>8	4	>8
Butanol fraction	4	8	4	8	4	>8	4	>8	8	>8
Residue fraction	8	>8	4	>8	4	>8	4	>8	8	>8

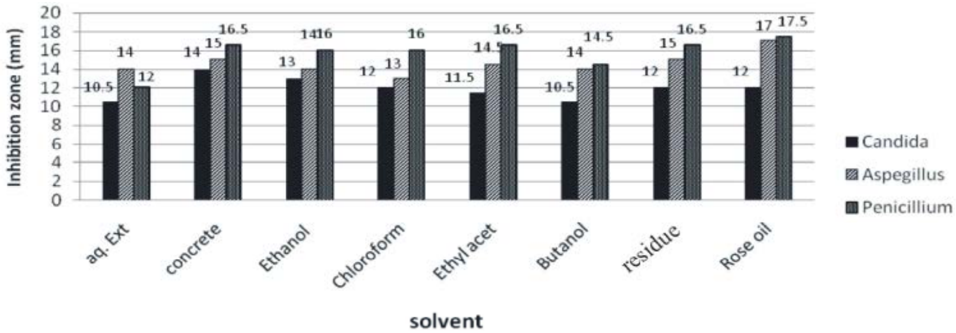

Fig. 1: Inhibition zones (mm) produced by rose oil and extracts of petals against *Aspergillus niger*, *Penicillium chrysogenum* and *Saccharomyces cerevisiae*.

Gram-positive bacteria were more sensitive than Gram-negative bacteria. While, Gram-positive bacteria were inhibited by 0.125- 2 mg/ml of extracts and killed by 0.5-4 mg/ml, Gram-negative, bacteria except for *A. baumannii*, were inhibited by concentrations ranging between 2 to 8 mg/ml and killed by 4 to >8 mg/ml extract.

The most sensitive Gram positive bacterium was *B. subtilis*. It was inhibited by a concentration ranging between 0.125 and 2mg/ml and killed by a concentration ranging between 0.5 and 4 mg/ml (Table 3).

While, *A. baumannii*, was the most sensitive Gram-negative bacterium, *K. pneumoniae* was the least sensitive one. *A. baumannii* was inhibited by 1 to 4 mg/ml and killed by 2 to 8mg/ml (Table 2). On the other hand, MICs and MBCs of *K. pneumoniae* ranged between 4 to 8 mg/ml and 8 to>8 mg/ml, respectively (Table 2). *M. phlei* was, generally speaking, more sensitive than Gram-negative bacteria and less sensitive than Gram-positive bacteria (Tables 2 and 3).

With regard to the antifungal activity of the tested rose oil and petal extracts, three microorganisms were tested; two molds (*P. notatum* and *A. niger*) and a yeast (*C. albicans*). The inhibition zones ranged between 10.5 to 17.5 mm. inhibition zones of *C. albicans* ranged between 10.5 and 14mm, while those of the *P. notatum* and *A. niger* ranged between, 12 to 17.5 and 11 to 17 respectively. *P. notatum* was the most sensitive and *C. albicans* was the least sensitive fungus (Fig. 1).

DISCUSSION

In this study *R. damascena* essential oil and different extracts of petals were evaluated for their antimicrobial activities against three Gram-positive bacteria, seven Gram-negative bacteria, one acid-fast bacterium and three fungi. Rose oil and all tested rose fractions exerted broad spectrum antibacterial activity against all tested bacteria and fungi.

C. albicans was the least sensitive and *P. notatum* was the most sensitive fungus. On the other hand, the aqueous extract was the least active and rose oil was the most active antifungal extract of petals. The concrete and other fractionated extracts were more or less similar in their activity against the tested fungi.

Generally speaking, ethyl acetate extracted fraction were relatively more active as antibacterial than the other tested fractions of petals. Gram-positive bacteria, *S. aureus*, *B. subtilis* and *St. pyogenes* were more sensitive than Gram-positive bacteria and had MICs and MBCs of 0.125-2 mg/ml and 0.5-4 respectively.

While, *A. baumannii*, was the most sensitive Gram-negative bacterium, *K. pneumoniae* was the least sensitive one. The MICs of both *B. subtilis* and *S. aureus* for different fractions were in most cases significantly ($p \geq 0.05$) less than those of *K. pneumoniae.*

M. phlei, on the other hand, was intermediate in its sensitivity to the extracted fractions compared to Gram-positive and Gram-negative bacteria. The higher susceptibility of the tested Gram-positive bacteria than Gram-negative bacteria to rose petal extracts is consistent with previous studies on the antibacterial activity of natural products [25-29]. Possibly this may be attributed to the fact that Gram-negative bacteria possess an outer membrane which acts as a barrier which prevents or decreases the penetration of numerous antimicrobials [30-32]. Lack of the outer membrane in Gram-positive bacteria, makes it more vulnerable to damaging molecules and this leads to the leakage of their cytoplasm contents [33].

The higher susceptibility of *A. baumannii* and *M. phlei* to essential oil and different extracts of petals *R. damascene* compared to the other tested Gram-negative bacteria is rather interesting. Both organisms are known to be less susceptible to antimicrobials [34-36]. Resistance of *A. baumannii* is attributed to the less permeable outer membrane and the ability to acquire resistance genes [35, 36]. On the hand, *M. phlei* is characterized by a highly hydrophobic cell wall with a mycolyl-arabinogalactan-peptidoglycan skeleton that leads to its impermeability to antimicrobials [36].

On the other hand, *K. pneumonia* was the least sensitive tested Gram-negative bacterium. This bacterium was also reported previously to have a lower susceptibility to the antibacterial activity of extracts of other plants like, *Nigella sativa* extracts and *Conocarpus erectus* extracts [29, 38]. The lower susceptibility of *K. pneumonia* may be attributed to the surrounding polysaccharides capsule [38], which might decreases the uptake of antimicrobials [39].

Although, the seven clinical Gram-positive and Gram-negative bacterial isolates included in this study were multidrug-resistant, this did not affect their susceptibility to *R. damascena* oil and petal extracts. The phenomenon of irrelevance of resistance to chemotherapeutic agents and the antibacterial activity of natural products has been previously reported [29, 37, 40]. This is presumably because the mechanisms of action of the phytoch-constituents of *R. damascene* are different to those of antibiotics.

It may be concluded that the essential oil and different extracts of *R. damascena* investigated in this study possess moderate broad-spectrum antimicrobial activity against Gram-positive, Gram-negative, acid-fast bacteria and fungi. The broad-spectrum antibacterial activity of the petals of *R. damascena* extracts, confirms the use *R. damascena* in folklore medicine. This study suggests that *R. damascene* petal extracts could possibly be used locally for treatment of skin infections or as gargles for treatment throat infections.

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