academicJournals

Vol. 8(24), pp. 2359-2367, 11 June, 2014
DOI: 10.5897/AJMR2014.6829
Article Number: F45F80445336
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antimicrobial activity of *Rosa damascena* petals extracts and chemical composition by gas chromatography-mass spectrometry (GC/MS) analysis

Eman M. Halawani

Biology Department, Faculty of Science, Taif University, KSA.

Received 12 April, 2014; Accepted 26 May, 2014

Antimicrobial activity of alcoholic and aqueous extracts from *Rosa damascena* was evaluated against 10 pathogenic microorganisms. Minimum inhibition concentration (MIC), Minimum bactericidal concentration (MBC) and the diameter of inhibition zone (DIZ) were determined by *in vitro* bioassays using hole-plate diffusion method and broth micro-dilution method (BMD) against *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus, Pseudomonas aeruginosa* ATCC 27853, *Psuedomonas aeruginosa*, *Escherichia coli* ATCC 25922, *E. coli*, *Streptococcus pneumoniae* ATCC 55143, *Acinetobacter calcaoceuticus, Salmonella enteritidis* and *Aspergillus niger* ATCC 16404. While hexane extracts showed very low activity against the test microorganisms, ethanol, methanol and water extracts significantly exhibited antimicrobial activity and inhibited the growth of Gram-positive and Gram-negative bacteria as well as *A. niger* at all tested concentrations. The most active antimicrobial effect was recorded for ethanol extract of *R. damascena* against *P. aeruginosa* ATCC 27853 at MIC and MBC of 62.5 µg/ml (DIZ = 34 mm), *E. coli* ATCC25922 at MIC and MBC of 62.5 µg/ml (DIZ = 30 mm). MIC and MBC data obtained from the antimicrobial studies were analyzed for significant difference at $p<0.05$ using one way analysis of variance (ANOVA). The extracted oil of Damascus rose petals were characterized by GC/MS; analysis reported that 30 compounds were present. The predominant components were citronellol (14.8- 29.0%), geraniol (11.3-16.2%) and nerol (11.6%) while the phenyl ethyl alcohol was 1.2%. This study sheds the light on the efficacy of plant extracts to combat pathogens which will help as natural antimicrobial agents.

Key words: *Rosa damascena*, antimacrobial activity, gas chromatography-mass spectrometry (GC/MS), Rose oil extracts.

INTRODUCTION

Infectious disease account for approximately one half of all deaths in tropical countries (Iwu et al., 1999) and they are considered a major threat to human health because of the unavailability of vaccines or limited chemotherapy. Ranked 5th in 1981, infectious diseases continue to be a growing public health concern (Kone et al., 2006) and

E-mail: Halawani.em@hotmai.com. Tel: +966505708387. Fax 00966127433699.

they became the 3rd leading cause of death in 1992, with an increase of 58% (Pinner et al., 1996). Most of the current antibiotics have considerable limitations in terms of antimicrobial spectrum, side effects and their widespread overuse has led to increasing clinical resistance of previously sensitive microorganisms and to the occurrence of uncommon infections (Cos et al., 2006). Plants used for traditional medicines contain a wide range of substances that can be used to treat chronic as well as acute infectious diseases (Diallo et al., 1999; Ofokansi et al., 2011), and have therefore become sources of important drugs and the pharmaceutical industries have come to consider traditional medicine as a source of bioactive agents that can be used in the preparation of synthetic medicines.

Rosa damascena mill L., commonly known as Damask rose (Kaul et al., 2000), 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 (Cai et al., 2005; Nikbakht et al., 2005). At present time, over 200 rose species and more than 18000 cultivars form of the plant has been identified (Gudin, 2000). There are evidences that Rosaceae family is an ancient plant (Nikbakht and Kafi, 2008; Yassa et al., 2009). Some fossils of rose are found in America that is 30 million years old (Yassa et al., 2009). The origin of Damask rose is the Middle East, but the origin of its fragrant oil and extracts is Greece (Arezoomandan et al., 2011). The highest quality rose water is produced in Taif Governorate, KSA. Kaaba (God House) in Mecca, Saudi Arabia, is washed yearly by unique and special rose water of Taif. Rose water is also of high value in the food industry and some special foods are prepared using this product (Nikbakht and Kafi, 2008). The *R. damascena* has also been used for medicinal purposes (Hongratanaworakit, 2009). Various products and isolated constituents from flowers, petals and hips (seed-pot) have been studied in a variety of *in vivo* and *in vitro* studies. It has been shown that *R. damascena* has wide spectrum antimicrobial activities. Essential oil, absolute and hydrosol are important products that showed these effects. Ulusoy et al. (2009) and Boskabady et al. (2011) showed that essential oil and absolute have strong antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *B. subtilis*, *Staphylococcus aureus*, *Chromobacterium violaceum* and *Erwinia carotovora* strains.

However, there is no detailed study concerning the antimicrobial activity of *R. damascena* performed so far. Most of the studies related to the damask rose have focused on the growing techniques, harvest time, and physical and chemical properties of oil. Therefore, the purpose of this study was to determine the *in vitro* antimicrobial activity against gram negative and gram positive bacteria as well as the chemical compositions of *R. damascena* volatile oil by using GC/MS to determine the major constituents of high quality rose oil.

MATERIALS AND METHODS

Preparation of extracts

The present study was conducted to determine the chemical composition (constituents) and antimicrobial activities of *R. Damascena* flower extracts. The flowers were collected in April, 2013 during the rose harvest season, from different local farms (AL-Hada and Al-Shefa), Taif governorate- Saudi Arabia. The green parts of roses were removed and the remains were cut into small pieces. Following the collection, part of the flowers was immediately frozen in closed containers at -80°C for further analysis. The rest of the flowers, 0.5 kg of fresh flowers from each accession were used immediately for alcoholic and water extraction. Fresh and dried parts of *R. damascena* (petals, flowers and seed pods) were either powdered or soaked freshly in five volumes of 50% ethanol, 80% methanol, and 100% hexane by stirring overnight, filtered through Whatman No. 1 filter paper after 72 hand centrifuged at room temperature at 5000 x g. The supernatants were evaporated to dryness at 45ºC under reduced pressure. Powdered *R. damascena* (500 g each) were extracted twice overnight with 2500 ml of distilled water at room temperature. The supernatant were collected and evaporated to dryness at 45ºC under reduced pressure. The yield of each extract was adjusted to be less than 10% (20 g of each alcoholic extract was dissolved in 100 ml distilled water containing 30% DMSO to get concentration 500 µg/ml) and stored in dark sterile bottles and became ready until use. The reconstituted alcoholic extracts were sterilized by filtering through 0.45 µm membrane filter and tested for sterility after membrane filtration by introducing 2 ml of the extract into 10 ml of sterile nutrient broth and incubated at 37°C for 24 h. A sterile extract was indicated by the absence of turbidity in the broth after the incubation period (Ronald, 1995).

Test microorganisms

A total of 10 microbial cultures belonging to nine bacteria and one fungus species, were used in this study. These microorganisms included *E. coli* (clinical isolet ,ur), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *P. aeruginosa* (clinical isolet, ETT), *S. aureus* (ATCC 25923), *S. aureus* (clinical isolet, Nasal), *S. pneumoniae* (ATCC55143), *S. enteritidis* (ATCC 13076), *A. calcaoceuticus* (clinical isolet, ur) and *A. niger* (ATCC 16404). These microorganisms were obtained from the Microbiology Unit, Al-Edwani Hospital, Taif Governorate, KSA. The organisms were maintained on nutrient broth, nutrient agar (Biolab, Hungary), potato dextrose agar and potato dextrose broth. The antibacterial assays were carried out using Mueller Hinton II Agar (Oxoid, England) and broth. The antifungal assays were carried out using, potato dextrose broth and potato dextrose agar.

Antimicrobial activity

All plant extracts were dissolved in sterile distilled water containing 30% DMSO to a final concentration of 500 µg/ml and sterilized by filtration by 0.45 µm Millipore filters. Antimicrobial tests were carried out in this report by agar well diffusion assays according to the NCCLS citeria. The antibacterial activity was carried out by employing 24 h cultures of *E. coli* (ur), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *P. aeruginosa* (ETT), *S. aureus* (ATCC 25923), *S. aureus* (Nasal), *S. pneumoniae* (ATCC55143), *S. enteritidis* (ATCC 13076), *A. calcaoceuticus* (ur) and *A. niger* (ATCC 16404). Activity of aqueous and alcoholic extracts of *R. damascena* (RD) was tested separately using Agar well diffusion method (Perez et al., 1990; Murray et al., 1995; Olurinola, 1996; Srinivasan et al., 2001; Dulger and Gonuz, 2004). The medium

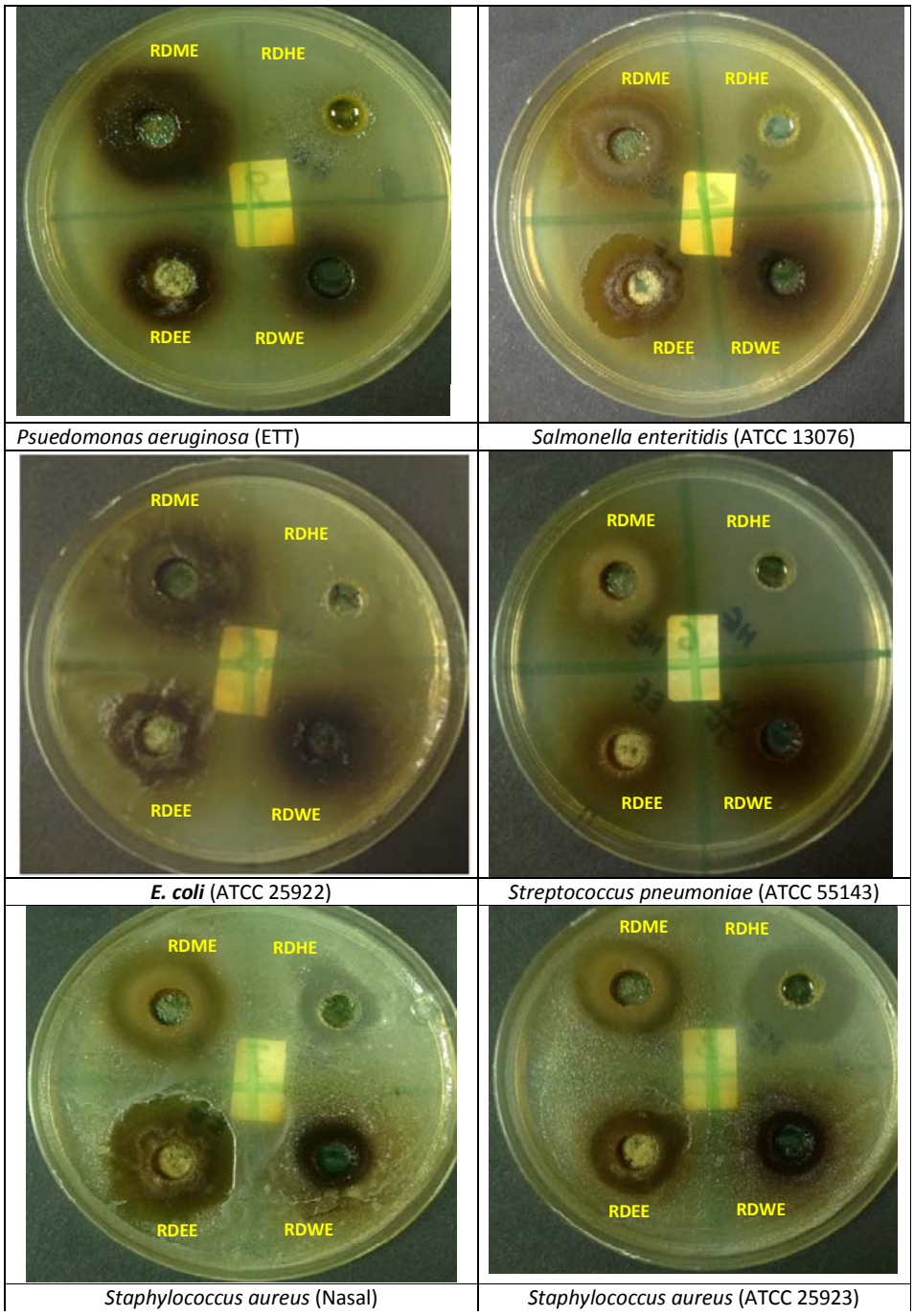

Figure 1. Agar well diffusion assay showing the antibacterial activity of different extracts of RD. Each well containing 100 µg of the each extract.

was sterilized by autoclaving at 120°C (15 lb/in^2). About 30 ml of the agar medium with the respective strains of bacteria was transferred aseptically into each sterilized Petri plate. The plates were left at room temperature for solidification. A well of 6 mm diameter was made using a sterile cork borer. Antibacterial assay plates were incubated at 37±2°C for 24 h. The diameter of the zone of inhibition was measured. Sterile ultrapure water and DMSO were used as negative control. Samples were tested in triplicate and results are expressed as mean ± standard deviation.

Determination of antifungal activity

The agar well diffusion method (Anesini and Perez, 1993) was modified. Potato dextrose agar (PDA) was used for fungal cultures (Figure 1). The culture medium was inoculated with the fungal strain *A. niger* ATCC 16404 separately suspended in potato dextrose broth. A total of 6 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (ethanol, methanol and hexane). Standard antibiotic (Nystatin, concentration 1 mg/ml)

was used as positive control and fungal plates were incubated at 28°C for 48 h. The diameters of zone of inhibition observed were measured. Antifungal activities were determined by measuring diameter of inhibition zone (DIZ) in mm. Each experiment was repeated thrice and the average values of antimicrobial activity were calculated (Shahidi, 2004).

Broth micro-dilution method (BMD)

MICs and MBCs of *R. damascena* extracts were determined by using BMD method as described by the NCCLS in flat-bottomed 96-well clear plastic tissue-cultured plates (NCCLS, 2003a). The MIC was assayed using two-fold BMD method in MH Broth in 96-well plates. Plates contained two fold dilutions of antimicrobial agents at the concentration ranges: 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 µg/mL. 1% DMSO was used as a solvent control. Plant extracts were diluted to twice the desired initial test concentration (1 mg) with Muller Hinton broth (MHB) (Oxoid, UK). All wells, except the first were filled with MHB (50 µL). Plant extract (100 µL) was added to the first well and serial two-fold dilutions were made down to the desired minimum concentration (32 µg). An over-night culture of bacteria suspended in MHB was adjusted to turbidity equal to 0.5 McFarland standards. The plates were inoculated with bacterial suspension (50 µL/ well) and incubated at 37°C for 24 h. Then the turbidity was observed visually by eyes. MICs and MBCs were determined for ethanol, methanol, hexane and aqueous extracts of *R. damascena*. MIC was determined as the lowest concentration of plant extract that inhibit the growth of each microorganism. MBC was determined as the lowest concentration of plant extract that prevent the growth of bacteria after sub-culturing on MH agar plates. To determine the MBCs, the suspensions (20 µl) were taken from each well without visible growth and inoculated in MH agar for 24 h at 37°C. To determine the Minimal Fungicidal Concentration (MFC), 100 µL of each dilution showing no growth was spread on PDA. The inoculated Petri dishes were incubated at 37°C for 48 h for fungal cultures. Tests were performed in triplicate for each test concentration.

Isolation of volatile oil

One hundred grams of dried flowers of each plant were ground in a blender and then subjected to hydrodistillation for 3 h according to the standard procedure described in the European Pharmacopoeia (2004). The oil was solubilized in *n*-hexane, dried over anhydrous sodium sulphate and stored at +4°C in the dark until tested and analyzed.

Gas chromatography-mass spectrometry (GC/MS) analyses

The essential oils were analyzed on a gas chromatograph (GC)-mass spectrometer (MS); Clarus 500 GC/MS (PerkinElmer, Shelton, CT) was used. The software controller/integrator was TurboMass, version 4.5.0.007 (PerkinElmer). An Elite-1 GC capillary column (30 m, 0.25-mmID, 0.25 DF, PerkinElmer) was used. The carrier gas was helium (purity 99.9999%) at a flow rate of 0.9 mL/min (initial 7.6 p.s.i. flow initial 36.2 cm/s, split; 50:1). Temperature conditions were: inlet line temperature, 270°C; source temperature, 210°C; trap emission, 100°C; and electron energy, 70 eV. The column temperature program was: 80°C hold for 5 min, increased to 150°C (rate, 5°C/min), and held for 5 min, increased to 270°C (rate, 20°C/min) and hold for 5 min. The injector temperature was 220°C. MS scan was from 45 to 350 *m/z*.

A sample volume of 50 µL was diluted with 500 µL of chloroform, vortexed for 2 s, and a volume of 1 µL was injected for GC-MS analysis. A volume of 50 µL from this diluted sample was transferred to total recovery vial 1-mL, mixed with 50 µL of MSTFA, capped, vortexed for 2 s, heated at 80°C for 30 min using heating block for half insertion of vial, cooled, and a volume of 1 µL was injected for GC-MS analysis.

The total ion chromatogram (TIC) was recorded from 45 - 350 *m/z*. The targeted peaks were extracted by the knowledge of major *m/z* fragments, averaged masses at peak top, and searched for matched compounds using mass spectrometry data bank NIST2008 database. The percentage composition of the essential oil was computed by the normalization method from the GC peak areas measurements.

Statistical analysis

Comparison of data was performed using the one way ANOVA and is presented as mean ± standard deviation. Comparison of MIC and MBC values, tests were made in triplicate for quantification. Values of $p<0.05$ were considered significant.

RESULTS

Antimicrobial activities of plant extracts

The results show that *Escherichia coli* ATCC 25922 was sensitive to all antibiotic disks tested but resistant only to AMC, *P. aeruginosa* ATCC 27853 was resistant to all antibiotic tested but sensitive only to CEZ. The rest of tested bacterial isolates were sensitive to all antibiotic disks tested. The antibacterial activity of aqueous and alcoholic extracts of flowers petals of *R. damascena* were observed using agar well diffusion method by measuring the diameter of the growth inhibition zone (Table 1).

The ethanolic extracts of *R. damascena* (RDEE) showed a positive significant antibacterial activity against all tested bacteria; diameter of zone of inhibition ranged between 15 mm for *A. niger* ATCC 16404 to 34 mm for *P. aeruginosa* (ATCC 27853). Methanolic extracts of *R. damascena* (RDME) showed moderate degree of activity against most bacterial organisms tested (diameter of zone of inhibition ranged) between 13 mm for *A. niger* ATCC 16404 to 25 mm for *Psuedomonas aeruginosa* (ATCC 27853) and *Salmonella enteritidis* (ATCC 13076), while the hexane extracts (RDHE) showed very less effect against tested bacteria. The ethanolic extracts of RDEE showed a highly significant antibacterial activity against *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922).

Hexane extracts of *R. damascena* (RDHE) showed the lowest degree of inhibition against most of the tested bacteria; diameter of zone of inhibition ranged between 9 mm for *A.niger* ATCC 16404 to 13 mm for *Psuedomonas aeruginosa* (ATCC 27853). The antifungal activity was determined by measuring the diameter of zone of inhibition recorded. RDEE, RDWE and RDME were found to have maximum antibacterial and antifungal activity in comparison to hexane extracts. RDEE possessed potent antifungal activity against *A. niger* showing diameter of zone of inhibition ~ 15 mm while methanolic extracts and Water extracts showed diameter

Table 1. MICs, MBCs and MFCs in µg/mL of RD extracts against nine bacteria and one fungal isolate.

Name of the organism	Test sample	DIZ (mm)	MIC	MBC/MFC	±SD
Acinetobacter calcaoceuticus (Ur)	RDEE	27	125.0	250.0	±62.5
	RDME	25	125.0	250.0	
	RDHE	15	250.0	250.0	
	RDWE	24	125.0	125.0	
Escherichia coli (Ur)	RDEE	25	62.50	125.0	± 193.4
	RDME	20	62.50	62.50	
	RDHE	10	500.0	500.0	
	RDWE	25	125.0	250.0	
Psuedomonas aeruginosa (ATCC 27853)	RDEE	**34**	62.50	62.50	±193.4
	RDME	25	125.0	125.0	
	RDHE	13	250.0	500.0	
	RDWE	20	250.0	250.0	
Salmonella enteritidis (ATCC 13076)	RDEE	25	62.50	125.0	±157.2
	RDME	25	250.0	250.0	
	RDHE	10	500.0	500.0	
	RDWE	25	250.0	250.0	
Escherichia coli (ATCC 25922)	RDEE	**30**	62.50	62.50	±235.9
	RDME	20	125.0	125.0	
	RDHE	10	250.0	500.0	
	RDWE	10	500.0	500.0	
Streptococcus pneumonia (ATCC 55143)	RDEE	27	62.50	125.0	± 176.7
	RDME	22	250.0	250.0	
	RDHE	10	250.0	500.0	
	RDWE	25	125.0	125.0	
Staphylococcus aureus (Nasal)	RDEE	**30**	62.50	62.50	±207.2
	RDME	20	125.0	250.0	
	RDHE	11	250.0	500.0	
	RDWE	28	62.50	62.50	
Staphylococcus aureus (ATCC 25923)	RDEE	28	62.50	62.50	±207.2
	RDME	20	62.50	125.0	
	RDHE	11	250.0	500.0	
	RDWE	20	125.0	250.0	
Psuedomonas aeruginosa (ETT)	RDEE	25	125.0	250.0	± 193.4
	RDME	20	125.0	125.0	
	RDHE	10	250.0	500.0	
	RDWE	20	125.0	250.0	
Aspergillus niger (ATCC 16404)	RDEE	15	125.0	250.0	±125.0
	RDME	13	250.0	250.0	
	RDHE	9	250.0	500.0	
	RDWE	13	250.0	250.0	

RDEE, *Rosa damascena* ethanol extract; RDHE, *Rosa damascena* hexane extract; RDME, *Rosa damascena* methanol extract; RDWE, *Rosa damascena* water extract; DIZ, diameter of inhibition zone; MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration; MBC, minimum bactericidal concentration; ±SD, standard deviation.

of zone of inhibition ~13 mm. Hexane extracts *R. damascena* showed very low antifungal activity against *Aspergillus niger* ATTC 16404 (diameter of zone of inhibition of 9 mm).

Water extracts of *R. damascena* showed also a highly significant antibacterial activity against tested organisms. The diameters of zone of inhibition ranged from to 13 to 28 mm.

Table 2. Compounds determined in the oil of *Rosa damascena* extracted by hydro-distillation.

Compound identified	Retention Time (min)	Content Average (%)
β-Linalool	5.65	0.012
***Phenyl ethyl Alcohol**	6.82	**1.276**
2.2-Phenylethyl trimethylsilyl ether	8.52	0.275
Benzoic acid trimethsilyl ester	8.87	0.139
***Citronellyl formate**	9.58	**1.421**
2,2,4,7,7-pentamethyl -3,6-Dioxa-2,7-disilaoctane	10.01	0.1943
2,2,11,11- tetramethyl-3,6,10 - Trioxa-2,11-disiladodecane	10.07	0.01
Trimethylsilane (Decyloxy)	10.21	0.335
Bis(trimethylsilyl)-1,2-Butanediol	10.27	0.622
***β-Citronellol**	10.74	**29.013**
***β-Citronellol,trimethylsilyl ether**	10.97	**14.83**
***Nerol, trimethyl ether**	11.4	**11.66**
***Geraniol**	11.49	**11.395**
***Geraniol, trimethyl silyl ether**	12.1	**16.271**
[2-methoxy-4-1 (1-propenyl)phenoxy]trimethyl- Silane	14.33	0.381
Geranyl propionate	17.08	0.24
Eugenol methyl ether	17.33	0.86
Germacrene D	17.46	0.636
Geranyl isobutyrate	18.09	0.054
σCadinene	18.46	0.036
Nerolidol	18.62	0.233
Geranyl isobutyrate	19.14	0.27
Oxalic acid,decyl 2-phenyl ester	19.45	0.497
Elemol	20.14	0.071
trans, trans-Farnesol, trimethylsilyl ether	20.37	0.375
1.γ-Eudesmol	20.7	0.123
4βH,5α-Eremophil-1(10)-ene, 11-(trimethylsiloxy)	25.24	0.023
1.Methyl pimar-7-en-18-oate	29.77	0.642
Methyl abietate	30.04	0.081

*Dominant compounds are indicated in bold

The results of minimum inhibitory concentrations (MICs) revealed that the *R. damascena* alcoholic extracts exhibited the best anti-bacterial activities towards Gram-negative and Gram-positive bacteria with MIC and MBC values between 62.5 to 250 µg/ml (Table 1).

The minimum fungicidal concentration (MFC) of *R. damascena* (RD) extracts in different solvents were evaluated against *A. niger* ATCC 16404. Ethanolic, methanolic and water extracts were found to have maximum antifungal activity in comparison to hexane extracts. Ethanolic extracts of RD (RDEE) showed maximum potency against *Aspergillus niger* ATCC 16404 at highest MFC value of 62.5 µg/ml while the MFC value of ethanolic and methanolic extracts were 125 µg/ml respectively. Thus, the MBC and MFC of *R. damascena* (RD) extracts showed a bactericidal and fungicidal behavior of these extracts. The anti-bacterial activity of *R. damascena* alcoholic and water extracts were higher than that of hexane extract.

In the current study, 0.2 ml of rose oil was obtained from 250 g of dried Taif rose petals processed through steam distillation and the oil obtained was observed to be pale yellow in color, and the yield of essential oil was 0.15% (v/w). GC/MS analysis reported that 30 compounds (Table 2) were present in the Taif rose essential oil of which β-Citronellol (29.013%) was recorded as the major component in rose oil followed by Geraniol, trimethyl silyl ether (16.271), β-Citronellol,trimethylsilyl ether (14.83%), Nerol, trimethyl ether (11.66%), Geraniol (11.395%), Citronellyl formate (1.42%) and Phenyl ethyl Alcohol (1.276%) (Figure 2).

DISCUSSION

Generally, the degree of the plant extract activity is revealed by the size of inhibition zone that is expressed by the diameter of the referred inhibition zone. Due to the

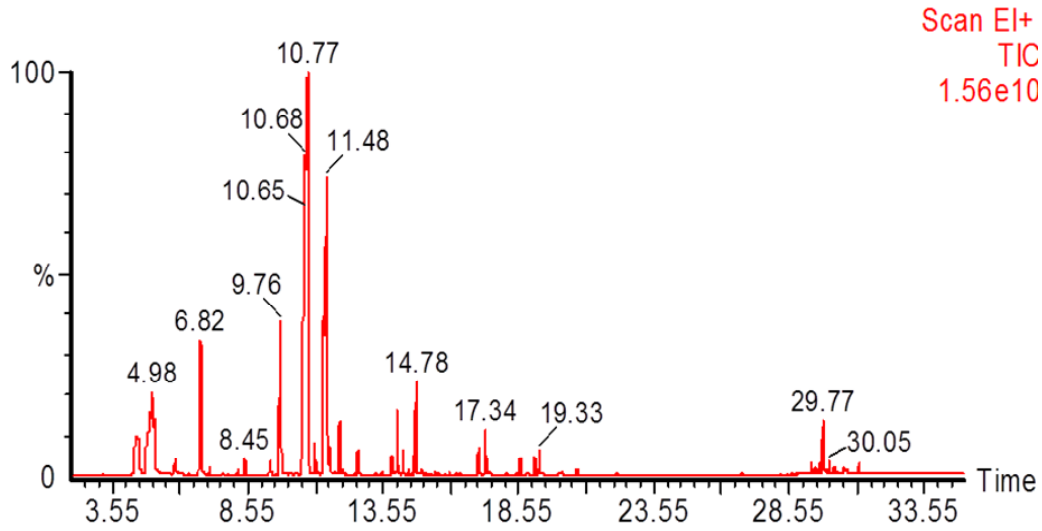

Figure 2. GC/MS chromatogram of *Rosa damascena* essential oil collected from Taif province.

simple nature of this assay, this technique is less suitable for more precise quantification purposes. MIC and MBC values were calculated to determine the susceptibility of a range of microbial species to a particular extract (Faleiro, 2011). The Gram-negative *Pseudomonas aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were the most sensitive strains (MIC of 62.5 µg/ml) for RDEE. Similarly, *Staphylococcus aureus* (ATCC 25923) was the most sensitive strain to RDME (MIC of 62.5 µg/ml). These results agreed with that obtained by Talib and Mahasneh (2010) who reported that butanol extract of *R. damascena* showed the highest activity (100% inhibition) against *S. typhimurium* and *B. cereus* (MIC 0f 62.5 and 250 µg/ml) respectively, while the aqueous extract of *R. damascena* was also active against *C. albicans* (MIC of 125 µg/ml). Also, in a study reported by Ulusoy et al. (2009) in Turkey, it was found that Rose absolute and essential oil had a strong antibacterial activity against *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *B. subtilis* (ATCC 6633), and *S. aureus* (ATCC 6538). Shohayeb et al. (2014) recorded a similar results that the essential oil and different extracts of Taify *R. damascen* possess brood spectram antimicrobial activity against gram positive and gram negative bacteria , acid fast bacteria and fungi with MIC and MBC range from 0.125 to 2 and 0.4 to 0.5 mg/ml, respectively .

The molecular mechanism of action of the *R. damascene* extracts on Gram-negative bacteria is unknown, but the ethanol extracts can probably inhibit the generation of adenosine triphosphate from dextrose and disrupt the cell membrane (Gill and Holley, 2006).

The high amount of hydrocarbons and the concurrent presence of monoterpenes (β-Linalool) could also contribute to inhibit the microbial DNA gyrase as reported by Cushnie and Lamb (2005).

It is well known that *R. damascene* components show also better antimicrobial effectiveness against Gram-positive bacteria. Due to the composition of outer membrane, plant extracts can alter not only such structures but penetrate within the cell, leading to those alterations, such as the denaturation of proteins and enzymes, the "unbalance" of the K+ and H+ ion concentration, until the modification of the entire cell morphology, which can lead to the death of the microorganism (Marrufo et al., 2013).

Phenolic compounds show generally a good antimicrobial effectiveness against Gram-positive bacteria; their effect is dependent on their amount: at low concentrations they are able to interfere with enzymes involved in the production of energy and at higher concentrations, they can induce the denaturation of proteins (Tiwari et al., 2009) until an irreversible modification of the cell and death.

The yield of essential oil was 0.15% (v/w) which agrees with the results of Moeina et al. (2010) who has obtained 0.16% (v/w). Our results of GC/MS analysis reported that 30 compounds were present in the Taif rose essential oil of which β-citronellol (29.013%) was recorded as the major component in rose oil followed by geraniol, trimethyl silyl ether (16.271), β-citronellol, trimethylsilyl ether (14.83%), nerol, trimethyl ether (11.66%), geraniol (11.395%), citronellyl formate (1.42%) and phenyl ethyl alcohol (1.276%).

These results agree with that obtained by Verma et al. (2011) who found that Citronellol (15.9-33.3%), Geraniol (8.3-32.2%), Nerol (4.0-9.6%), nonadecane (5.5-16.0% and heneicosane (2-.6-7.9%) were reported as the major components in rose oil. Also, Ulusoly et al. (2009) reported that Citronellol and Geraniol were the major compounds of Turkish *R. damascena* essential oils. The results of GC/MS of Iranian *R. damascena* reported by Yassa et al. (2009)

showed some similarity to our results in which Linalool (3.68%), Nerol (3.05%), Geraniol (15.5%), nonadecane (18.56%), tricosane (16.68%), and n-hexatriacontane (24.6%) were the major components of *R. damascena.* Babu and Kaul (2005) reported that Bulgarian rose oil composed of Citronellol (30.31%), Geraniol (16.96%), Phenyl ethyl alcohol (12.60%), Nerol (8.46%), hexacosane (3.70%), nonadecane (2.7%), Linalool (2.15%), Ionone (1.00%), ecosane (1.65%), docacosane (1.27%), farnesol (1.36%), neryal acetate (1.41%), citronellyl propionate (1.38%), geraniol (1.35%), pinene (0.60%), myrceen (0.46%), cis rose oxide (0.55%), decanal (0.51%), terpine-4-ol (0.55%), caryophyllene+citronellyl act (0.81%), isoborneol (0.57%), and heptadecane (0.92%).

The comparison of the results with the literature showed significant differences for oils, which can be attributed to ecological factors, genetic differences or the development stages of the plant parts analyzed.

Conclusion

Data obtained in this work could be useful in determining the major constituents of the flower essential oil of *R. damascene* which indicate its high quality. The results of antimicrobial activities of it can contribute to confirm the popular uses of these plants in Saudi Arabia in folk practices and to suggest new practical uses as natural aromatic mouth wash and wound disinfectant.

Conflict of Interests

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

ACKNOWLEDGEMENTS

This research was financially supported by Taif University, Grant 1/434/2154. The author is grateful to Dr. Hyam Abdulgader for her nice corporation.

REFERENCES

Anesini C, Perez C (1993). Screening of plant use in Argentine folk medicine for antimicrobial activity. J. Ethnopharmacol. 39:119-128.

Arezoomandan R, Kazerani HR, Behnam-Rasooli M (2011). The Laxative and Prokinetic Effects of *Rosa damascena* mill in rats. Iran J. Basic Med. Sci. 14(1):9-16.

Babu KG, Kaul KV (2005). Variation in essential oil composition of rose-scented geranium (Pelargonium sp.) distilled by different distillation techniques. Flavour Fragr. J. 20:222-231.

Boskabady MH, Vatanprast A, Parsee H, Ghasemzadeh M (2011). Effect of aqueous-ethanolic extract from *Rosa damascena* on guinea pig isolated heart. Iran J. Basic Med. Sci. 14:116-121.

Cai YZ, Xing J, Sun M, Zhan ZQ, Corke H (2005). Phenolic antioxidants (hydrolyzable tannins, flavonols, and anthocyanins) identified by LC-ESI-MS and MALDI-QIT-TOF MS from *Rosa chinensis* flowers. J. Agric. Food Chem. 53:9940-9946.

Cos PM, Sindambiwe LW, Vlietink AJ, Berghe DV (2006). Bioassays for antimicrobial and antifungal activities. In: Biological screening of plant constituents. Edited by Mahabir P, Gupta S, Swami H, Karan V. Trieste. International Centre for Science and High Technology. 19-28.

Cushnie TP, Lamb AJ (2005). Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents 5:343-356.

Diallo D, Hveen B, Mahmoud MA, Betge G, Paulson BS (1999). An ethnobotanical survey of herbal drugs of Gourma district Mali. Pharm. Biol. 37:80-91.

Dulger B, Gonuz A (2004). Antimicrobial activity of certain plant used in Turkish Traditional Medicine. Asian J. Plant Sci. 3 (1): 104-107.

European Pharmacopoeia (2004). 5th ed.; Council of Europe: Strasbourg Cedex, France. I:217-218.

Faleiro ML (2011). The mode of antibacterial action of essential oils. Science against microbial pathogens: communicating current research and technological advances. pp. 1143-1156.

Gill AO, Holley RA (2006). Inhibition of membrane bound ATPases of *Escherichia coli* and Listeria monocytogenes by plant oil aromatics. Int. J. Food Microbiol. 111:361-379.

Gudin S (2000). Rose: genetics and breeding. Plant Breed. Rev. 17:159-189.

Hongratanaworakit T (2009). Relaxing effect of rose oil on humans. Nat. Prod. Commun. 4: 291-296.

Iwu MM, Duncan AR, Okunji CO (1999). New antimicrobials of plant origin: Perspectives on new crops and new uses. Alexandra, ASHS Press.

Kaul VK, Singh V, Singh B (2000). Damask rose and marigold: prospective industrial crops. J. Med. Aromat. Plant Sci. 22: 313-318.

Kone WM, Atindehou KK, Kacou-N'douba A, Dosso M (2006). Evaluation of 17 medicinal plants from Northern Cote d'Ivoire for their in vitro activity against Streptococcus pneumoniae. Afr. J. Tradit Complement. Altern. Med. 4:17-22.

Marrufo T, Nazzaro F, Mancini E, Fratianni F, Coppola R, De Martino L, Bela Agostinho A, De Feo V (2013). Chemical Composition and Biological Activity of the Essential Oil from Leaves of *Moringa oleifera* Lam. Cultivated in Mozambique. Molecules 18:10989-11000.

Moeina M, Karamib F, Tavallalib H, Ghasemia Y (2010). Composition of the Essential Oil of Rosa damascene Mill. From South of Iran. Iran. J. Phama. Sci. Winter 6(1):59-62.

Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken HR (1995). Manual of Clinical Microbiology, 6th Edition. ASM Press, Washington. DC. 15-18.

Nikbakht A, Kafi M (2008). A study on the relationships between Iranian people and Damask Rose (Rosa damascena) and its therapeutic and healing properties. Acta. Hortic. (ISHS). 790:251-254

Nikbakht A, Kafi M, Mirmasoudi M, Babalar M (2005). Micropropagation of Damask rose (*Rosa damascena* Mill.) cvs Azaran and Ghamsar. Int. J. Agric. Biol. 7(4):535-538.

Ofokansi KC, Eze AO, Uzor PF (2011). Evaluation of the antimicrobial activity of the aqueous and methanolic leaf extracts of *Mitacarpus villosus* with amoxicillin. Afr. J. Pharm. Res. Dev. 3(1):43-47.

Olurinola PF (1996). A Laboratory Manual of Pharmaceutical Microbiology. Idu, Abuja, Nigeria. 69-105.

Perez C, Pauli M, Bazerque P (1990). An antibacterial assay by agar well diffusion method. Acta Bio. Et Med. Exp. 15:113-115.

Pinner RW, Teutsch SM, Simonsen L, Klug LA, Graber JM, Clarke MJ, Berkelman RL (1996). Trends in infectious diseases mortality in the United States. JAMA 275:189-93.

Ronald MA (1995). Micro-organisms in our World. Mosby Year Book, Inc. St. Louis. p. 765.

Shahidi Bonjar GH (2004). Evaluation of Antibacterial properties of Iranian Medicinal plants against Micrococcus aureus, Serratia marcescens, Klebsiella pneunomiae and Bordella bronchoseptica. Asian J. Sci. 3(1):82-86.

Shohayeb M, Abdel-Hammed E, Bazaid S, Magrabi I (2014). Antibacterial and antifungal activity of Rosa damascene MILL. Essential oil, different extracts of Rosa petals. Glob. J. Pharmacol. 8(1):01-07.

Srinivasan D, Nathan S, Suresh T, Perumalsamy LP (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J. Ethnopharmacol. 74:217-220.

Talib WH, Mahasneh AM (2010). Antimicrobial, Cytotoxicity and

Phytochemical Screening of Jordanian Plants Used in Traditional Medicine. Molecules 15: 1811-1824.
Tiwari BK, Valdramidis VP, O'Donnel CP, Muthukumarappan K, Bourke P, Cullen PJ (2009). Application of natural antimicrobials for food preservation. J. Agric. Food Chem. 57:5987-6000.
Ulusoy S, Boşgelmez-Tinaz G, Seçilmiş-Canbay H (2009). Tocopherol, carotene, phenolic contents and antibacterial properties of rose essential oil, hydrosol and absolute. Curr. Microbiol. 59:554-558.
Verma RS, Padalia RC, Chauhan A (2011). Chemical investigation of the volatile components of Shade-dried petals of damask rose (*Rosa damascena*). Arch. Biol. Sci. Belgrade 63 (4): 1111-1115.
Yassa N, Masoomi F, Rohani-Rankouhi SE, Hadjiakhoondi A (2009). Chemical composition and antioxidant activity of the extract and essential oil of Rosa damascena from Iran, Population of Guilan. DARU J. Pharm. Sci. 17:175-180.