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The Antimicrobial Potential of Royal Jelly against some Pathogenic Bacteria and Fungi

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Abstract

The antimicrobial activity of frozen and powdered Chinese royal jelly (CRJ) and fresh Jordanian (JRJ) against human pathogenic bacteria and fungi has been investigated in this study using the well diffusion method. The frozen aqueous extract of CRJ was shown to be the most effective preparation against *Salmonella typhimurium* (14028), but was significantly inactive against *Escherichia coli* (25922). Dry CRJ was significantly highly effective against *Enterobacter aerogenes* (35029) and *Proteus vulgaris* (33420), but was much less effective against *Klebsiella oxytoca* (13182). The aqueous extracts of Fresh JRJ significantly inhibited the growth of *E. coli* (0157), *E. coli* (25922), *Staphylococcus aureus* (25923), *K. oxytoca* (13182), *Klebsiella pneumoniae* (7700), and *E. aerogenes* (35029). However, the fresh JRJ was not detectably active against *Pseudomonas aeruginosa* (27253). The aqueous extracts of JRJ were more effective against *Candida albicans* (10231) compared with frozen and dry CRJs. In contrast, JRJ was less effective than CRJ against *Aspergillus brasiliensis* (16404). The ethanolic JRJ extract was less effective than the fresh and dry CRJs against *A. brasiliensis* and had an intermediate activity compared to the frozen and dry CRJ against *C. albicans*. The present study shows that the aqueous and ethanolic extracts of RJ obtained from two different geographical sources possess significant antibacterial and antifungal activities.

Keywords: Antimicrobial activity, Fresh Jordanian Royal Jelly, Frozen Chinese Royal Jelly, Dried Chinese Royal Jelly

1. Introduction

Royal jelly (RJ), which is a white milky highly viscous substance secreted from the mandibular and hypopharyngeal glands of the worker honeybee *Apis mellifera* (Apidae), is important for the growth and development of honeybee queens (Budavari *et al*., 1996; Lombardi *et al*., 1998). The queen's larva floats in RJ placed in a frame composed of beeswax (Shakhoon), frames always filled with an excessive amount of RJ to feed the queen larvae (Leung *et al*., 1968; Gojmerac, 1993). Honeybee queen is genetically similar to other female bees in the hive, but phenotypically different due to differentiation caused by the queen's RJ regime (Crane, 1999; Brevets, 2009).

Royal jelly (RJ) is acidic (pH 3.1–3.9), with a high buffering capacity ranging between pH 4 and pH 7 (Sauerwald *et al*., 1998). Generally, fresh RJ contains 60 % – 70 % water, 9 % – 18 % protein, 7 %–18 % sugar, and 3 % – 8 % lipids, vitamins, free amino acids, and oligosaccharides (Sabatini *et al*., 2009). Moreover, it contains Mn, P, Fe, S, Ca, Mg, Na, Zn, Cu and K as well as trace elements with biological functions, such as Sn, Ba, Sr, Bi, Cr, Cd, Pb, Tl, Mo, W, Hg, Sb, Ni, Ti, V, Co, Al and Te (Stocker *et al*., 2005). RJ also contains B complex vitamins such as B1, B2, B6 (Moreschi, and Almeida-Muradian, 2009) and biotin (Nandhasri *et al*., 1990). Moreover, RJ possesses the following activities: antibacterial (Eshraghi, and Seifollahi, 2003), immune regulatory (Okamoto *et al*., 2003), anti-lipid peroxidation (Guo *et al*., 2005), and antitumor (Nakaya *et al*., 2007). RJ can also be useful to improve metabolism in human beings (Guo *et al*., 2007). The major fatty acid present in RJ is 10-hydroxy-2-decenoic acid (10-HDA), which plays an important role in increasing the activity of the immune system and possesses anticancer and antibacterial activities (Eshraghi, and Seifollahi, 2003; Popescu, and Marghitas, 2007). The beneficial effects of RJ cannot be attributed to an individual component (Bonvehi, 1991; Budavari *et al*., 1996). RJ likely maintains homeostasis in humans, because of the similar balance between the concentrations of its components and those in the body (Iannuzzi, 1990; Budavari *et al*., 1996; Lombardi *et al*., 1998; Parfitt, 1999).

There has been a fast increase in the number of antibiotic-resistant pathogenic bacteria (Nugent *et al*., 2010; WHO, 2012), which hindered efforts to maintain pathogen-free curative abilities and staff. This increased the severity of bacterial illnesses and nosocomial infections. The primary causes of increasing antibiotic resistance could be attributed to the misuse of antibiotics (Nugent *et al*., 2010; Food and Drug Administration, 2011). More attention should, therefore, be placed on exploring other options other than the common antibiotics, such as using RJ, honey (Noori *et al*., 2013), and propolis to prevent a further build-up in antibiotic resistance.

Numerous studies show the wide range of important medical applications of RJ. For example, these studies address the ability of RJ to inhibit microbial growth

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(Weston, and Brocklebank, 2000; Lees, 2002), suppress allergic reactions (Leung *et al*., 1997; Lombardi *et al*., 1998; Oka, 2001), lower blood cholesterol (Shen *et al*., 1995; Vittek, 1995), and prevent cell damage in patients with cancer or those infected with human immunodeficiency virus (Manfredi, and Chiodo,2000; Takahashi *et al*., 2001). Furthermore, RJ plays a role in wound- healing and the acceleration of cell growth (Fujii *et al*., 1990). RJ is active against gram-positive and gram-negative bacteria (Fujiwara *et al*., 1990; Fontana *et al*., 2004), and may serve to confer broad immunity upon the queen bee against pathogenic bacteria. The strength of the antibacterial properties of RJ may be related to the presence of 10-HAD in the ether soluble fraction (Kitahara *et al*., 1995; Genc, and Aslan, 1999).

Illnesses caused by antibiotic-resistant strains of bacteria have become unresponsive to normal treatments. This prevents the effective control of infectious diseases in addition to the increase in the the total costs of health care. Thus, it is necessary to find new treatments and increase funding for more suitable ones (WHO, 2012).

The present study is aimed at evaluating the antimicrobial activities of frozen and powdered CRJ compared with those of fresh JRJ to determine the potential suitability of RJ as an alternative or supplement to conventional antibiotics.

2. Material and Methods

Fresh Jordanian Royal Jelly (JRJ), frozen JRJ, and dried JRJ are bio-assayed against different bacteria and fungi strains. The JRJ samples were collected during May and June of 2014 from the Langstroth hives housing colonies of the most common honeybee race in Jordan, *Apis mellifera syriaca*, located at the As-Salt and Amman apiaries. The samples were collected using the artificial cups method of Grout (1992). Larvae, aged approximately twenty-four hours, were transferred into beeswax queen cups, and were placed in queenless rearing colonies. About ten honeybee colonies were used in each location (Amman and Al-Salt locations). The colonies were fed with sufficient amount of honey and pollen grains during the production process. A closed honeybee brood was added to colonies every three to five days of production to make sure that there was enough number of nurse bees to produce enough amount of good quality royal jelly. The produced RJ (subsequent batches) was collected after two days of grafting, and was added to glass vials and stored at–18°C**.** The frozen and dried Chinese RJ samples were purchased from local markets.

2.1. Bioassays

Standardized pure cultures of four gram-positive and seven gram-negative pathogenic bacterial strains and two pathogenic fungal strains (Microbiologics Inc., Minnesota, USA) were used in the bioassays (Table 1). The microorganisms were cultured in a nutrient broth, and were then kept in an incubator at 4°C. Each microorganism was cultured again in this manner for three successive days before performing the experiments.

Table 1. Human pathogenic bacteria and fungi used in the study

Microbe	Species	Gram stain
Pathogenic Bacteria	*Staphylococcus aureus* ATCC (25923)	+ve*
	Escherichia coli ATCC (0157)	−ve
	Escherichia coli ATCC (29522)	−ve
	Klebsiella oxytoca ATCC (13182)	+ve
	Klebsiella pneumoniae ATCC (7700)	−ve
	Pseudomonas aeruginosa ATCC (27253)	−ve
	Methicillin resistant *Staphylococcus aureus* ATCC (29974)	+ve
	Salmonella typhimurium ATCC (14028)	−ve
	Salmonella paratyphi ATCC (13076)	−ve
	Proteus vulgaris ATCC (33420)	+ve
	Enterobacter aerogenes ATCC (35029)	−ve
Pathogenic Fungi	*Candida albicans* ATCC (10231)	fungus
	Aspergillus brasiliensis ATCC(16404)	fungus

−ve: Nigative +ve: Positive,

2.2. Extraction of RJ

2.2.1. Aqueous Extract

The aqueous extracts of RJ were prepared as described by Suzuki (1990) with a slight modification according to Osman (2008), in which 10.0g of RJ was suspended and extracted with 10mL of distilled water with shaking at 300rpm. The extract was stored after preparation in a tightly closed dark bottle in an incubator at 4°C until analysis.

2.2.2. Ethanolic Extract

The ethanolic extracts were prepared as described by Valdes Gonzales *et al*., (1985), in which 10g of RJ was placed in 10 mL of undiluted ethanol to obtain a 30 % extract. Completely- closed dark bottles were used to store the extract in an incubator at 4°C.

2.3. Well Diffusion Assay

An eighteen-hour bacterial culture was diluted with sterile physiological saline solution 0.85 % (w/v) to prepare an inoculum of approximately 10^6 colony-forming units per ml (Wayne, 2002). 100 µL of the solution of bacteria culture was spread onto the surface of solid Brain Heart Infusion Agar (BHIA, Oxoid) plates and was incubated for twenty minutes at room temperature. Wells were created into each plate using pasture pipettes (5-mm diameter borosilicate glass, Fisher Scientific) and 35 µL of the RJ extract was added to each well. The plates were incubated at 37°C for twenty-four hours for twenty minutes at room temperature to allow the extract to diffuse through the agar. The inhibition zone was measured, and the assay was performed twice for each extract. Water and ethanol were used as an ineffective solvent to the fractions. Penicillin antibiotic was used as the positive control for tested bacterial strains, and Nalidixic acid and Nystatin were used as the positive control for the tested fungal strains. The Minimum Inhibitory Concentration of the royal jelly samples was determined for each pathogen.

2.4. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical comparisons of the results were performed

by analysis of variance using (SAS, 2012). The differences in mean values were determined using Fisher's Least Significant Differences (LSD) method at 5 %.

3. Results

The results showed that using the fresh aqueous extract of CRJ gave the highest activity against *Salmonella typhimurium* ATCC (14028) but it was less effective against *Escherichia coli* ATCC (0157) and *E. coli* ATCC (29522) (Table 2). The aqueous extract of dry CRJ highly suppressed the growth of *Enterobacter aerogenes* ATCC (35029), *Proteus vulgaris* ATCC (33420), and *S. typhimurium* ATCC (14028), but it was less active against *Klebsiella oxytoca* ATCC (13182), *Staphylococcus aureus* ATCC (25923), *E. coli* ATCC (0157), and methicillin-resistant *S. aureus* ATCC (29974). The aqueous extract of fresh JRJ resulted in significant inhibition of the growth of *E. coli* ATCC (29522). Moreover, when a comparison was done among the three tested RJ on the same strain of bacteria, it was found that the fresh JRJ was highly effective against *S. aureus* ATCC (25923), *E. coli* ATCC (0157), *E. coli* ATCC (29522), *K. oxycota* ATCC (13182), and *Klebsiella pneumonia*e ATCC (7700), but was less effective against *S. paratyphi* (13076) (Table 2). In contrast, the aqueous extract of dried CRJ gave a highly significant activity against *K. pneumoniae* ATCC (7700), *Pseudomonas aeruginosa* ATCC (27253), methicillin-resistant *S. aureus* ATCC (29974), *S. paratyphi* (13076), *P. vulgaris* ATCC (33420), and *E. aerogenes* ATCC (35029). The results showed that the aqueous extract of fresh CRJ was highly active against *K. oxycota* ATCC (18182), and *S. typhimurium* ATCC (14028) compared with the other tested RJ.

The aqueous extract of JRJ was more effective against *Candida albicans* ATCC (10231) than the fresh and dry CRJ extracts. In contrast, the JRJ was less effective than both types of the C RJ extracts against *Aspergillus brasiliensis* ATCC (16404) (Table 3).

The ethanolic extract of the fresh CRJ was most effective against methicillin-resistant *S. aureus* ATCC (29974) and least effective against *E. coli* ATCC (0157), *E. coli* ATCC (29522), and *S. paratyphi* ATCC (13076) (Table 4). Dry CRJ was effective against *K. oxytoca* ATCC (18182), *K. pneumoniae* ATCC (13883), *P. aeruginosa* ATCC (27253), *S. typhimurium* ATCC (14028), and *S. paratyphi* ATCC (13076). The fresh JRJ was effective against *E. coli* ATCC (0157) and *E. coli* ATCC (29522) and was ineffective against *K. pneumoniae* ATCC (13883), *P. aeruginosa* ATCC (27253), and methicillin-resistant *S. aureus* ATCC (29974) (Table 4).

The ethanolic extract of JRJ was superior to the CRJs against three bacterial species, and was most effective against *E. coli* ATCC (29522) (Table 4), but it was less effective than the fresh and dry CRJs against *A. brasiliensis* ATCC (16404); its activity was in between of fresh and dry CRJ against *C. albicans* ATCC (10231) (Table 5). Its worthy to note that *A. brasiliensis* ATCC (16404) was resistant to both of the tested antibiotics (Nalidixic acid and Nystatin) while *C. albicans* ATCC (10231) was resistant only to Nalidixic acid, but susceptible to Nystatin.

Weak negative relationships were obtained between the activities of the JRJ aqueous and ethanol extracts and the fresh CRJ (Table 6). Moreover, there was significant differences between the activities of the ethanol extract of the JRJ and the fresh CRJ. Moreover, there was no significant relationship or a negligible relationship between the JRJ and the Dry CRJ for both aqueous extracts and ethanolic extract (Table 6).

Table 2. The antibacterial activities of an aqueous extract of fresh (frozen) and dried Chinese RJ compared with those of an aqueous extract of local fresh Jordanian RJ

Bacteria	Inhibition zone in (cm) ± standard error			
	Fresh Chinese R.J. (aqueous extract)	dried Chinese R.J. (aqueous extract)	Fresh Jordanian R.J (aqueous extract)	Penicillin
Staphylococcus aureus ATCC(25923)	1.12 ±0.07 $^{b\,C}$	1.20 ±0.06 $^{b\,C}$	1.75 ±0.08 $^{a\,C}$	1.0 ± 0.07^{b}
Escherichia coli ATCC (0157)	1.07 ±0.05 $^{c\,C}$	1.38 ±0.20 $^{b\,C}$	2.03 ±0.06 $^{a\,B}$	0.5 ± 0.04^{d}
Escherichia coli ATCC (29522)	0.95 ±0.20 $^{c\,C}$	1.72 ±0.11 $^{b\,B}$	2.43 ±0.10 $^{a\,A}$	0.7 ± 0.04^{d}
Klebsiella oxytoca ATCC (13182)	1.25 ±0.27 $^{a\,C}$	0.15 ±0.06 $^{b\,D}$	1.43 ±0.06 $^{a\,C}$	0.0 ± 0.0^{b}
Klebsiella pneumoniae ATCC (7700)	1.13 ±0.07 $^{b\,C}$	1.68 ±0.16 $^{a\,B}$	1.58 ±0.12 $^{a\,C}$	0.0 ± 0.0^{c}
Pseudomonas aeruginosa ATCC (27253)	1.13 ±0.06 $^{b\,C}$	1.85 ±0.04 $^{a\,A}$	0.88 ±0.07 $^{b\,D}$	0.0 ± 0.0^{c}
Methicillin resistant *Staphylococcus aureus* ATCC (29974)	1.05 ±0.07 $^{b\,C}$	1.29 ±0.18 $^{a\,C}$	0.97 ±0.04 $^{b\,D}$	0.5 ± 0.03^{c}
Salmonella typhimurium ATCC (14028)	2.03 ±0.06 $^{a\,A}$	1.70 ±0.10 $^{b\,B}$	1.53 ±0.10 $^{b\,C}$	0.0 ± 0.0^{c}
Salmonella paratyphi ATCC (13076)	1.43 ±0.07 $^{b\,B}$	1.88 ±0.07 $^{a\,A}$	0.95 ±0.07 $^{c\,D}$	0.0 ± 0.0^{c}
Proteus vulgaris ATCC (33420)	1.52 ±0.08 $^{b\,B}$	2.05 ±0.04 $^{a\,A}$	1.45 ±0.13 $^{b\,C}$	0.3 ± 0.0^{c}
Enterobacter aerogenes ATCC (35029)	1.63 ±0.15 $^{b\,B}$	2.07 ±0.12 $^{a\,A}$	1.67 ±0.07 $^{b\,C}$	0.5 ± 0.05^{c}

* Values indicated by lowercase letters in the same row are significantly different (Fisher's LSD, $\alpha = 0.05$).

** Values with different uppercase letters in the same column are significantly different (Fisher's LSD, $\alpha = 0.05$).

Table 3. The antifungal activities of the aqueous extract of fresh (frozen) and dried Chinese royal jelly compared with that of local fresh Jordanian RJ

No.	Fungi	Inhibition zone in (cm) ± standard error		
		Fresh Chinese R.J. (aqueous extract)	Dried Chinese R.J. (aqueous extract)	Fresh Jordanian R.J (aqueous extract)
1	*Aspergillus brasiliensis* ATCC (10231)	1.77 ±0.05 $^{a\,A}$	1.66 ±0.08 $^{a\,A}$	1.05 ±0.07 $^{b\,B}$
2	*Candida albicans* ATCC (16404)	1.38 ±0.06 $^{b\,B}$	0.92 ±0.09 $^{c\,B}$	1.79 ±0.10 $^{a\,A}$

* Values indicated by lowercase letters in the same row are significantly different (Fisher's LSD, $\alpha = 0.05$).

** Values with different uppercase letters in the same column are significantly different (Fisher's LSD, $\alpha = 0.05$).

Table 4. The antibacterial activities of the ethanolic extract of fresh (frozen) and dried Chinese royal jelly compared with local fresh Jordanian RJ

No	Bacteria	Fresh Chinese R.J. (ethanol extract)	dried Chinese R.J. (ethanol extract)	Fresh Jordanian R.J (ethanol extract)	Penicillin
1	*Staphylococcus aureus* ATCC (25923)	1.47 ±0.07 $^{a\,C}$	1.45 ±0.13 $^{a\,C}$	1.38±0.13 $^{a\,D}$	0.1 ± 0.07 ^{b}
2	*Escherichia coli* ATCC (0157)	1.07 ±0.05 $^{c\,D}$	1.25 ±0.14 ^{bC}	1.37±0.14 $^{a\,D}$	0.5 ± 0.04 ^{d}
3	*Escherichia coli* ATCC (29522)	1.17 ±0.07 $^{c\,D}$	1.83 ±0.21 $^{b\,B}$	2.08±0.08 $^{a\,A}$	0.7 ± 0.04 ^{d}
4	*Klebsiella oxytoca* ATCC (13182)	1.73 ±0.08 $^{b\,B}$	1.90 ±0.06 $^{a\,B}$	1.68±0.11 $^{b\,C}$	0.0 ±0.0 ^{c}
5	*Klebsiella pneumoniae* ATCC (7700)	2.03 ±0.18 $^{b\,A}$	2.27 ±0.11 $^{a\,A}$	0.73±0.08 $^{c\,E}$	0.0 ±0.0 ^{d}
6	*Pseudomonas aeruginosa* ATCC (27253)	1.67 ±0.07 $^{b\,B}$	1.93 ±0.06 $^{a\,B}$	0.92±0.06 $^{c\,E}$	0.0 ±0.0 ^{d}
7	*Methicillin* resistant *Staphylococcus aureus* ATCC (29974)	2.15 ±0.09 $^{a\,A}$	1.88 ±0.08 $^{b\,B}$	1.12±0.05 $^{c\,E}$	0.5 ± 0.03 ^{d}
8	*Salmonella typhimurium* ATCC (14028)	1.88 ±0.05 $^{b\,A}$	2.13 ±0.10 $^{a\,A}$	1.72±0.07 ^{bC}	0.0 ±0.0 ^{c}
9	*Salmonella paratyphi* ATCC (13076)	1.63 ±0.09 $^{c\,B}$	2.25±0.09 $^{a\,A}$	1.87±0.08 $^{b\,B}$	0.0 ±0.0 ^{c}
10	*Proteus vulgaris* ATCC (33420)	1.93 ±0.06 $^{a\,A}$	1.90±0.06 $^{a\,B}$	1.42±0.11 $^{b\,D}$	0.3 ± 0.0 ^{c}
11	*Enterobacter aerogenes* ATCC (35029)	1.67 ±0.09 $^{b\,B}$	2.08±0.07 $^{a\,A}$	1.90±0.08 $^{a\,B}$	0.5 ± 0.05 ^{d}

* Values with different lowercase letters in the same row are significantly different ($P < 0.05$)

** Values with different uppercase letters in the same column are significantly different ($P < 0.05$)

Table 5. The antifungal activities of the ethanolic extract of fresh (frozen) and dried Chinese RJ compared with those of local fresh Jordanian RJ

No.	Fungi	Inhibition zone in (cm) ± standard error		
		Fresh Chinese R.J. (ethanol extract)	dried Chinese R.J. (ethanol extract)	Fresh Jordanian R.J (ethanol extract)
1	*Aspergillus brasiliensis* ATCC (10231)	1.87±0.08 $^{a\,A}$	1.47±0.03 $^{b\,A}$	1.08±0.08 $^{c\,B}$
2	*Candida albicans* ATCC (16404)	1.97±0.12 $^{a\,A}$	1.45±0.10 $^{b\,A}$	1.85±0.10 $^{a\,A}$

* Values with different lowercase letters in the same row are significantly different ($P < 0.05$)

** Values with different uppercase letters in the same column are significantly different ($P < 0.05$)

Table 6. Correlations among the antibacterial activities of aqueous and ethanolic extracts of Jordanian fresh RJ, Chinese fresh RJ, and Chinese dry RJ extracts

	Extraction	Correlation	Chinese fresh royal jelly	Chinese dry royal jelly
Jordanian fresh royal jelly	Aqueous	Pearson	−0.223	0.000
	Ethanol	Pearson	−0.305*	0.023

The 11 bacterial species tested are listed in Table 4. *Significant correlation, $\alpha = 0.05$.

4. Discussion

Jordan has a relatively slight rainy season extending from November to March with almost no rain for the remainder of the year. The mean daily temperatures in Amman, the capital city of Jordan, range from 10°C in January to 32°C in August; however, the great north-south Jordan Rift Valley is nearly always much warmer. The mean temperature for the summer months is 40°C. RJ is produced in the Jordan Valley region during the period from March to May, and during the period from May to July in mountainous regions. (the average temperature from March to July is 32ºC). Unsuitable weather conditions, in particular very high temperatures, affect the foraging activity of honeybees, which will be reflected negatively on the amount of pollen grains and nectar that are available and could be collected by bees. Beekeepers depend mainly on the pollen grains and nectars that are present in an area to produce high-quality royal jelly with good quantity. During the production of RJ, adding sugar syrup or supplements is not recommended. Jordan produces relatively little amounts of RJ, and this, in fact, is what makes it expensive. Beekeepers, therefore, import RJ from other countries, mainly China.

RJ kills numerous bacteria and other microbes and inhibits gram-positive and gram-negative bacteria (Fujiwara *et al*., 1990). RJ is the only natural source of

acetylcholine which possesses antibacterial and antimicrobial properties serving as a beneficial treatment for a wide range of health conditions (Colhoun, and Smith, 1960) The potency of the antibacterial components of RJ might be related to 10-HDA, which is the most important active ingredient in RJ. The HDA content can be considered an index for estimating the quality of RJ (Bărnuţiu *et al.*, 2011). Protein and peptides present in RJ can participate in the defense mechanism of honeybees against microbial pathogens through the direct inactivation of microorganisms, as well as through the induction of cytokines participating in the regulation of the transcription of genes encoding defensive proteins and peptides (Fujiwara *et al*., 1990; Bărnuţiu *et al.*, 2011).

Staphylococcus aureus and *E. coli* were bio-chosen as targets in the present study as they cause different infections to humans. *S. aureus* is a common agent in skin infections, food poisoning, and toxic shock syndrome [://www.nlm.nih.gov/medlineplus /staphylococcal infections.html]. *E. coli*, a resident of the body's natural intestinal flora, can cause infections and produce toxins, similar to *S. aureus*, it can cause food and water-borne poisoning [http://www.mayoclinic.com/health/e-coli/DS01007]. To treat such infections, RJ has the potential to prevent food and surface contamination, and it can also be used as a post-infection treatment. Verifying the antimicrobial spectrum of this natural product may lead to the use of RJ as an effective natural antibiotic, antiseptic, and disinfectant. Eshraghi, and Seifollahi, (2003) examined the effects of RJ on *E. coli* (ATCC 29532), *S. griseus* (ATCC 11746), *S. aureus* (ATCC 14776), and *Streptomyces* strains S.46,S.F8, and S.66, and found that the application of the ether soluble fraction of RJ was effective against these pathogens. These findings agree with the results obtained from the present study.

Diverse bacteria cause wound contamination and colonization as well as clinical infections. *E. coli, Streptococcus uberis, Enterococcus faecalis, K. pneumoniae, S. aureus, Micrococcus luteus, S. epidermidis*, and *P. aeruginosa* are repeatedly isolated from skin wounds of animals and humans. Methicillin-sensitive and -resistant *S. aureus* are the main strains concerned in the difficult-to-treat skin infections and underlying tissue infections associated with gram-positive bacteria (Halcón, and Milkus, 2004). *S. epidermidis* infections are commonly obtained in hospitals because of the contamination of medical incision sites with microorganisms from the hospital personnel or patients (Vuong, and Otto, 2002). Infection with *P. aeruginosa* is the most serious complication in burn patients (Nasser *et al*., 2003; Altoparlak *et al*., 2005), followed by infections with *K. pneumoniae, S. aureus, E. coli*, and other pathogenic microorganisms (Nasser *et al*., 2003). The results show that the infections with *K. pneumoniae* ATCC (7700), Methicillin resistant *S. aureus* ATCC (29974), *S. typhimurium* ATCC (14028) and *P. vulgaris* ATCC (33420) could be treated efficiently with the ethanolic extract of fresh Chinese RJ. The ethanolic extract of fresh Jordanian RJ was highly active against *E. coli* ATCC (29522).

Monica (2014) found that the *S. epidermidisis* is the most bacterium affected by RJ, producing a 29.0-mm mean of inhibition area. This result is consistent with the data reported here, while JRJ was effective as an aqueous extract against *Staphylococcus*. Furthermore, Jordanian and Chinese RJ were active as antibiotics against *Staphylococcus* compared with the penicillin control. The frequent use of Penicillin as antibiotic may cause the bacteria strains to build up resistance against this medicinal product.

All tested RJ types were effective either against gram-positive and/or gram-negative bacteria strains, with no specific trend. Also, it was found that to treat infections caused by *A. brasiliensis* ATCC (16404), one can use the aqueous extraction of both fresh and dried CRJ. In contrast, to control *C. albicans* ATCC (10231), patients can use the aqueous extract of JRJ. Other studies evaluated the antibacterial properties of RJ. For example, RJ kills *Streptomyces griseus, S. aureus*, and *E. coli* (Eshraghi, and Seifollahi, 2003). Further, RJ has strong antibacterial activities at concentrations>200 µg/mL (Boukraâ *et al.*, 2009), and the RJ ointment has a better healing effect on mucositis in hamsters compared with propolis and honey ointments, contributing to the resolution of mucositis (Suemaru *et al.*, 2008). RJ and propolis contain antibacterial constituents, which could believably be prescribed to treat slight bacterial infections, and be adopted as standard first-line cures for mild illnesses (Bărnuţiu *et al*., 2011, Al-Abbadi *et al*., 2015).

In conclusion, the present study shows that the aqueous and ethanolic extracts of RJ, prepared from two different geographical sources, possess significant antibacterial and antifungal activities. The differences in their effectiveness may be attributed to the different chemical compositions of these RJs which come from different geographical areas, and which have passed through different manufacturing and storing processes.

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