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An Evaluation of the Antimicrobial Activity of *Commiphora myrrha* Nees (Engl.) Oleo-gum Resins from Saudi Arabia

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The present work was aimed to evaluate oleo-gum resins aqueous, methanol and chloroform extracts of *Commiphora myrrha* for antimicrobial activity against four types of bacteria and twelve species and strains of fungi. All of the oleo-gum resin extracts irrespective of their types inhibited the growth of all microbes to varying degrees. Aqueous extract showed the least antibacterial and antifungal activity against all of the pathogens used especially with regard to gram positive bacteria *Staphylococcus aureus* (11 mm) and *Escherichia coli* (11 mm) in concentration of 100 mg mL^{-1} as compared to methanol or chloroform. Less or no activity was observed against *Trichophyton concentricum* (7.01 mm) and 4.01 mm inhibition zone for *Candida rugosa* in the same concentration used. The oleo-gum resins methanol extracts in different concentrations were significantly inhibitory to the growth of the different tested fungal dermatophytes. Reduction in mycelial weight of fungi was directly correlated with concentration of extract. The concentration of 200 mg mL^{-1} of myrrha was the most inhibitory against *Epidermophyton floccosum* with 2 g mycelial fresh weight while the less inhibition in the growth was obtained in *Candida albicans* and it was 6.61 g in the same extract concentration. The other used fungal or bacterial groups showed varying degrees of activity pending on the type of extract used. The Minimum Inhibitory Concentration (MIC) values of methanol and aqueous extracts of oleo-gum resins of myrrh showed that the highest values were obtained in methanol extract for *Trichophyton concentricum* and lowest MIC values for the same extract for the bacterium *Staphylococcus aureus*. In the aqueous extract of myrrha the highest and lowest MIC values were found for *Bacillus subtilis* and *Pseudomonas aeruginosa*. Phytochemical analyses showed the presence of Sesquiterpenes (Isoprenoids i.e., Terpenoids) and Furanosesquiterpenes as major constituents of the oleo-gum resins of the plant. These results confirm the antibacterial and antifungal activity of gum resins and support the traditional use of the myrrh in therapy of bacterial infections.

Key words: *Commiphora myrrha*, traditional medicine, antimicrobial activity, Saudi Arabia

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INTRODUCTION

The incidence of bacterial and fungal infections has markedly increased in recent years. Several factors have contributed to this increase. These include greater use of immunosuppressive drugs, prolonged use of broad-spectrum antibiotics, widespread use of indwelling catheters and the acquired immunodeficiency syndrome (AIDS). Drug resistance in bacteria is increasing and the pace at which new antibiotics are being produced is slowing. It is now almost common place to hear about methicillin resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant Enterococci (VRE), multi-drug resistance in *Mycobacterium tuberculosis* (MDRTB) strains and Multi-Drug-Resistant (MDR) ram negative bacteria. So-called new and emerging pathogens add to the gravity of the situation (Saadabi and Moglad, 2011).

In the past 60 years, antibiotics have been critical in the fight against infectious diseases caused by bacteria and fungi. Antimicrobial chemotherapy has been a leading cause for the dramatic rise of average life expectancy in the 20th century. However, disease causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem (Lotfy *et al*., 2006). Wound infections, gonorrhea, tuberculosis, pneumonia, septicemia and childhood ear infections are just a few of the diseases that have become hard to treat with antibiotics. One part of the problem is that bacteria and other microbes that cause infections are remarkably resilient and have developed several ways to resist antibiotics and other antimicrobial drugs. Another part of the problem is due to increasing use and misuse of existing antibiotics in human, veterinary medicine and in agriculture.

Microbial development of resistance as well as economic incentives has resulted in research and development in the search for new antibiotics in order to maintain a pool of effective drugs at all times (Groove and Randall, 1955). While the development of resistant strains is inevitable, the slack ways that we administer and use antibiotics has greatly exacerbated the process (Saadabi and Moglad, 2011).

Unfortunately, because of the inappropriate use of antibiotics in human and veterinary medicine, certain strains of bacteria and fungi developed the ability to produce substances which block the action of antibiotics or change their target or ability to penetrate cells (Newall *et al*., 1996). To substitute synthetic antibiotics, many of today's modern and effective drugs have their origin in traditional folk medicine (Saadabi and Moglad, 2011). Plants have been used to treat human, animals and plant diseases from time immemorial. Also herbal medicines have been known to man for centuries (Almagboul *et al*., 1988; Michie and Cooper, 1991; Newall *et al*., 1996; Azaizeh *et al*., 2003; Hawar, 2008; Saadabi and Moglad, 2011; Gadir and Ahmed, 2014). Therapeutic efficacy of many oleo-gum resins for many disorders has been described by practitioners of traditional medicine (Wanner *et al*., 2010; Shuaib *et al*., 2013; Singh *et al*., 2013). Myrrh *Commiphora myrrha* has been used for centuries as incense and for medicinal purposes (Claeson *et al*., 1991; Michie and Cooper, 1991; Al-Harbi *et al*., 1997; Al Faraj, 2005). Medicinally, it has been used as an astringent, antiseptic, antiparasitic, antitussive, emmenagogue and antispasmodic agent. Myrrh has also been used in a variety of infectious diseases, including leprosy and syphilis and to treat cancers (Al-Harbi *et al*., 1994). Myrrh is also a part of African, Middle Eastern and Chinese traditional medicine. The Arabic term "Murr" means "Bitter" and describes myrrh's taste and balsamic odor (Michie and Cooper, 1991; El Ashry *et al*., 2003).

In Saudi Arabia a few reports has been documented concerning antibacterial and antifungal activity of myrrh oleo-gum resins (Saadabi *et al*., 2006; Saadabi, 2006; Abdallah *et al*., 2009; Omer *et al*., 2011; Abdallah and Khalid, 2012; Adam and Selim, 2013). The paucity of pharmacological and chemical data of Saudi myrrh prompted an investigation into its antimicrobial activity. Therefore, the present study was aimed to finding out the antimicrobial activities of myrrh oleo-gum resins and their efficacy against different fungal and bacterial strains.

MATERIALS AND METHODS

Plant material and preparation of the extracts: The study and procedures of laboratory work were carried out in the College of Applied Medical Sciences, Shaqra University. Kingdom of Saudi Arabia between November and December, 2014. *Commiphora myrrha* oleo-gum resins fresh samples used in this study were obtained from Riyadh city. Samples were identified and authenticated by the Department of Botany, University of Khartoum, Sudan and a voucher specimens were deposited at the departmental Herbarium.

Ten gram of the coarsely powdered oleo-gum resins of *Commiphora myrrha* plant were successively soxhlet-extracted with $CHCl_3$ and MeOH for 24 h. The methanol and chloroform extracts were evaporated under vacuum and the residues were separately dissolved or suspended in the same extracting solvent (10 mL) and kept in refrigerator till use. In addition, water extract was prepared by adding distilled water to 10 g of coarsely powdered oleo-gum resins plant material in a conical flask and left to soak overnight. The residue was then filtered and the final volume was adjusted to 10 mL with distilled water and the solution used immediately (Saadabi, 2006).

Bacterial strains: Four strains of bacteria namely *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* were used. The bacteria were cultured on nutrient broth (Oxoid) at 37°C for 24 h.

Fungal strains: Six fungal strains were isolated from clinical cases obtained from the General Hospital of Shaqra city. These fungi were *Epidermophyton floccosum*, *Microsporum audouinii* , *Trichophyton rubrum* , *Trichophyton concentricum*, *Trichophyton tonsurans*. In other sets of experiments different strains of *Candida* spp. were performed viz. *Candida albicans*, *C. glabrata*, *C. rugosa*, *C. parapsilosis*, *C. tropicalis* and *C. dubliniensis*. Each organism was cultured on Sabouraud's Dextrose Agar (SDA) medium incubated at 25°C for 7 days, to obtain inoculums for testing.

Antibacterial bioassay: Each extract; water, methanol and chloroform was tested against the four types of bacteria using the cup-plate agar diffusion method (Groove and Randall, 1955) and the inhibition zones were measured.

Determination of antifungal bioassay: Sterile, filter paper discs of 6 mm diameter were impregnated with about 0.1 mL disc^{-1} of extract which have been dissolved in dimethyl sulphoxide (DMS) and placed in duplicates onto the Sabouraud's dextrose agar plates, seeded with 0.2 mL of fungal suspension. The plates were then incubated at 37°C for 10-14 days (Saadabi *et al*., 2012). The zone of inhibition around each disc was measured in mm.

Influence of oleo-gum resins methanol extract on the growth of different fungal dermatophytes: To determine the influence of myrrha oleo-gum resins methanol extract on the growth of six different fungal dermatophytes, aliquots of different dilutions of the extracts (100, 150 and 200 mg mL^{-1}) were separately added on to 175 mL Czapek-Dox liquid medium in conical flask. Flasks containing Czapek-Dox medium alone (200 mL) served as control. The same flasks were then inoculated with *Epidermophyton floccosum*, *Microsporum audouinii*, *Trichophyton rubrum*, *Trichophyton concentricum*, *Trichophyton tonsurans* and *Candida albicans* then incubated at 25°C. Each set of treatments was replicated 5 times. After 15 days the fungal mats were harvested through Whatman filter paper No. 2 (which removes mycelium), then gently pressed between the folds of blotting paper to remove the excess amount of water and weighed to determine the mycelial fresh weight.

Determination of Minimum Inhibitory Concentration (MIC): To determine MIC agar well diffusion method was adopted according to the method reported by Saadabi *et al.* (2012). In accordance with such method different concentrations of the *Commiphora myrrha* oleo-gum resins samples were prepared to obtain 100, 150 and 200 mg mL^{-1}. Three drops of overnight cultures of the test organisms i.e., *Epidermophyton floccosum*, *Microsporum audouinii*, *Trichophyton rubrum*, *Trichophyton concentricum*, *Trichophyton tonsurans*, *Candida albicans*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were inoculated into the dilutions and incubated at 25 and 37°C for 24 h for 4 days using sterile molten Mueller-Hinton agar for bacteria and Sabouraud's dextrose agar media for fungi.

Phytochemical screening: Phytochemical screening was carried out for Myrrh samples using the method adopted by Crombie *et al*. (1990).

RESULTS AND DISCUSSION

As a general rule, plant extracts are considered active against both bacteria and fungi when the zone of inhibition is greater than 6 mm or in the category of moderate growth inhibition or more (Groove and Randall, 1955; Saadabi *et al*., 2006). Extracts of different types obtained from *Commiphora myrrha*, oleo-gum resins drastically suppressed the growth of the tested organisms (Table 1-4). The aqueous extract was characterized by least activity when compared to that of methanol and chloroform extracts. Methanol extract is

Table 1: Antifungal Activity of *Commiphora* myrrha, oleo-gum resins against different fungal isolates

		Fungal isolates*					
Extract type	Concentration	*Cladosporium* sp.	*Trichophyton tonsurans*	*Trichophyton concentricum*	*Trichophyton rubrum*	*Microsporum audouinii*	*Epidermophyton floccosum*
Chloroform	100 mg mL^{-1}	11.15±0.11	8.31±0.45	7.10±0.15	9.00±0.41	12.23±0.12	10.17±0.87
	150 mg mL^{-1}	12.23±1.14	9.22±1.33	8.11±1.22	10.44±1.11	12.10±1.66	10.86±0.97
	200 mg mL^{-1}	20.14±0.22	15.32±0.13	13.14±1.66	17.25±0.14	23.33±0.96	18.17±0.76
Methanol	100 mg mL^{-1}	12.13±1.14	9.67±0.45	8.32±0.16	10.11±1.75	13.62±1.02	11.00±0.21
	150 mg mL^{-1}	14.18±0.55	10.27±0.25	9.00±1.81	11.14±1.17	13.85±0.16	12.49±0.55
	200 mg mL^{-1}	23.14±0.15	18.17±1.36	18.17±1.14	19.00±1.89	22.23±0.39	20.00±0.94
Aqueous	100 mg mL^{-1}	8.17±0.25	8.41±0.15	7.01±0.85	9.00±0.34	11.00±0.21	9.12±0.44
	150 mg mL^{-1}	10.30±1.35	9.18±1.44	8.85±0.18	10.95±0.88	12.14±0.18	10.11±0.39
	200 mg mL^{-1}	15.36±0.44	15.66±1.01	13.77±1.31	17.30±1.34	21.22±0.24	17.28±0.15
Nystatin	100 µg mL^{-1}	23.50±0.25	20.61±0.44	20.42±0.31	20.22±0.31	25.88±0.52	16.00±0.01
Amphotericin B	100 µg mL^{-1}	26.10±0.23	23.11±0.10	23.00±0.22	23.66±0.21	28.71±0.11	19.30±0.37

*Data is presented as Mean±SD of zone of inhibition, inhibition zones are the mean of three replicates

Table 2: Antifungal activity of *Commiphora myrrha*, oleo-gum resins against different *Candida* spp. fungal isolates

		Fungal isolates					
Extract type	Concentration	*Candida albicans*	*C. glabrata*	*C. rugosa*	*C. parapsilosis*	*C. tropicalis*	*C. dubliniensis*
Chloroform	100 mg mL^{-1}	8.11±0.01	5.11±0.35	2.22±0.13	6.10±0.23	9.99±0.55	7.18±0.44
	150 mg mL^{-1}	9.13±1.22	6.25±1.23	5.44±1.66	7.14±1.22	9.11±1.55	7.44±0.16
	200 mg mL^{-1}	17.31±0.12	12.42±0.63	10.12±1.22	14.35±0.34	17.13±0.16	12.27±0.22
Methanol	100 mg mL^{-1}	6.14±1.88	6.77±0.15	5.42±0.18	7.13±1.45	10.99±1.12	8.00±0.11
	150 mg mL^{-1}	11.23±0.33	7.77±0.15	6.00±1.51	8.18±1.18	10.77±0.06	9.19±0.25
	200 mg mL^{-1}	20.15±0.12	15.15±1.16	15.10±1.18	16.00±1.44	19.83±0.19	17.00±0.54
Aqueous	100 mg mL^{-1}	5.33±0.15	5.66±0.45	4.01±0.66	6.00±0.14	8.00±0.82	6.62±0.41
	150 mg mL^{-1}	7.22±1.45	6.44±1.34	5.15±0.14	7.81±0.66	9.04±0.19	7.11±0.19
	200 mg mL^{-1}	12.26±0.24	12.76±1.09	10.17±1.11	14.20±1.33	19.22±0.54	14.18±0.65
Nystatin	100 µg mL^{-1}	20.40±0.45	17.61±0.84	17.12±0.61	17.82±0.71	22.78±0.72	13.07±0.41
Amphotericin B	100 µg mL^{-1}	23.30±0.13	20.71±0.26	20.10±0.32	20.17±0.44	26.09±0.87	16.21±0.44

*Data is presented as Mean±SD of zone of inhibition, inhibition zones are the mean of three replicates

Table 3: *In vitro* antibacterial activity of *Commiphora myrrha*, oleo-gum resins

	Zone of inhibition (mm)*			
Extract type	*Pseudomonas aeruginosa*	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus subtilis*
Chloroform	16^{a}	14^{a}	17^{a}	15^{b}
Methanol	21^{b}	22^{b}	21^{b}	19^{a}
Aqueous	12^{c}	11^{c}	11^{c}	13^{c}
Ampicillin 100 µg mL^{-1}	18	25	23	21

*Concentration of extracts 0.1 mL/cup (100 mg mL^{-1}), inhibition zones are the mean of three replicates. Means followed by the same letter in columns are not significantly different ($p<0.05$)

Table 4: Influence of *Commiphora myrrha*, oleo-gum resins methanol extract on the growth of different fungal dermatophytes

	Mycelial fresh weight grown on czapek-dox medium (g)*					
Clinical fungal isolates	0 (control)	100 (mg mL^{-1})	150 (mg mL^{-1})	200 (mg mL^{-1})	DSD (at 1%)	LSD (at 5%)
Candida albicans	18.9	9.41	8.13	6.61	1.433	0.754
Epidermophyton floccosum	15.55	10.12	6.45	2.00	1.300	1.31
Microsporum audouinii	15.00	13.01	10.66	5.01	1.399	1.50
Trichophyton concentricum	14.22	7.50	5.00	3.00	1.233	1.511
Trichophyton tonsurans	13.00	8.22	6.77	3.15	2.441	1.851
Trichophyton rubrum	10.77	9.66	6.66	3.00	1.251	0.641
CD at 1%	1.801	1.644	1.435	2.022		
CD at 5%	1.232	1.144	1.555	1.447		

*Values are means of 5 replicates, LSD: Least significant difference, DSD: Direct stream digital

Table 5: Minimum inhibitory concentration (MIC) (mg mL^{-1}) of *Commiphora myrrha*, oleo-gum resins*

Test organism	Methanol extract	Aqueous extract
Candida albicans	1.50^{a}	4.0^{a}
Epidermophyton floccosum	1.50^{a}	8.0^{b}
Microsporum audouinii	8.50^{b}	11.0^{b}
Trichophyton concentricum	14.00^{c}	11.5^{b}
Trichophyton tonsurans	9.00^{c}	9.0^{b}
Trichophyton rubrum	6.50^{b}	9.0^{b}
Bacillus subtilis	11.00^{c}	14.0^{c}
Pseudomonas aeruginosa	0.15^{a}	0.15^{a}
Staphylococcus aureus	0.14^{a}	2.0^{a}
Escherichia coli	0.18^{a}	4.0^{a}

*Values followed by different letters in vertical columns are significantly different using Duncan's Multiple Range Test ($p \leq 0.005$). Concentrations used are 10, 15 and 20 mg mL^{-1}

superior in activity followed by chloroform. The maximum inhibition zones were found in the three types of extracts of the resins against fungi with less inhibition zones in bacteria (Table 1-4). In case of fungi, chloroform extract and methanol extract at concentration of 200 mg mL^{-1} were effective against *Microsporum audouinii* (23.33 mm), *Cladosporium* sp. (20.14 mm), *Epidermophyton floccosum* (18.17 mm), *Trichophyton rubrum* (17.25 mm) of chloroform extract. The same fungal group in methanol extract of the same concentration were 22.23, 23.14, 20 and 19 mm, respectively (Table 1). In water extract the inhibition zones were 21 mm for *Microsporum audouinii*, 15.36 mm for *Cladosporium* sp. 17.28 mm for *Epidermophyton floccosum* and 17.30 mm for *Trichophyton rubrum*.

Methanol extract of myrrha gum was found effective against all of the microbial groups tested either fungi or bacteria (Table 1-5). On the other hand, oleo-gum resins of myrrh was found very suppressive against all of the four tested bacterial species (Table 3). The maximum inhibition zone determined was 22 mm in case of *Escherichia coli* followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* (21 mm) and *Bacillus subtilis* (19 mm) in methanol

Table 6: Phytochemical analysis of *Commiphora myrrha*, oleo-gum resins samples

Constituents	Level*
Sesquiterpenes (Isoprenoids i.e., Terpenoids)	+++
Sterols	+++
Steroids	+++
Furanosesquiterpenes	++
Oxidase enzymes	++
Ethanol-soluble resins	++
Tannins	+++
Acidic polysaccharides	++
Alcohol-soluble resin	+
Volatile oils	±
Lindestrene	-
Curzerenone	-

*:+: Low concentration, ++: Medium concentration, +++: High concentration, ±: Traces, -: not detectable

extract followed by chloroform and aqueous extract (Table 3). The least inhibition zones were obtained in water extract (11 mm) in *Staphylococcus aureus* and *Escherichia coli*. For fungal species, methanol extracts are suppressive in mycelial fresh weight (g) of the different fungal strains when the extracts were added and grown on Czapek-Dox medium and suppression increased when concentration is increased (Table 4).The minimum value of mycelial fresh weight was obtained when methanol extract was added in the concentration of 200 mg mL^{-1} to the culture of *Epidermophyton floccosum* with 2 g mycelial fresh weight (Table 4).

The Minimum Inhibitory Concentration (MIC) values of methanol and aqueous extracts of oleo-gum resins of myrrh showed that the highest MIC values were obtained in methanol extract for *Trichophyton concentricum* (14 mg mL^{-1}) and lowest MIC values for the same extract were 0.14 mg mL^{-1} for the bacterium *Staphylococcus aureus* (Table 5). In the aqueous extract of myrrha the highest and lowest MIC values were 14 mg mL^{-1} for *Bacillus subtilis* and 0.15 mg mL^{-1} for *Pseudomonas aeruginosa* (Table 5). It was clearly noticed that the myrrha oleo-gum resins of the plant *Commiphora myrrha*, had a broad spectrum activity against all of the bacteria and fungi in the two of the tested extracts.

When the obtained results were compared to antibiotics findings; it could be concluded that extracts of the different types obtained from *Commiphora myrrha* was less effective than the standard antibiotics used (Table 1-3). Furthermore, oleo-gum resins of *Commiphora myrrha* were phytochemically screened and the results are shown in Table 6. The oleo-gum showed the presence of Isoprenoids (Terpenoids), sterols, steroids and tannins in high levels concentration. Other constituents such as furanosesquiterpenes, oxidase enzymes, ethanol soluble resins and acidic polysaccharides were found in a moderate concentration while unspecified alcoholic-soluble resins were found in low concentration. However, the significance of this finding remains the area of further investigations as far as the chemical constituents of the oleo-gum resins of *Commiphora myrrha* plant are concerned.

These results are in close agreement with other findings obtained by other workers elsewhere (Lotfy *et al*., 2006; Saadabi and Moglad, 2011; Abdallah *et al*., 2009; El Ashry *et al*., 2003; Zhu *et al*., 2003; Dolara *et al*., 2000; Hawar, 2008; Gadir and Ahmed, 2014; Ali, 2007) especially in a sense of Myrrh activity . For example an *in vitro* study of 2 sesquiterpenes derived from myrrh (furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one) discovered antibacterial activity against *Pseudomonas aeruginosa* (minimum inhibitory concentration (1.4 mg mL^{-1}), *Staphylococcus aureus* (MIC 0.18 mg mL^{-1}) and *Escherichia coli* (MIC 2.8 mg mL^{-1}). Additionally, these sesquiterpenes demonstrated antifungal activity against *Candida albicans* (MIC 1.4 mg mL^{-1}). Local anesthetic activity was also noted in mammalian nerve cells (Dolara *et al*., 2000; Adam and Selim, 2013). In a Clinical case report, a paste containing 800 mg of bee propolis and 50 g of myrrh mixed in honey was applied topically to the wound. Every 3 days, the paste was made and refrigerated. The wound was cleaned daily followed by application of the paste. After 4 weeks of treatment the wound healed completely. Although myrrh's exact role in wound healing is difficult to determine because of many confounding factors in this case report, data suggest there may be a potential role for topically applied products containing myrrh (Lotfy *et al*., 2006; Gallo *et al*., 1999; Fraternale *et al*., 2011).

Also, it is not possible to make a direct correlation between the observed activity of the oleo-gum resins *in vitro* and the actual effects when used *in vivo* for the diseases observed by the indigenous people and traditional healers. Therefore, it is important that the oleo-gum resins should also be investigated to evaluate the significance of these extracts, clinical role and the medical system of indigenous people. Further research is also necessary to isolate and characterize their active compounds for pharmacological testing. The present study identifies oleo-gum resins *of Commiphora myrrha* Saudi Myrrh as potential source of biological antimicrobial, since it showed a high activity against wide spectrum of bacteria and fungi which enables only human pathogenic fungi and bacteria to be killed without any side effects and/or bacterial resistance as current synthetic antibiotics are doing and this specificity appears as additional point in the natural antibiotics research.

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