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Full Length Research Paper

Antibacterial activity of oleo-gum resins of *Commiphora molmol* and *Boswellia papyrifera* against methicillin resistant *Staphylococcus aureus* (MRSA)

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The petroleum ether, ethyl acetate, methanol and water extracts of the oleo-gum resins of two Arabian medicinal plants, *Commiphora molmol* Engl. ex Tschirch and *Boswellia papyrifera* Hochst were investigated for their antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA). The phytochemical investigation demonstrated the presence of phenolic compounds, alkaloids and saponins in the methanol extracts which is lacking in the petroleum ether and ethyl acetate extracts. The methanol extracts exhibited the highest antibacterial activity whereas the ethyl acetate extracts exhibited some degree of activity and the petroleum ether and water extracts exhibited no or least activity. The minimum inhibitory concentration (MIC) ranged between 31.25 and 250 µg/ml for oleo-gum resin methanol extract of *C. molmol* and of *B. papyrifera* ranged from 62.5 to 500 µg/ml respectively.

Key words: *Commiphora molmol*, *Boswellia papyrifera*, *Staphylococcus aureus*, MRSA, antibacterial activity.

INTRODUCTION

In the past decade, there has been renewed attention and interest in the use of traditional medicine globally (WHO, 2002). Traditional medicine was a source of discovering many important medical pharmaceuticals (Gilani and Atta-ur-rahman, 2005). Recently, however, plant derived compounds offer potential source of new antimicrobial, anticancer and anti-HIV agents among other pharmaceuticals (Gurib-Fakim et al., 2005). Antimicrobial compounds derived from plants may inhibit microorganisms by a different mechanism than the pre-sently used antibiotics and may have value in treatment of resistant microbial strains (Eloff, 1997). Resistance to antibiotics has been so tremendous that, incidences of outbreaks of multi-resistant bacteria in the past 10 years increased dramatically with no effective antibiotics to treat them (Walsh and Amyes, 2004).

The oleo-gum resins of *Commiphora molmol* Engl. Ex Tschirch. (known as myrrh) and *Boswellia papyrifera* Hochst (known as frankincense) are obtained from the bark of trees belonging to family *Bruseraceae*. They have been in use in traditional medicine in many Arab countries for ages. *C. molmol* is originally found in Northern Africa, Arabia and Northern Somalia (Hanus et al., 2005). Early Muslim writers recorded many medicinal uses for the oleo-gum resin of *C. molmol*. It has been used to treat wounds, intestinal disorders, diarrhea, cough and chest aliments (Ghazanfar, 1994). Addi-tionally, the oleo-gum resin of C. molmol is used to treat gingivitis (Serfaty and Itid, 1988), inflammations (Kimura et al., 2001) and fascioliasis (Massoud et al., 2001). Rahman et al. (2008) reported that the anti-bacterial terpenes obtained from the oleo-resin of *C. molmol* effective against several strains of *Staphylococcus aureus*.

Boswellia papyrifera is originally found in East Africa (Sudan, Eritrea and Ethiopia) and is considered to be the source of frankincense since immemorial (Langenheim, 2003). Medical applications of the oleo-gum resin of *B. papyrifera* include: treating cough (Michie and Cooper, 1991), vaginal infections (Thomas, 2000) and inflammations (Frank and Unger, 2005). Camarda et al. (2007) reported that the essential oils obtained from *B. papyrifera* exhibited considerable activities against some fungal

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strains.

Methicillin-resistant *S. aureus* (MRSA) infections are among the leading causes of hospital acquiring infections in the world (Midha, 1997). The first clinical isolate of MRSA was reported in 1961, just 1 year after the launch of methicillin. Since that time, MRSA infections have gradually spread (Hiramatus et al., 2001). Resistance of MRSA to antibiotics is due to production of an altered penicillin-binding protein (PBP2a) and affects all β-lactam antibiotics (Petinaki et al., 2001). For this reason, there have been global worries, about the danger of the spread of MRSA which accounts for 40 - 70% of the *S. aureus* infections in intensive care units (Sahm et al., 1999). Thus, new anti-MRSA agents have strongly sought and researched on the agents that have been carried out in the recent decade (Yamamoto and Kurazono, 2007). A number of studies suggested that certain essential oils may be effective in preventing the spread of the organisms (Dryden et al., 2004; Nelson, 1997) or be used in dressings to treat burns (Edwards-Jones, 2004). In relation to past knowledge and studies as antiseptics and antimicrobial activity, this study is aimed at investigating the anti-MRSA activity potential of the oleo-gum resins extracts from 2 plants used in Arabian traditional medicine namely: *C. molmol* and *B. papyrifera*.

MATERIALS AND METHODS

Plant materials

The oleo-gum resin of *C. molmol* was purchased from traditional medicine shops from Al-Rass town, Saudi Arabia and the oleo-gum resin of B. papyrifera was purchased from traditional medicine shops from Khartoum town, Sudan. Both oleo-gum resins of *C. molmol* and *B. papyrifera* were crushed into fine powder using sterile mortar and pestle.

Preparation of oleo-gum resin extracts

The powder form of oleo-gum resins of *C. molmol* and *B. papyrifera* were subjected to successive solvent extraction according to the method proposed by Darout et al. (2000) and Jothivel et al. (2007). The extraction was performed using the following solvents in increasing order of polarity: petroleum ether, ethyl acetate, methanol and de-ionized distilled water, respectively. The solvents were then distilled, evaporated and vacuum dried.

Phytochemical screening

The various extracts of oleo-gum resins of *C. molmol* and *B. papyrifera* were subjected to the following tests in order to study their phytochemical constituents. Prior to experiments, all petroleum ether, ethyl-acetate and methanol extracts of both oleo-gum resins (*C. molmol* and *B. papyrefira*) were dissolved in 70% acetone. Water extracts were prepared by dissolving in distilled sterile water to a final concentration of 50 mg/ml. Identification of various phytochemical compounds were determined as follows: Carbohydrates using Molisch's test, proteins using Biuret's test, lipids using oily spot test (Joshi and Saraswat, 2004), phenolic compounds using ethanolic ferric chloride test, alkaloids using Dragendroff's test (Clarke, 1975), saponins using frothing activity test, anthraquinones using Bornträger test (Stahl, 1973) and steroids were identified by adding 3 drops of acetic anhydride and 1 drop of concentrated sulphuric acid to 1 ml from each of the test solutions. Change in colour from deep green to brown to dark brown is an indication of the presence of steroids (Clarke, 1975).

Bacterial strains

7 clinical strains of methicillin-resistant *S. aureus* clonotypes (MRSA-1, MRSA-2, MRSA-3, MRSA-4, MRSA-5, MRSA-6 and MRSA-N32064) were obtained from the Microbiology Laboratory Culture Collection, School of Bioscience and Biotechnology, Faculty of Science and Technology, National University of Malaysia. *S. aureus* (ATCC 25923) was used as a reference control of non-MRSA microorganism. Bacterial strains were sub-cultured in brain heart infusion broth and incubated at 37°C for 18 - 24 h.

Antibiotics

6 commercial antibiotics were used as reference antibiotics and tested against MRSA strains by the disc diffusion method: methicillin 5 µg (Oxoid®, UK), vancomycin 30 µg (DIFCO®, USA), penicillin G 60 µg (Oxoid®, UK), ampicillin 10 µg (Bioanalyse®, USA), chloramphenicol 30 µg (Bioanalyse®, USA) and tetracycline 30 µg (Oxoid®, UK).

Antimicrobial screening

The standard Kirby-Bauer disk diffusion method described by NCCLS (2002) was adopted with modification to test for the antibacterial activity of the prepared extracts. Stock solutions of 500 mg/ml were prepared from the dried oleo-gum resin extracts of *C. molmol* and *B. papyrifera* (previously prepared) as follows: 0.5 g from each dried plant extracts was dissolved in 1 ml of DMSO (10%, v/v in water). Test bacteria were sub-cultured onto brain-heart infusion agar (Becton Dickinson Comp., USA) and incubated at 35°C overnight. Then, 4 - 5 colonies were isolated with an inoculating loop transferred to a tube of sterile saline and vortex thoroughly. The bacterial suspension was compared and adjusted to the 0.5 McFarland standards to prepare culture stocks (about 5 × 107 CFU ml-1). Within 15 min, a sterile cotton swab was dipped into the bacterial suspension and streaked over sterile plate containing Mueller Hinton agar and left for a while to set. Then, sterile filter paper (6 mm in diameter) was impregnated with 15 µl of extract (7.5 mg/disc) from previously prepared stock of 500 mg/ml. After 20 min, the plate was gently turned upside down and incubated at 35°C for 16 - 18 h. The diameter of inhibition zones (in mm) were measured and recorded. Commercial antibiotics were applied to serve as positive control and diluent, DMSO 10% (v/v) as negative control.

Determination of minimum inhibitory concentration (MIC)

MIC of the methanolic extracts of oleo-gum resins of *C. molmol* and *B. papyrifera* which showed the highest antibacterial activity in the disc diffusion assay were determined based on a micro-well dilution method (Karaman et al., 2003) and described with some minor modifications as follows: the inocula of micro-organisms were prepared from 12 h broth cultures, suspensions were adjusted to 0.5 McFarland standard turbidity, Stock solutions (500 mg/ml) were diluted with nutrient broth (Oxoid®, UK) in serial tenfold dilutions until 500 µg/ml, followed by serial 2 fold dilutions using nutrient broth to make dilutions ranging from 500 - 7.8 µg/ml. The 96-well plates were then prepared by dispensing 95 µl of nutrient broth and 5 µl of the inoculum into each well and a set of 100 µl from the dilutions of methanol extracts (500 - 7.8 µg/ml) were added into 6

Table 1. Phytochemical analysis in different extracts of oleo-gum resins of *C. molmol* and *B. papyrifera*.

Plant	Extract	Carbohydrates	Proteins	Lipids	Phenolics	Alkaloids	Saponins	steroids	Anthraquinones
C. mol mol	Petroleum ether	+	-	+	±	-	±	+	-
	Ethyl acetate	+	-	+	+	-	-	±	-
	Methanol	+	-	+	+	+	+	-	-
	Water	+	±	-	-	-	-	-	-
B. papyrifera	Petroleum ether	+	+	+	-	-	-	-	-
	Ethyl acetate	+	-	+	-	±	±	-	-
	Methanol	+	±	+	+	+	+	-	-
	Water	+	±	-	-	-	-	-	-

(+) indicates presence, (–) absence and (±) weak positive reaction of plant constituents respectively.

6 consecutive wells, respectively. In the last well, 195 µl of nutrient broth and 5 µl of the inoculum were added on each strip and used as negative control. The final volume in each well was 200 µl. Then, tetracycline (Oxoid®, UK) was used as standard drug for the positive control. It was diluted using nutrient broth to make dilutions ranging from 500 to 7.8 µg/ml and 200 µl from each dilution was added to the last 6 consecutive wells. Finally, the plate was covered with a sterile plate sealer, mixed on plate shaker at 300 rpm for 20 s and incubated at 35°C for 20 h.

Microbial growth was determined by absorbance at 595 nm using a microplate reader (Model 680, Bio-Rad). MIC is recorded as the lowest concentration of the extract to inhibit the growth of micro-organisms. This is confirmed by placing 5 µl of samples from clear wells on nutrient agar.

RESULTS AND DISCUSSION

Phytochemical analysis reveals presence of the carbohydrates in all extracts (Table 1). Phenolic compounds, alkaloids and saponins were detected particularly in the methanolic extracts of *C. molmol* and *B. papyrifera* and to a lesser degree in ethyl acetate and petroleum ether extracts. However, water extracts exhibited none of those compounds. The result in this study is in agreement with the findings of Brieskorn and Noble (1983) that proves the presence of phenolic compounds in the resins of *C. molmo*l. Both n-octanol and n-octyl acetate, along with the diterpenic components incensole and incensole acetate, were the characteristic compounds of *B. papyrifera* oleo-gum resin oil (Camarda et al., 2007). Adelaku et al. (2001) found that the methanolic extract of stem park of *Boswellia dalzielliii* consist of saponins and lacked anthraquinones or alkaloids. However, in our study, alkaloids were also detected. This may be due to differences in species and the part of plant that was extracted.

The antibacterial activities of different extracts of oleo-gum resins of *C. molmol* and *B. papyrifera* were detected against *S. aureus* and MRSA strains (Table 2). The methanolic extract manifested the highest antibacterial activity and ethyl acetate showed some degree of antibacterial activity. Petroleum ether and water extracts showed weak or no antibacterial activity. The control did not produce any inhibitory activity against the organisms. It is known that, methanol is a better solvent for more consistent extraction of antimicrobial substances from medicinal plants compared to other solvents, such as hexane, ethanol and water (Ahmed et al., 1998; Karaman et al., 2003). No zone of inhibition is seen when diluent DMSO (10%) is applied to the disk for all the tested bacteria. The findings of this study are in agreement with El-Ashry et al. (2003) who showed that there is a different *Commiphora* species have a considerable antimicrobial activity against some gram positive and gram negative bacteria. Recently, it was found that *C. molmol* has antibacterial activities against some strains of *S. aureus*, *Salmonella enterica* and *Klebsiella pneumoniae* (Rahman et al., 2008). Additionally, our results are in line with Camarda et al. (2007), who stated that 2 *Boswellia* species oleo-gum resins demonstrated presence of antibacterial activities. *B. carteri* against the methicillin resistant, *S. aureus* (MRSA), *P. aeruginosa* and *B. rivae* against *E. coli* (Camarda et al., 2007). Interestingly, *B. papyrifera* and *B. rivae* essential oils was found to be active against staphylococcal and *C. albicans* biofilms (Schillaci et al., 2008).

Indeed, little is known about the sensitivity of MRSA stains to plant extracts as they were poorly documented in literature. The interest mainly focusing in the mechanism of resistance to antibiotics (Hiramatus et al., 2001; Fernando et al., 2005) and in developing new anti-MRSA antibiotics apart from medicinal plants (Yamamoto and Kurazono, 2007). However, medicinal plant extracts might be a promising source of new antibacterial agents, particularly against MRSA.

Table 2. Antibacterial activities of *C. molmol* and *B. papyrifera* extracts.

Plant	Extract	Mean zone of inhibition (mm)*							
		***S. aureus* ATCC 25923**	**MRSA-1**	**MRSA-2**	**MRSA-3**	**MRSA-4**	**MRSA-5**	**MRSA-6**	**MRSA-N32064**
C. molmol	Petroleum ether	6.3 ± 0.1	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0
	Ethyl acetate	9.5 ± 0.2	8.0 ± 0.2	7.8 ± 0.1	8.0 ±0.5	8.1 ± 0.1	8.0 ±0.5	7.3 ± 0.3	7.8 ± 0.1
	Methanol	10.1 ± 0.1	10.8 ±0.7	9.8 ± 0.4	8.8 ± 0.1	9.6 ± 0.6	8.6 ± 0.3	8.8 ± 0.4	9.0 ± 0.5
	Water	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0
B. papyrifera	Petroleum ether	6.5 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0
	Ethyl acetate	7.6 ± 0.1	7.6 ± 0.3	7.6 ± 0.1	7.8 ± 0.1	7.6 ± 0.3	7.5 ± 0.2	7.6 ± 0.3	7.8 ± 0.1
	Methanol	8.0 ± 0.0	10.6 ±1.1	10.0 ± 0.7	10.1 ± 0.6	9.8 ± 0.4	9.1 ± 0.6	8.8 ± 0.1	9.6 ± 0.4
	Water	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0

*Data presented as mean ± S.E.M., n = 3. dose = 7.5 µg/disk.

Table 3. The minimum inhibitory concentrations (MIC) of oleo-gum resin methanol extracts from *C. molmol* and *B. papyrifera* against *S. aureus* and MRSA isolates.

Extract	Minimum Inhibitory Concentrations (MIC) in µg/ml							
	***S. aureus* ATCC 25923**	**MRSA-1**	**MRSA-2**	**MRSA-3**	**MRSA-4**	**MRSA-5**	**MRSA-6**	**MRSA-N32064**
C. molmol	31.25	125	250	31.25	125	125	31.25	62.5
B. papyrifera	500	500	500	62.5	62.5	500	500	500

The MIC values obtained in this study from methanol extract of *C. molmol* ranged from 31.25 - 250 µg/ml, while methanol extract of *B. papyrifera* ranged from 62.5 - 500 µg/ml (Table 3). According to Salvat et al. (2004), plant extracts with MIC's less than/or around 0.5 mg/ml (500 µg/ml) indicate good antibacterial activity. Based on this, it is concluded that methanol extracts of *C. molmol* and *B. papyrifera* exhibited good antimicrobial activity against *S. aureus* and MRSA stains. Notably, the MIC of methanol extract of *C. molmol* was the lower values, so its activity may be relatively higher than that of *B. papyrifera*. However, high MIC values may indicate that active compounds in the extracts may be present in low concentrations due to the method of extraction itself. From another research, the *B. carteri* resin oil demon-strated the high degree of activity against the methicillin-resistant *S. aureus* with MIC 3.52 µg/ml. Therefore, further future investigations using fractionation of the methanol extracts of *C. molmol* and *B. papyrifera* are required.

Table 4 showed different degrees of obvious effect on MRSA isolates towards 3 commercial antibiotics namely vancomycin, chloramphenicol and tetracycline. Of interest was the observation showing increased resistance of MRSA-2 towards tetracycline. Recently, vancomycin, which has been the main treatment for serious pulmonary infections due to MRSA is no longer be considered for pharmacological reasons as an optimal therapy for pneumonia (Ferrara, 2007). These findings are in consistent with (Brumfit and Hamilton-Miller, 1989) who cited that MRSA clinical strains are commonly resistant to wide classes of antibiotics. When comparing the antibacterial potency of methanol extracts of *C. molmol* and *B. papyrifera* in Table 4, we found that the extracts at least were better than or equally effective to penicillin and ampicillin.

Interestingly, Rahman et al. (2008) has recently shown that the crude extract of the oleo-resin of *C. molmol* displayed potentiation of ciprofloxacin and tetracycline against various *S. aureus* strains. This seems worthy to be studied intensively in

Table 4. Antibacterial activities of commercial antibiotics and oleo-gum resin methanol extracts from *C. molmol* and *B. papyrifera* against tested bacteria.

Microorganism	Mean zone of inhibition (mm)*							
	Methicillin 5 µg /disk	Vancomycin 30 µg /disk	Penicillin G 60 µg /disk	Ampicillin 10 µg /disk	Chloramphenicol 30 µg /disk	Tetracycline 30 µg/disk	Methanol extract of *C. molmol* (7.5 µg/disk)	Methanol extract of *B. papyrifera* (7.5 µg/disk)
S. aureus ATCC 25923	24.6 ± 0.6	17.8 ± 1.0	10.6 ± 0.3	6.8 ± 0.1	25.6 ± 0.8	22.3 ± 0.8	10.1 ± 0.1	8.0 ± 0.0
MRSA-1	6.0 ± 0.0	18.0 ± 1.0	7.6 ± 0.1	6.5 ± 0.2	23.3 ± 0.3	14.3 ± 1.2	10.8 ± 0.7	10.6 ± 1.1
MRSA-2	6.0 ± 0.0	17.6 ± 0.8	8.3 ± 1.3	6.0 ± 0.0	21.0 ± 3.0	12.8 ± 1.4	9.8 ± 0.4	10.0 ± 0.7
MRSA-3	6.0 ± 0.0	18.3 ± 0.8	6.0 ± 0.0	6.0 ± 0.0	17.3 ± 1.4	14.3 ± 1.2	8.8 ± 0.1	10.1 ± 0.6
MRSA-4	6.0 ± 0.0	17.6 ± 0.8	8.3 ± 0.3	7.3 ± 0.3	22.6 ± 1.2	16.6 ± 2.1	9.6 ± 0.6	9.8 ± 0.4
MRSA-5	6.0 ± 0.0	20.0 ± 0.5	7.8 ± 0.1	6.5 ± 0.2	21.6 ± 2.6	13.1 ± 0.6	8.6 ± 0.3	9.1 ± 0.6
MRSA-6	6.0 ± 0.0	21.8 ± 1.3	10.0 ± 2.0	6.0 ± 0.0	18.1 ± 0.6	14.3 ± 0.8	8.8 ± 0.4	8.8 ± 0.1
MRSA-N32064	6.0 ± 0.0	17.8 ± 0.9	6.0 ± 0.0	6.0 ± 0.0	13.5 ± 1.0	14.6 ± 0.3	9.0 ± 0.5	9.6 ± 0.4

* Diameter of disk = 6.0 mm, data presented as mean ± S.E.M., n =3.

further investigations.

Conclusion

In conclusion, oleo-gum resin methanol extracts from both plants have shown some degree of anti-bacterial activity against *S. aureus* and MRSA strains. The oleo-gum resin extracts, particularly methanol extracts contains some active principles phenolic compounds, alkaloids and saponins.

The comparable activity of methanol extract to some antibiotics may help to discover new chem-ical classes of antibiotics that may be used for the topical treatment of disorders resulted from *S. aueus* and MRSA pathogens. Folk and traditional medicine cine is a promising field for discovering new therapeutics.

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