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# Antimicrobial Activity of *Commiphora myrrha* Against Some Bacteria and *Candida albicans* Isolated from Gazelles at King Khalid Wildlife Research Centre

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## ABSTRACT

Ethanolic and ether extracts of *Commiphora myrrha* were evaluated for their antimicrobial activity against two Gram negative organisms (*Escherichia coli* and *Pseudomonas aeruginosa*), two Gram positive organisms (*Bacillus subtilis* and *Staphylococcus albus*) and fungi represented by *Candida albicans* isolated from gazelles held at King Khalid Wildlife Research Centre, Thumamah. The method used in evaluation of the antimicrobial activity was the two-layer agar diffusion method. The ethanolic extract of *C. myrrha* exhibited antimicrobial activity against the Gram negative organisms investigated together with *S. albus*. On the other hand, the ether extract showed antimicrobial activity against Gram positive organisms investigated and against *Candida albicans*, with the antifungal activity being greater. The minimum inhibitory concentration of the ethanolic extract against *P. aeruginosa* and *E. coli* was found to be 20 and 40 mg $mL^{-1}$, respectively. While the minimum inhibitory concentration of the ether extract against both *S. albus* and *C. albicans* was found to be 10 and 40 mg $mL^{-1}$ for *B. subtilis*, respectively.

**Key words:** *Commiphora myrrha*, antimicrobial, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus albus*

## INTRODUCTION

Plant extracts have been used for a wide variety of purposes for thousands of years (Jones, 1996). *Commiphora myrrha* is a member of family *Burseraceae*, known locally as morr Hijazi or myrrh and commercially as Arabian myrrh or Karam. Myrrh is the dried gum-resin from a number of closely related, small, thorny trees of the genus *Commiphora*, probably originating in the highlands of Yemen (Wadi Hadramaut). It is widely distributed in the Kingdom of Saudi Arabia and it is grown in Jizan area on Red Sea coast, a distinct so bare and dry that is called Tihama meaning very hot hill. It is also found in Somalia and other coast African countries (Mugahid, 1981; Vollensen, 1985). It is used in traditional medicine as antiseptic, carminative, anti-inflammatory, tonic in dyspepsia and emmenogogue (Tarig *et al*., 1985). It also used as remedy

for spongy gums, aphthous stomatitis and indolent ulcer (Satyavati *et al*., 1969). The Southern most countries on the Arabian Peninsula, today Yemen and Oman, have exported myrrh (and olibanum) for incense ceremonies in the temples and principalities of the Orient.

The constituents of this plant are terpenes, sesquiterpenes, aldyhydes, euginol, resin commophoric acids, volatile and essential oils, salts and proteins (Provan and Waterman, 1988; Al-Harbi *et al*., 1994). The antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Reynolds, 1996; Lis-Balchin and Deans, 1997). Hammer *et al*. (1999) studied the antimicrobial activity of essential oils and extracts of 52 plants. Mothana and Lindequist (2005) and Mothana *et al*. (2009) studied the antimicrobial, anticancer and antioxidant activities of extracts from several plant species from the Island Soqotra, among which they evaluated the antimicrobial and antifungal activity of *Commiphora parvifolia* and *C. ornifolia*. On a different study Abbas *et al*. (2007) evaluated the extracts of *C. opobalsamum* as antitumour, antimicrobial, anti-inflammatory, antioxidant and antimalarial as well as its estrogenic activity. The effect of *C. mukul* extracts on the cardiac function was evaluated by Ojha *et al*. (2008) and they reported significant improvement of cardiac function and prevention of myocardial ischemic impairment was associated with the use of this plant extracts.

In this study the effect of ethanolic and ether extracts of *C. myrrha* as antibacterial against some Gram-positive and Gram-negative organisms and as antifungal against *Candida albicans* isolated from gazelles at King Khalid Wildlife Research Centre is evaluated.

## MATERIALS AND METHODS

One kilogram of *C. myrrha* oleo-gum resin was finely ground and successively extracted with petroleum ether (60-80°C) for 18 h and with ethanol for 14 h using sexhlet apparatus. The oleo-gum resin of *Commiphora myrrha* extraction was performed at the Department of Veterinary Medicine, Pharmacology and Toxicology, University of Khartoum during 2008. The method used to evaluate antimicrobial activity of the plant extracts was the two-layer technique described by Stokes and Waterworth (1972). The media used was Muller Hinton agar medium (Laboratories Britania S.A. Lopotos, Buenos Aires, Argentine). The composition of Muller Hinton agar is 300 g $L^{-1}$ beef extract, 175 g $L^{-1}$ acid casein hydrolase, 1.5 g $L^{-1}$ starch and 15 g $L^{-1}$ agar. Bacteriological evaluation was done at the Veterinary Research Laboratory at King Khalid Wildlife Research Centre, Saudi Arabia.

Thirty seven grams of the medium were dissolved in one liter of warm distilled water and the reconstituted medium was sterilized by autoclaving at 121°C for 15 min. The medium was then distributed in sterile Petri dishes (95 mm in diameter) in 10 mL volumes and allowed to cool forming the base layer. Two Gram-positive organisms, *Bacillus subtilis* and *Staphylococcus albus* and two Gram- negative organisms *Escherichia coli* and *Pseudomonas aeruginosa* were grown in nutrient broth (Oxoid) at 37°C for 10 h. *Candida albicans* was grown in Sabouraud Dextrose Agar (SDA) at 37°C for 4-5 days. These organisms were isolated from gazelles raised at King Khalid Wildlife Research Centre, Thumamah. A fresh Mueller Hinton agar medium was prepared the same way as the base layer and was inoculated with the test organisms including *C. albicans* at $10^6$ cells $mL^{-1}$. The inoculated medium was distributed evenly in 10 mL volumes into the surface of the base layer. The plates were stored in the refrigerator at 4°C till use. On each inoculated plate, 5 cups (8 mm diameter) cut using sterile cork porer. Concentrations of 10, 20, 40, 80 and 100 mg $mL^{-1}$ of the ethanolic and the ether extracts were made up and 200 μL of each concentration was placed in one

of the cups in each plate by mean of sterile Pasteur pipette. The extracts were allowed to diffuse for 3 h before incubating the plates for 18 h at 37°C. Oxytetercycline (Beecham, UK) was used as the reference drug at similar concentrations to those of extracts. Three replicates were made from each concentration. The diameter of inhibition zones resulting from the activity of the extracts were measured in mm and comparative activity was recorded.

## RESULTS AND DISCUSSION

The ethanol extract of *C. myrrha* exhibited anti-bacterial activity against *E. coli*, *P. aeruginosa* and *S. albus* (Fig. 1) but did not show activity against *B. subtillis* and *C. albicans*. The Minimum Inhibitory Concentration (MIC) of ethanolic extract against both *E. coli* and *S. albus* was found to be 40 mg $mL^{-1}$, while that against *P. aeruginosa* was shown to be 20 mg $mL^{-1}$. The ether extract, on the other hand, was highly active against Gram positive organisms (*B. subtillus*, *S. albus*) and *C. albicans* (Fig. 2) but did not show any activity against Gram negative organisms used in the present investigation (*E. coli* and *P. aeruginosa*). The minimum inhibitory concentration (MIC) of the ether extract against both *S. albus* and *Candida abicans* was found to be 10 mg $mL^{-1}$ unlike that MIC shown for *B. subtilis* which was 40 mg $mL^{-1}$. In both ethanolic and ether extract the activities of the extracts increased with the increase of the concentration of the extract.

The ethanolic and ether extracts activities against the bacteria and the yeast used in this study were found to show lower activity compared to the control antibiotic (oxytetracycline) used (Table 1). However, unlike the oxytetratcycline used the ether extract showed consistent activity against *C. albicans*.

The present investigation have shown that the petroleum ether extract exhibited activity against *S. albus*, *B. subtilis* and *C. albicans* with no activity against *E. coli* or *P. aeruginosa*. The ethanolic extract was highly active against *E. coli* and *P. aerguinosa* and *S. albus*. This might possibly explain the common use of *C. myrrha* for the treatment of infections in the mouth such as mouth ulcers, gingivitis, phyorrhea, as well as the catarrhal problems of pharyngitis and sinusitis.


Fig. 1: Activity of ethanolic extract of *Commiphora myrrha* oleo-gum resin against *Staphylococcus albus*. The increase in the zone of inhibition is proportional to the increase of the extract

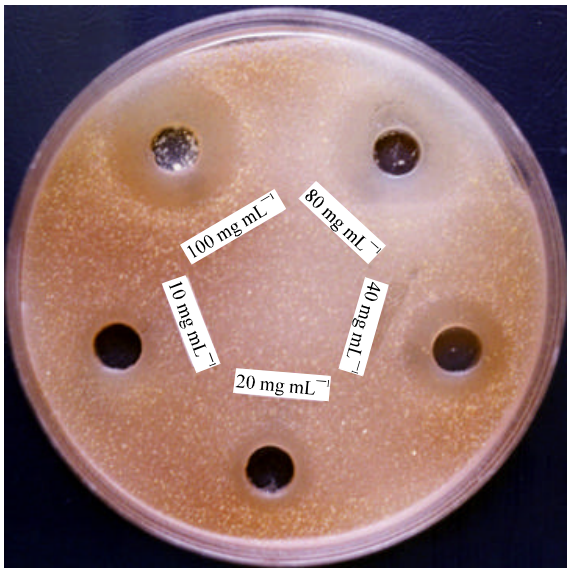


Fig. 2: Activity of ether extract of *Commiphora myrrha* oleo-gum resin against *Candida albicans*. The increase in the zone of inhibition is proportional to the increase of the extract

Table 1: Antimicrobial activities of ethanolic and ether extracts of *Commiphora myrrha*, zone of inhibition is measured in mm

| | | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| Test organism | Extract/antibiotic | 10 mg mL$^{-1}$ | 20 mg mL$^{-1}$ | 40 mg mL$^{-1}$ | 80 mg mL$^{-1}$ | 100 mg mL$^{-1}$ |
| *Escherichia coli* | Ethanolic extract | 0 | 0 | 1.7±0.6 | 2.7±0.6 | 3.7±0.6 |
| | Ether extract | 0 | 0 | 0 | 0 | 0 |
| | Oxytetracycline | 9±2 | 11.3±3 | 17.7±1.5 | 19.7±1.5 | 21.3±1.5 |
| *Pseudomonas aeruginosa* | Ethanolic extract | 0 | 0.7±0.6 | 1.3±1.1 | 2.3±2.1 | 4.7±1.5 |
| | Ether extract | 0 | 0 | 0 | 0 | 0 |
| | Oxytetracycline | 4±1 | 5.6±0.6 | 8.3±1.5 | 9.7±1.5 | 11.7±1.5 |
| *Bacillus subtilis* | Ethanolic extract | 0 | 0 | 0 | 0 | 0 |
| | Ether extract | 0 | 0 | 3.3±1.5 | 6.7±0.6 | 11±1 |
| | Oxytetracycline | 7±1.2 | 10±2 | 12±1 | 14.7±1.5 | 19.7±1.5 |
| *Staphylococcus albus* | Ethanolic extract | 0 | 0 | 3.3±1.5 | 3.3±1.5 | 3.7±0.6 |
| | Ether extract | 3.3±1.2 | 5.7±1.2 | 8.7±1.5 | 10.7±1.2 | 12.7±0.6 |
| | Oxytetracycline | 5.2±1.4 | 7.3±0.9 | 12.4±1.2 | 15.2±0.9 | 20.3±1.1 |
| *Candida albicans* | Ethanolic extract | 0 | 0 | 0 | 0 | 0 |
| | Ether extract | 2±1 | 3.7±0.6 | 4.7±0.6 | 6.7±0.6 | 10.3±1.2 |
| | Oxytetracycline | 0 | 0 | 0 | 0 | 0 |

The high activity of the ether extract against *Candida albicans* coincides with the routine use of *C. myrrha* in Ayurveda for oral and vaginal hygiene, parasite treatment, antiseptic action and as a natural antibiotic (Nadkarni, 1992; Treadway, 1998). The use of ether extract may also help in treating laryngitis, respiratory complaints, thrush and in footbath for athletes' feet because of its anti-fungal properties demonstrated in the present investigation.

The method of antimicrobial activity assessment and choice of test organisms may vary from one plant species to anther (Janssen *et al*., 1987). Hammer *et al*. (1999) reported antimicrobial activity of *C. myrrha* oil against many organisms using two different methods. Unlike what we have

demonstrated in the present investigation, Hammer *et al.* (1999) found that oil from *C. myrrha* inhibits Gram positive organisms only. Ethanolic and ether extracts of *C. myrrha* used in this investigation showed remarkable activity against Gram positive and Gram negative organisms as well as against *C. albicans*. This can be attributed to changes in the composition of plant extract from different geographical regions (probably different plant species), methods used for assessment of the activity as well as the method used for extraction (Janssen *et al.*, 1987; Sivropoulou *et al.*, 1995; Reynolds, 1996). The oil preparation used by Hammer *et al.* (1999) may contain certain extracts of *C. myrrha*.

Several plant constituents were found to have anti-bacterial activity and/or antifungal properties (Oliver-Beaver, 1986). These include e.g., phenol from *Acardium occidentale*, quinones from *Drosera indica*, alkaloid from *Argemone mexicana*, flavinoids from *Conscora decusta* and terpinoids from *Borreria verticillala*. The sesquiterpene (Furano *seco*-A-ring sesquiterpene curzerenone and other sesquiterpene mixtures) which were isolated from *Commiphora molmol* were found to be responsible of the activity against *S. aureus* (Hsieh *et al.*, 1998; Gibbons, 2004). The minimum inhibitory concentration was found to be 0.7 μg $mL^{-1}$ and this concentration compared very well with ciprofloxacin activity against the same strain (Dolara *et al.*, 2000). Tucker (1986) earlier claimed that myrrh was used for treating wounds and as a local eye medication, which was probably due to the activity of sesquiterpenes present in myrrh. Hsieh *et al.* (1998) and Dolara *et al.* (2000) investigations are in line with what has been reported in the present investigation. However, the minimum inhibitory concentration for the ether extract in the present study was 10 mg $mL^{-1}$ whereas the ethanolic extract was 40 mg $mL^{-1}$. This finding suggests that the sesquiterpenes concentration is higher in the ether extracts of *C. myrrha* compared to the ethanolic extracts. Unlike what has been reported in the present study, Kubmarawa *et al.* (2007) in Nigeria, reported activity of *Commiphora kerstingii* extracts against *C. albicans*, *B. subtilis* and *E. coli* but not against *S. aureus* and *P. aeruginosa*. This is probably due to difference in species and geographical region. Ali *et al.* (2008) and Suleiman *et al.* (2010) evaluated the oil extracts from *Commiphora kua* from Socotra and *Commiphora harveyi* extracts against the phyopathogenic fungus *Cladosporium cucumerinum* and the animal pathogen *Cryptococcus neoformans* respectively, in both studies antifungal activities were reported and this is support with what we found in case of *C. albicans*. These findings are in support to our study indicating the antifungal activity of *Commiphora* sp.

This study confirms that ethanolic and ether extracts of *C. myrrha* possess in vitro antibacterial and antifungal activity. In case such extracts are to be used for food preservation or medicinal purposes, issues of safety and toxicity must be taken into account. However, further investigation is needed in this important area to explain the active constituents present in this plant extract in inhibiting bacterial and fungal growths.

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