The antimicrobial and antioxidant activities of *Commiphora molmol* extracts

Mohaddese MAHBOUBI* and Nastaran KAZEMPOUR

Department of Microbiology, Medicinal Plant, Research Center of Barij, Kashan, Iran.

*Corresponding author, M. Mahboubi, Telephone: +98 86 44465187, E-mail: mahboubi1357@yahoo.com; mahboubi@barijessence.com

Received: 16. March 2015 / Accepted: 02. June 2015 / Available online: 03. November 2016 / Printed: December 2016

Abstract. *Commiphora molmol* oleoresin (Family Burseraceae) is traditionally used for treatment of many ailments such as ulcers, abscesses and wounds. The aim of this study was to evaluate the antimicrobial and antioxidant activities of *C. molmol* extracts in vitro (essential oils and ethanol extract). The antimicrobial activities of extracts were evaluated by disc diffusion and micro-broth dilution assays. The antioxidant activities of extracts were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the amount of phenolic and flavonoid contents were determined by spectrophotometric methods. The phenolic and flavonoid contents of *C. molmol* ethanol extract were higher than its essential oils and showed higher antioxidant activity (IC50 625 µg/ml). The antioxidant activities of extracts were lower than Butylated hydroxytoluene (BHT) (IC50 20 µg/ml).

The antimicrobial activity of *C. molmol* essential oil was higher than its ethanol extract. Gram positive bacteria and yeast were more sensitive to *C. molmol* extracts. The antimicrobial and antioxidant activities of extracts were lower than Butylated hydroxytoluene (BHT) (IC50 20 µg/ml).

Key words: *Commiphora molmol*, myrrh, extract, antioxidant, antimicrobial activity.

Introduction

Myrrh as yellowish aromatic oleoresin exudates from *Commiphora molmol* bark (Burseraceae family) has traditional history in treatment of some diseases. The exudates is used topically for treatment of ulcers, abscesses and wounds (Samuelsson et al. 1992, Lans et al. 2006), headaches and backaches (Bagatti 1946), fungal infections, relieving cramps, muscular pains and spasms (Lee & Law 1991, Lee & Lam 1993, Leow 1997), snake bites (Omeish 1999).

There are many investigations on myrrh oleoresin such as antimicrobial agent (Romero et al. 2005, Rahman & Gibbons 2007, Alzahrani et al. 2011, Kim et al. 2011, Abdallah & Khalid 2012, Kuete et al. 2012, Adam & Selim 2013), antiparasitic effects (El-Shafie et al. 2011, Massoud et al. 2013). The anti-diabetic (Bakhotmah & Alzahrani 2010), antioxidant (El-Ashmawy et al. 2006, Ashry et al. 2010), antiinociceptive (Tipton et al. 2003), immunoenhancer (Haffor 2010) and anti-inflammatory activities (Vissiennon et al. 2013) of myrrh extracts were confirmed. Although, myrrh is well documented worldwide chemically and biologically, but little is known about the biological activities of myrrh extracts in Iran, particularly as anti-spellage agent.

This investigation aimed to evaluate the antimicrobial, antioxidant, total phenolic and flavonoid contents of myrrh extracts (essential oil, ethanol extract) against food spoilage microorganisms.

Materials and methods

Plant materials

*Commiphora molmol* Engl. oleo-gum resin was purchased from grocery store and was identified in the Agriculture Department, Medicinal Plant, Research Center of Barij, Kashan, Iran and authenticated under the number (2221). For extraction of essential oil, 100 g of myrrh oleo-gum resin was mixed with 1200 ml water and was boiled in a Clevergen type apparatus for 3 h, the yield of oil was 2.4% w/w.

For preparation of myrrh ethanol extract, the oleo-gum resin was mixed with 96% ethanol, then it was shaken for 24 h, the supernatant was filtered and dried under the vacuum. The viscous extract was kept in the cold place until the analysis.

Total phenolic (TP) and Total Flavonoid (TF) contents of extracts

TP contents of each extract were analyzed spectrophotometrically using the Folin-Ciocâlteu’s reagent. In brief, 0.1 ml of each extract (1 mg/ml) and 0.5 ml of Folin-Ciocâlteu’s reagent (10%) were mixed. After 3-8 min, 0.4 ml of 7.5% (w/v) sodium carbonate solution was added and left for an hour at ambient temperature, the absorbance of reaction mixture was measured at 757 nm and TP was calculated (mg/g of dry extract as Gallic acid) using the standard curve of gallic acid and was reported.

The modified aluminum chloride colorimetric method was used for determination of TF content. 0.5 ml of diluted standard solution of each extract were separately mixed with 1.5 ml of ethanol (95%), then 0.1 ml of aluminum chloride (10%), 0.1 ml potassium acetate (1 M) and 2.8 ml distilled water were mixed and left for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The TF content of each extract (mg/g of dry extract as Quercetin) was determined (Mahboubi et al. 2013).

Determination of Antioxidant activity by DPPH method

In brief, a serial dilution of each extract (30-300 µg/ml) was prepared. 2 ml of each dilution were added to 2 ml of DPPH methanol solution (60 µg/ml) and mixed. The absorbance of samples was determined at 517 nm for 70 min. Inhibition of free radical DPPH in percent (I%) was calculated as follow:

\[
I\% = \left[ \frac{A_{blank} - A_{sample}}{A_{blank}} \right] \times 100
\]

Where A<sub>blank</sub> is the absorbance of the control reaction, and sample is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC50) was calculated by plotting inhibition percentages against concentrations of the sample. BHT as the reference antioxidant was used. All tests were carried out in triplicate and IC50 values were reported as means (Mahboubi et al. 2013).

Microbial strains and antimicrobial evaluation

Staphylococcus aureus ATCC 25925, Bacillus cereus ATCC 1247, Escherichia coli ATCC 8739, Salmonella typhimurium ATCC 14028 and fungi Candida albicans ATCC 10231, Aspergillus niger ATCC 16404 were used in this study.

For evaluating the antimicrobial activity by disc diffusion method, bacteria (10<sup>5</sup> CFU/ml) and fungi (10<sup>5</sup> CFU/ml) suspensions were prepared in Brain Heart Infusion (BHI) and Sabouraud dextrose broth using standard routine spectrophotometric methods (adjusted to 0.5 McFarland).

Using a sterile cotton swab, the microbial suspensions were cultured on appropriate media. Subsequently, sterile blank discs (6 mm in diameter) were saturated with different concentrations of extracts and were put on the cultured media. The plates were incubated at 37 °C for 24 h. The inhibition zones (IZ) diameters were measured in...
millimeters (mm) and average of IZ was recorded as means ± SD (Standard Deviation) (NCCLS 2012).

The minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values of the extracts were determined by micro broth dilution assay (NCCLS 2009). The extracts were twofold serially diluted with 10% DMSO which contains 51.2-0.1 mg/ml of each extract. These dilutions were prepared in a 96-well microtiter plates. MOPS-buffered RPMI 1640 (fungi), Mueller-Hinton broth were used as broth media. After shaking, 100 µl of extract dilutions was added to each well. The above microbial suspensions was diluted (1×10^6 CFU/ml for bacteria; 10^4 CFU/ml for fungi) and then 100 µl was added to each well and incubated at 35±2 ºC. MICs were defined as the lowest concentration of dilution that inhibits bacteria and fungi after 24, 48 h, respectively. MLC values were the first well that showing no growth on solid media. The experiments were performed for three times in the same conditions.

Results

TP and TF contents of myrrh extracts showed the higher total phenolic content (3.9%) for myrrh ethanol extract, followed by myrrh oil (3.3%). The TF content of myrrh ethanol extract was 0.8% quercetin per dry extract while this amount was 0.2% for myrrh oil (Table 1).

![Figure 1. The antioxidant activity of myrrh extracts by DPPH radical scavenging assay.](image)

| Table 1. The chemical attributes of Myrrh extracts. |
|---------------------------------|----------------|----------------|
| TPC (%) | TFC (%) | IC50 (µg/ml) |
| myrrh extract | 3.90 | 0.80 | 625 |
| myrrh essential oil | 3.30 | 0.20 | 750 |
| BHT | - | - | 20 |

TPC (Total phenolic content):% gallic acid/dry extract
TFC (Total Flavonoid Content):%quercetin/dry extract
IC50:The sample concentration providing 50% inhibition

The lower IC50 was for myrrh ethanol extract (625 µg/ml), followed by myrrh essential oil (750 µg/ml). The IC50 for BHT as positive control was 20 µg/ml (Fig. 1).

The antimicrobial evaluations showed that the antimicrobial activity of myrrh oil was higher than that of myrrh ethanol extract. The inhibition zone diameters of myrrh oil against different microorganisms were in the ranges of 6-12.8 mm, and for myrrh ethanol extract were in the ranges of 6-12.5 mm.

The MIC and MLC values of myrrh oil were in the ranges of 0.8-6.4 and 0.8-12.8 mg/ml while these amounts were in the ranges of 0.8-6.4 and 3.2-12.8 mg/ml for myrrh ethanol extract, respectively (Table 2).

| Table 2. The antimicrobial activity of Myrrh extracts against food spoilage microorganisms. |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|
| Myrrh oil | Myrrh ethanol extract |
| Disc (mg) | (mg/ml) | Disc (mg) | (mg/ml) |
| MIC | MLC | MIC | MLC |
| S. aureus | 8.1 | 8.9 | 11.8 | 0.8 | 0.8 | 6 | 7.9 | 11.1 | 3.2 | 6.4 |
| B. cereus | 6.5 | 9.8 | 12.8 | 0.8 | 0.8 | 9.4 | 12.5 | 15.6 | 6.4 | 12.8 |
| E. coli | 6.3 | 6.9 | 10.6 | 1.6 | 3.2 | 6.2 | 6.7 | 8.1 | 1.6 | 3.2 |
| Sh. flexneri | 6 | 6 | 9.6 | 0.8 | 1.6 | 6 | 6 | 8.9 | 6.4 | 12.8 |
| S. typhimurium | 6 | 6 | 6 | 3.2 | 6.4 | 6 | 6 | 8.5 | 6.4 | 12.8 |
| C. albicans | 6 | 6.5 | 10.7 | 0.8 | 1.6 | 6 | 6 | 8.9 | 0.8 | 1.6 |
| A. niger | 7.2 | 8.6 | 9.9 | 6.4 | 12.8 | 6 | 6.8 | 7.5 | 6.4 | 12.8 |

MIC= Minimal Inhibitory Concentration
MLC= Minimal Lethal Concentration

Discussion

According to our results, there are a positive correlation between phenolic and flavonoid contents of extracts and their antioxidant activities. Myrrh ethanol extract with high phenolic and flavonoid contents and low IC50 showed the higher antioxidant activity. There are many reports on positive correlation of phenolic and flavonoid contents and antioxidant activity. The positive relation between total phenolic content of extract and antioxidant activity were confirmed for Capparis spinosa fruit methanol extract (Mahboubi & Mahboubi 2014) and S. nigra flowers methanol extract (Mahboubi et al. 2012).

In spite of the positive correlation between the phenolic and flavonoid contents of extracts and antioxidant activities, there is negative correlation between the antimicrobial activities of these extracts and their phenolic and flavonoid contents.

Myrrh oil with lower contents of phenolic and flavonoid ethanol extract, respectively.

S. aureus and B. cereus (MIC=MLC= 0.8 mg/ml) showed more sensitivity to myrrh oil, followed by C. albicans (MIC, MLC=0.8, 1.6 mg/ml) and E. coli (MIC, MLC=1.6, 3.2 mg/ml). S. typhimurium (MIC, MLC=3.2, 6.4 mg/ml) and A. niger (MIC, MLC=6.4, 12.8 mg/ml) showed less sensitivity to myrrh oil.

C. albicans with MIC and MLC values of 0.8 and 1.6 mg/ml had more sensitivity to myrrh ethanol extract, followed by E. coli (MIC, MLC=1.6, 3.2 mg/ml), S. aureus (MIC, MLC=3.2, 6.4 mg/ml), B. cereus, S. typhimurium and A. niger (MIC, MLC=6.4 and 12.8 mg/ml).
compounds had higher antimicrobial activity than that of its ethanol extract. Although the antimicrobial activity of phe-
monic compounds has been confirmed (Nitiema et al. 2012, Alves et al. 2013), but the compounds, other than its phenolic
contents are responsible for the antimicrobial activity of myrrh.
Gram positive were more sensitive to myrrh oil than Gram negative microorganisms. Our results are not accord-
ing to the results of Adam and Selim, 2013 (Adam & Selim 2013) showed the lower antimicrobial activity for myrrh oil than that of methanol extract. B. cereus
and S. aureus had slight sensitivity to myrrh oil (Adam & Selim 2013). The results of above study showed methanol
extract showed the best antimicrobial activity than its essential oil or ethanol extract.
It has been shown the vapor of myrrh oil inhibited the germination of A. niger spores strongly (Ali 2007) while our
results showed that myrrh oil had less activity to A. niger and myrrh ethanol extract had higher activity against Can-
dida albicans. In compatible with our results, the anti-
candidal activity of methanol extract was confirmed against C. albicans (Omer et al. 2011).

Conclusion
The antimicrobial activity of each myrrh extract was related to the type of pathogens. The antimicrobial activity of C. molmol essential oil was higher than its ethanol extract. Gram positive bacteria and yeast showed more sensitivity to C. molmol extracts. More experimental and clinical studies are required to demonstrate myrrh antimicrobial and antioxi-
dant activities.

Acknowledgement. This study is supported by Medicinal Plants Research, Center of Barij, Kashan, Iran.

References