ejpmr, 2017,4(11), 449-453

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

AN IN-VITRO STUDY TO TEST ANTIMICROBIAL EFFECTS OF COMMIPHORA MYRRHA IN COMPARISON TO BIOCIDES

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Article Received on 01/09/2017 **Article Revised on 22/09/2017** **Article Accepted on 12/10/2017**

ABSTRACT

Introduction: Commiphora myrrha (C. myrrha) belongs to the family Burseraceae. Myrrha extract is produced from the oleo-resin gum obtained from the bark of Commiphora molmol, a variable species found in Arabia and Africa. It has been commonly used for treatments of inflammations, as an antipyretic, anti-septic, for infections and ulcers. The aim of this study was to investigate the antimicrobial effects of C. myrrha in comparison to known biocides. **Method:** C. myrrha resin gum purchased from local markets in Qassim, Saudi Arabia, was washed in clean water to remove debris and dust, then dried in shade and ground to make Myrrha powder. The aqueous and ethanol extracts of C. myrrha powder was evaluated for antibacterial and antifungal activity using the agar disc diffusion and well diffusion methods; against two gram positive, one gram negative and one fungal organism. The diameter of the inhibition zones was measured in millimeters and compared with the antimicrobial activity of known biocides. **Results**: The 10% ethanol extract of C. myrrha exhibited maximum inhibition with 11.3 mm for Staphylococcus aureus and was comparable to the inhibition zones found with biocides such as Amikacin, Tetracycline, and Amoxicillin. As the serial concentration of the extract increased the inhibition zone increased for S. aureus. However, C. myrrha extracts did not have inhibitory effects on Streptococcus species, E. coli, and Candida albicans. **Conclusion:** Commiphora myrrha ethanol and aqueous extracts have demonstrated a species-specific antibacterial effect, particularly on Staphylococcus aureus as compared to antibiotics such as Amikacin, Amoxicillin and Tetracycline.

KEYWORDS: Commiphora Myrrha, Antimicrobial Activity, Biocides.

INTRODUCTION

There are several causes of human disease and one of the most intriguing cause is microbial infection caused by bacteria, viruses, and fungi. These organisms can cause mild, moderate and/or severe infection. Indiscriminate use of antibiotics is one of the major cause of developing drug resistant bacteria; there is a dire need for new antibiotics. As opposed to chemically synthesized antimicrobials with side effects, herbal extracts provide a safe and less toxic alternative to combat infections.

For ages, nature has provided many herbs and plants which dominate traditional medicine to treat illness and is still widely used all over the world. Many researchers in the field of Naturopathy have indicated the importance of using plant extracts for treatment as it may have low to no side-effects as compared to commercial antimicrobials. Commiphora myrrha is one of the oldest known medicines which has been widely used for various purposes in households of the Middle East for centuries.

Commiphora myrrha belongs to Bruseraceae family and are commonly known as "Myrrha". Myrrha is one of the important medical plant. It has been used commonly for traditional treatments in Arab countries. There are several known beneficial effects of C. myrrha such as antibacterial, anti-inflammatory and anti-fungal. The use of natural plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments as an alternative to commercial biocides. The aim of this study was to investigate the antimicrobial activity of C. myrrha and compare the results with known biocides.

BACKGROUND

Traditionally used medicinal plants have received the attention of the pharmaceutical and scientific

communities. This involves the isolation and identification of the secondary metabolites produced by the plants and used as the active principles in medical preparations to combat human disease (Taylor et al., 2001 cited in O. A. Aiyegoro, et al, 2009). The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Van Wyk BE and Gericke N, 2000). Plant based antimicrobials represent a vast untapped source for medicine and disease control (O. A. Aiyegoro, et al, 2009).

Commiphora species are small trees or shrubs with short, thorny branches. True myrrha is produced by C. myrrha, a variable species found in southern Arabia and northeast Africa (mainly in Somalia) and as far south as northeast Kenya (Lumír O. Hanuša, et al. 2005).

Figure 1: C. Myrrha Resin.

Myrrha has been approved in USA by Food and Drug Administration as a safe natural flavoring agent in foods and beverages and as fragrance in cosmetics (Hall RL, Oser BL, 1965). Myrrha consists of water-soluble gum, alcohol-soluble resins and volatile oil. The gum contains polysaccharides and proteins, while the volatile oil is composed of steroids, sterols and terpenes. Myrrha's characteristic odor is derived from furanosesquiterpenes (Lumír OH, et al 2005).

Medicinal Properties: The approved modern therapeutic applications for myrrha are based on its long history of use in well-established systems of traditional and conventional medicine, inclusive of case studies, in vitro studies, pharmacological studies in animals, and on phytochemical studies of its volatile oil, gum and resin fractions (Blumenthal et al., 1998). Myrrha is one of the most effective herbal medicines in the world for sore throats, canker sores and gingivitis. Myrrha is used in India to treat mouth ulcers, pharyngitis, respiratory catarrh (Karnick, 1994). Myrrha is also used to treat many disorders associated with the female reproductive cycle, particularly dysmenorrhea and amenorrhea, and to help relieve some of the uncomfortable symptoms of menopause (Frawley D, and Lad V, 1986). Also, to treat rheumatoid arthritis, heart ailments, neurological disorders, skin infections, and obesity (Bhatt, et al. 1989).

The antifungal activity of Myrrha (Commiphora molmol) essential oil tested against three storage fungi, Aspergillus flavus, A. niger and Penicillium citrinum showed inhibitory effect of the oil against tested fungi with the increasing concentrations. Minimum inhibitory concentration (MIC) using agar dilution method was 4% (v/v) for A. niger and A. flavus and above 4% (v/v) for P. citrinum. Concentrations (0.5-2) % (v/v) of oil also affected the sporulation of these fungi causing reduction or complete inhibition of sporulation process (Batool Z. Ali, 2007).

The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments but several studies have also reported several types of contamination of herbal medicines which include microorganisms and toxins produced by microorganisms, pesticides and toxic heavy metals. (P. Talaly, P. Talaly 2001) that researchers need to be aware of during experimentation. Therefore, much research is required to be conducted to elicit scientific information of herbal products for disease control.

METHODOLOGY

An in-vitro experimental study was done to test the antimicrobial activity of Commiphora Myrrha and compared with biocides. The study was performed in the Microbiology laboratory of Qassim University in 2017. The plant-samples, Commiphora myrrha were collected from local markets in Unaizah city, Al Qassim region. C. myrrha samples were washed in running tap water to remove debris and dust particles; dried in the shade; then powdered using an electric blender; and the resulting C. myrrha powder was stored in sterile containers.

Aqueous Extract: Ten grams of myrrha powder was soaked in 100 ml of distilled water in a conical flask and the crude preparation was left for 24 hours in the shaker. The mixture was filtered with sterile filter paper in a conical flask. The collected extracts were then stored in a refrigerator at 5°C.

Ethanol extraction: Ten grams of myrrha powder was homogenized in 100 ml of ethanol (96%) and distilled water (80:20 V: V) for 10 min, then left in dark glass bottles for 72 hours for tissue maceration. The extracts were filtered with sterile filter paper. The final extracts were collected in dark glass bottles and exposed to 60°C in water bath for 3 min for ethanol evaporation. The collected extracts were then stored in refrigerator at 5°C.

Media: Two media were used to evaluate the antimicrobial activity; Nutrient Agar for bacterial subculture and Sabouraud Dextrose Agar for the fungal species.

Microbial isolates source: Three bacteria were isolated (Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae) and one fungi (candida albicans) from Microbiology laboratory in Qassim University. The

strains were kept at 4°C and sub cultured at 37°C for 24 hours on nutrient agar for bacterial species and Sabouraud Dextrose Agar for fungal species.

Antimicrobial Activity Assay: Antimicrobial activity of C. myrrha (aqueous and ethanol) extracts were evaluated by paper disc diffusion method and agar well diffusion with various concentrations such as: 10% (100mg/ml), 5% (50mg/ml), 2.5% (25mg/ml) and 1% (10mg/ml) for each extract.

Agar disc diffusion: For evaluating the antimicrobial activity by disc diffusion method. The disc made from sterile filter paper (6mm in diameter) was soaked in aqueous and ethanol solution (100 µl in each disc) with the following concentrations 10%, 5%, 2.5% and 1%. A bacterial suspension in sterile normal saline was prepared with reference to the 0.5 McFarland Standards. Turbidity of the bacterial suspension was compared with 0.5 McFarland standard solutions, followed by lawn culture of the bacterial suspension on Mueller-Hinton agar plates using sterile cotton swab and then placed in an incubator for 15 minutes. Then, the prepared aqueous and ethanol discs were placed on these cultures and the plates incubated at 37°c for 24 hours. The antimicrobial activity was determined by measuring the diameter of the inhibition zone (mm) exhibited in the cultured plates.

Agar well diffusion: An agar-well diffusion method was employed for evaluation of antibacterial activity. A bacterial suspension in sterile normal saline was prepared regarding the 0.5 McFarland Standards. Turbidity of the bacterial suspension was compared with 0.5 McFarland standard solutions, followed by lawn culture of the bacterial suspension on Mueller-Hinton agar plates using sterile cotton swab and then placed in an incubator for 15 minutes. 2 cm^2 holes were made and the extract was filled (100 µl) in holes with the following concentrations 10%, 5%, 2.5% and 1% The extracts were allowed to diffuse for half hour then incubated at 37°c for 24 hours. And diameter of inhibition zone around each well was measured in mm.

Determination of antimicrobial activity: The inhibition zones around each disc was measured in millimeters (mm). Three standard antibiotics discs (Amikacin, Tetracycline, Amoxicillin) and one standard antifungal disc (clarimazole) were used to compare with C. myrrha antimicrobial activity.

Statistical Analysis: Each experiment was repeated in triplicate sets and the mean values for the inhibition zones was used to represent the antimicrobial activity of the myrrha extracts. The results from the antibiotic and antifungal disks were recorded as S (sensitive), I (intermediate sensitive) or R (resistant).

RESULTS

The antimicrobial activity by using different concentrations of C. myrrha (Ethanol and Aqueous) extract were tested on four microbes using the agar disc diffusion method, due to its quality, ease of performance and clarity of results. The results were determined by measuring the inhibition zones in millimeters. The results recorded for the triplicate set of ethanol extract showed that C. myrrha ethanol extract exhibited antimicrobial activity for staphylococcus aureus. There was no inhibition zone found for streptococcus, E. coli and Candida albicans in all the triplicate sets.

The mean inhibition zone of C. myrrha ethanol extract (**Figure 2**) revealed maximum inhibition (11.3 mm) for Staphylococcus aureus with the 10% concentration of the extract. It should be noted that as C. myrrha concentration increased the inhibition zones also increased.

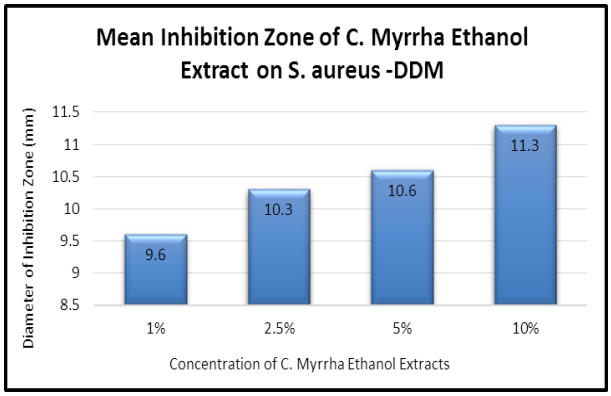

Figure 2: Mean Inhibition Zone of C. Myrrha Ethanol Extract on S. aureus by the Disc Diffusion Method (DDM).

Result for aqueous extracts of C. myrrha shows that C. myrrha aqueous extract has antimicrobial activity for the staphylococcus aureus. There was no inhibition zone found for streptococcus, E. coli and Candida albicans in all the triplicate sets. The mean inhibition zone of C. myrrha aqueous extract (**Figure 3)** revealed the sensitivity of Staphylococcus aureus in various concentration. As seen with the ethanol extract, the aqueous extract of C. myrrha also showed a linear increase in the inhibition zones as the concentration of the extract increased.

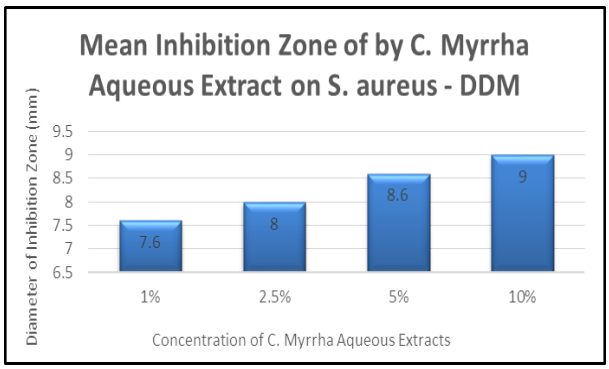

Figure 3: Inhibition Zone Exhibited by C. Myrrha Aqueous Extract on S. aureus by the Disc Diffusion Method (DDM).

According to Clinical and Laboratory Standards Institute (CLSI) tetracycline showed high degree of susceptibility to Staph. aureus and E. coli while moderate effect against S. Pneumoniae, Amikacin was susceptible to Staph. aureus and it showed no antibacterial activity against E. coli and S. Pneumoniae. Amoxicillin was susceptible to Staph. aureus, E. coli and S. Pneumoniae. Clarimazole was Susceptible to candida albicans.

The maximum inhibition exhibited by C. myrrha extracts on S. aureus was compared with the inhibition zones exhibited by biocides. The comparative results revealed that the inhibition zones produced by known biocides such as (amikacin, Amoxicillin, tetracycline) was larger than all concentrations of C. myrrha extracts. The C. myrrha (Aqueous, Ethanol) did not inhibit the growth of E. coli, streptococcus and candida albicans. When tested by agar diffusion method the results obtained in the study showed that C. myrrha had significant activity against Staphylococcus aureus while with Streptococcus, E. coli, and Candida albicans there was no discernible inhibition zone.

Well Diffusion Method

The antimicrobial activity by using different concentrations of C. myrrha (Ethanol and Aqueous) extract were tested on four microbes using the well diffusion method. The results (**Figure 4)** for the triplicate set of ethanol extract showed that C. myrrha ethanol extract has antimicrobial activity for Staphylococcus aureus and no inhibition zone found for Streptococcus, E. coli and Candida albicans.

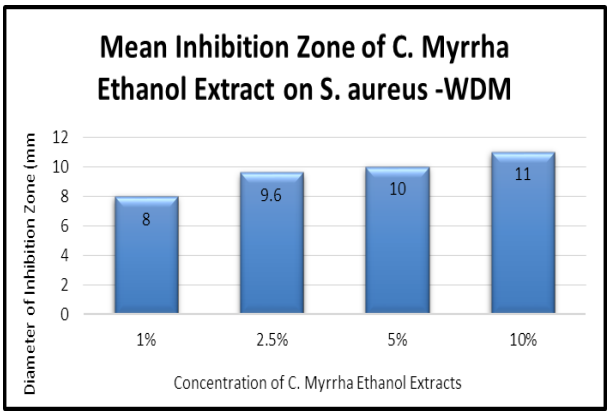

Figure 4: The Mean Inhibition Zone (mm) by several concentrations of C. myrrha ethanol extract on S. aureus by the Well Diffusion Method (WDM)

The mean inhibition of C. myrrha ethanol extract on S. aureus was observed to be highest with 10% concentration (11mm) and lowest with 1% concentration (8mm). It was noted that there was a linear increase in the inhibition zone with increase in the concentrations of C. myrrha extract.

DISCUSSION

Herbal drugs are used by physicians for hundreds of years as indigenous systems of medicine and about 80% of the world population still use them for primary health care. Currently, in addition to antibiotics and chemically synthesized drugs, the trend to look for alternative medicine in nature is increasing as the natural resources are less toxic to the overall health of humans. This study was performed to investigate the antimicrobial activity of C. myrrha on different microbes. C. Myrrha was effective in exhibiting the antibacterial activity against Staphylococcus aureus.

Sooad et al (2012), studied the antimicrobial activity of C. myrrha, against S. pyogenes, S. aureus and P. aeruginosa and E. coli. Highest antibacterial activity was observed with methanol extract against S. aureus with 19 mm inhibition zone and the aqueous extract showed 14mm of inhibition zone. Our study had comparable results with the inhibition zone for S. aureus ranging from 9.6 to 11.3 mm for ethanol extract. However, the aqueous extract showed smaller inhibition zones ranging from 7.6 to 9 mm by the disc diffusion method and by the agar well diffusion methods C. myrrha inhibited S. aureus with inhibition zones from 8 to 11mm with ethanol extract.

Rehab A (2014) investigated using cold and hot aqueous extract of C. myrrha against E. coli and candida albicans and reported that there were no significant inhibitions against both microbes. Our study results for C. myrrha aqueous extract and ethanol extract with no significant inhibition for candida albicans by the two methods of antimicrobial sensitivity assay (disc diffusion and well diffusion methods) correlates well with the results of Rehab A (2014).

There is little to no information in the scientific literature regarding the antibacterial activity of C. Myrrha on Streptococcus pneumonia. Probably due to the inactivity of C. Myrrha on this species of bacteria as found in our study, researchers may have excluded this species in their experiments.

N. Chandrasekhar Nath, et al (2013) studied the effects of C. Myrrha on Klebsiella, Enterococci, Escherichia coli, Pseudomonas aeruginosa that cause UTI. Their study demonstrated that the ethanol extract of C. myrrha inhibited Enterococci but there was no inhibition zone for other organisms. Our study results seem to corroborate their findings, in that the ethanol and aqueous extract exhibited no significant inhibition zone particularly for E. coli by agar diffusion and well diffusion methods.

This study confirms that C. myrrha has antimicrobial activity against S. aureus but for other organisms there was no discernible inhibitory effect. It is often stated that bacteria cannot develop resistance to herbal medicines. However, recent reports suggest that microbes can overcome bactericidal or bacteriostatic activities of herbal drugs (Vadhana P et al, 2015). This could be an attributable factor for C. myrrha to have lost its

antimicrobial activity against E. coli, Streptococcus pneumonia and Candida albicans as reported in this study. Another plausible explanation could be that C. myrrha is an oleo resin gum and the oil extracts may have greater potency to inhibit bacterial growth than the aqueous or ethanol extracts of this gum. However, further research on the antimicrobial activity of C. Myrrha is warranted with the use of more sophisticated microbiological techniques that minimizes human error, laboratory contaminations, and allows for controlled experiments that could produce robust results.

CONCLUSION

Commiphora myrrha extracts have demonstrated antibacterial effects particularly on Staphylococcus aureus as compared to antibiotics such as Amikacin, Amoxicillin and Tetracycline in our study. The ethanol extracts of Commiphora myrrha was found to be more effective than the aqueous extracts against Staphylococcus aureus; but was not effective against E. coli; Streptococcus and the fungal species, Candida albicans; indicating that C. myrrha could be considered a bioactive compound that is species specific. C. myrrha has bioactive potential and it may be considered a natural alternative to antibiotic therapy, particularly for Staphylococcus aureus infections. Thus, C. myrrha could be effectually used as a natural remedy for Staphylococcus infections in place of antibiotics and thereby avoiding the repeated use of synthetic antibiotics and endure its related side effects.

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