Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 3, Issue 7, July, 2022

BIOLOGICAL ACTIVITY OF MYRRH EXTRACT AGAINST SOME PATHOGENIC BACTERIA: GC-MS ANALYSIS OF EXTRACT

Nisreen W. Mustafa
Electron Microscope Unit, College of Pharmacy,University of Basrah,
nisrenw@yahoo.com

Fouad H. Kamel
Erbil Medical Technical Institute, Erbil Polytechnic University-Erbil-Iraq

Zaid N. Elia
Medical Laboratory Technology Department, Erbil Polytechnic University Erbil-Iraq

Abstract
Myrrh is a gum- resin exudate obtained by cutting in Commiphora myrrha trees belong Burseraceae. The bactericidal activity of Myrrh hexane extract tested against Staphylococcus aureus (S. aureus) , Escherichia coli (E.coli) and Pseudomonas aeruginosa (Ps. aeruginosa) . Present study was aimed to determine the active groups of myrrh and their biological activity.
Where oil extract exhibited better activity against S.aureus followed by Ps. aeruginosa and E.coli bacteria; inhibition diameters of S.aureus growth were (0.0,20,26,28 mm) for the concentrations of oil used (100%, 75%, 50%, 25 %) , respectively . While the diameter of the growth inhibition zone of Ps. aeruginosa were (0.0, 15, 23, 26 mm) respectively; and for E.coli were less (0.0, 12, 20, 23mm). The concentration 25% of hexane extract significantly inhibited bacterial growth comparing with other concentrations .
Gas chromatography/mass spectrophotometer (GC/MS) analysis of Myrrh showed major identified compounds of the hexane extract included identification of 7 compounds representing Benz furan, 6-ethenyl 4,5,6,7-tetrahydro-3,6 dimethyl-5-isopropenyl-, trans(33.37%), 3,5,8a-trimethyl-4,4a,8a,9-tetrahydronaphtho [2,3-b] furan (25.626%),(4aS,8aS)3,8a-Dimethyl-5-methylene-4,4a,5,6,8a,9-hexahydronaphtho[2,3-b]furan(9.55%),4,4'Dimethyl-2,2'-dimethylenebicyclohexyl 3,3'-diene(5.59%) ,Cyclohexane,1-ethenyl-1-methyl-2-(1-methylethenyl)-4 (1-methylethylidene) (3.39%),Cyclohexane,1-ethenyl-1 methyl-2,4-bis(1-methylethenyl)-,[1S (1.alpha.,2.beta.,4.beta.)] (3.24%) and 3,5,8a-trimethyl-4,4a,8a,9tetrahydronaphtho[2,3-b] furan(2.67%).While other small ingredients (16.54). These results referred to hexane Myrrh oil is a promising antibacterial and that can be formulated in suitable dosage forms.

Keywords: Essential oil , Ps. aeruginosa, E. coli , S. aureus Myrrh, GC/ MS and Multi-resistant clinical isolates.

Introduction

Myrrh is a resinous substance mixed with oils, and is extracted by scraping the stem of the myrrh tree. Myrrh tree grows in many Arab countries, including: Oman, Yemen, Saudi Arabia and Somalia, and the

ISSN: 2776-1010 Volume 3, Issue 7, July, 2022

most important characteristic of its transparent brown color and pungent taste, its types: African myrrh and Hijazi myrrh [1]. The use of myrrh has been known in folk and Chinese medicine throughout the ages [2]. The myrrh plant has been used since ancient times in mummification, and in the manufacture of perfumes and incense, in addition to using myrrh oil that is extracted using steam distillation, and it has multiple therapeutic and cosmetic benefits, because of its anti-inflammatory properties and antioxidant [3]. The use of myrrh herb and myrrh oil for wounds is one of the benefits of myrrh that were known in ancient folk medicine [4]. Myrrh helps heal wounds and scars of the skin and treat skin infections. The ancient Greeks used it to treat soldiers' wounds in battle and found that myrrh oil may help treat scars and wounds, as well as fight microbes that infect the skin and cause wound infections. Myrrh is used in medicine for ulcers,indigestion, colds, asthma, coughs, arthritis pain, lung congestion, cancer, convulsions,leprosy and syphilis, and it can also help reduce swelling (inflammation) and kill bacteria [5].

Direct application of murrh to the mouth help to reduce pain, swelling, gingivitis, mouth ulcers, loosening of the teeth and chapped lips bad breath. It is also applied topically for bed sores, hemorrhoids, boils wounds and cuts [6]. Myrrh oil may help get rid of the fungi that cause some skating diseases, and myrrh oil has been found to be more effective in fighting oxidation caused by free radicals, which helps protect cells from free radical damage [7]. While in beverages and foods, myrrh is exploited as a flavoring ingredient [8]. In manufacturing, myrrh is used as incense cosmetics fixation and in embalming [9, 10].

A follicle is an inflammation that occurs in the superficial or deep part of a hair follicle. It appears as pustules and red papules or acne-like nodules and may be accompanied by formation of pus in the skin superficial layers [11]. Inflammation of the hair follicles occurs in places covered by hair from the skin, and to a greater extent in areas where there is friction between parts of the body: such as the thighs, buttocks, neck and armpits, and may affect the scalp and buttocks[12]. Inflammation may affect a small number of follicles and may be widespread, and the duration of infection varies from short temporary to long-term chronic depending on the cause and severity of the infection. Antifungals, topical antibiotics such as clindamycin and erythromycin, and oral antibiotics are used to kill the bacteria causing the infection. Topical permethrin or oral metronidazole is used in case of infection caused by a parasite [13]. It was approved in this study to detect common bacteria in infections and to adopt bitter herbs for treatment, either directly or in the form of powders. Here a modified method has exhibited to demonstrate the potent antibiotic effectiveness of myrrh against non-developing bacterial cells, this is of great importance because the inability to inhibit growth-free cells is one of the fundamental reasons why most of the antibiotics in use today are not effective.

Material and Methods

Extraction of myrrh essential oil

Myrrh was obtained from Basra markets , (500 mg) of Myrrh powder was extracted using hexane by shaking at room temperature for 4 hr. with occasional mixing. Myrrh mixture was subjected to

ISSN: 2776-1010 Volume 3, Issue 7, July, 2022

distillation for 5 hr using a Clevenger apparatus. oil extract was refrigerated at 4 °C in a sealed vial until use [14].

Bacterial culture

Three standard bacterial species were obtained from the clinical microbiology laboratories in Media center-Erbil (S. aureus, E.coli and Ps. aeruginosa) which identified by Vitek 2 Compact system and approved to estimate the bactericidal efficacy of the extracted oil. The number of tested bacteria was adjusted to a 0.5 McFarland scale containing 1.5 x 10^8 CFU/ml with the tested strains, then diluted with sterile broth to obtain a concentration of 10^7 CFU/ml, the modified suspensions should be prepared [15].

Determination of bactericidal activity by viable count technique

The agar-well diffusion method was applied to determine the antimicrobial activity. Then , a stock solution of the sample was made by mixing the extract in dimethyl sulfoxide (DMSO). Standard bacteria activated in brain heat infusion agar then 6 mm diameter wells were created. 100 µl of the extract oil of (25, 50, 75, and 100 %) were diluted with (DMSO) and peppermint in to the pours, the tests were done in three replicates and all plates were stored at 37° C overnight. The diameter of growth inhibition zone was measured compared to the positive (Ampicillin) control group whereas distilled water sample as negative control and all test plates were incubated at 37 °C for 24-48 hr. [16-17].

Gas chromatography/mass spectrometry (GC/MS) analysis of the essential oil and hexane extract

An Agilent 5977A gas chromatograph (Agilent Technologies, USA) with a column HP-5MS (30 m × 0.25 mm and 0.25 µm film thickness) was used for analysis of the essential oils. The injector and detector temperatures were 230 °C. Helium gas (99.99%) was used as carrier at a flow rate of 1 ml/min. Manual split injection (1 µl) was applied. Ionization voltage was 70 eV and temperature was set at 230 °C [18].

Statistical analysi

One way ANOVA test (SPSS 22) was used to analyze the results. Statistical significant was considered at $p<0.05$.

Result

Hexane extract of Myrrh yielded 33.6 ± 2 % w/w and distillation yielded 6.4 ± 0.1% essential oil.

Table (1) summarizes the effectiveness of the Myrrh oil extract on three isolated pathogenic microbial growth. The efficacy of the oil extract concentrations (100%, 75%, 50%, 25 %) were more effective against S. aureus , the diameter of inhibition zones were (0.0,20,26,28 mm) respectively followed by Ps. aeruginosa and E.coli . The diameter of the inhibition zone of Ps. aeruginosa were (0.0, 15, 23, 26 mm) while for E.coli were less (0.0, 12, 20, 23mm), respectively . The concentration 25% recorded significant different comparing with other concentrations in inhibition zones diameters of all included bacterial strains .

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 3, Issue 7, July, 2022

Table (1) Effects of Myrrh oil extracting on the microbial activity

Oil Con. µl in DMSO	Diameter zone of Inhibition microbes (m. m.)		
	S.aureus	Ps. aeruginosa	E.coli
25%	28a	26a	23.66a
50%	26a	23.33b	20.66b
75%	20.67b	14.83c	12c
100%	0.00c	0.00d	0.00d
P value	0.00	0.00	0.00

GC/ MS analysis of Murray essential oil

GC/MS analysis of Myrrh in the hexane extract showed major identified compounds included 7 compounds representing Benz furan, 6- ethenyl-4,5,6,7- tetrahydro -3 ,6-dimethyl- 5- isopropenyl-, trans- (33.37%), 3,5,8a-trimethyl-4,4a,8a,9- tetrahydronaphtho [2,3-b] furan (25.626%),(4aS,8aS)-3, 8a-Dimethyl-5-methylene-4,4a,5,6,8a,9- hexahydronaphth o[2,3-b]furan(9.55%),4,4'-Dimethyl-2,2'-dimethylenebicyclohexyl- 3,3'-diene (5.59%), Cyclohexane,1-ethenyl-1-methyl-2-(1-methylethenyl) -4-(1- methylethylidene)-(3.39%), Cyclohexane,1-ethenyl- 1-methyl-2,4-bis (1- methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-(3.24%) and 3,5,8a-trimethyl-4,4a,8a,9-tetrahydronaphtho [2,3-b] furan(2.67%). While Other small ingredients (16.54) as in Table (2) and Fig. (1).

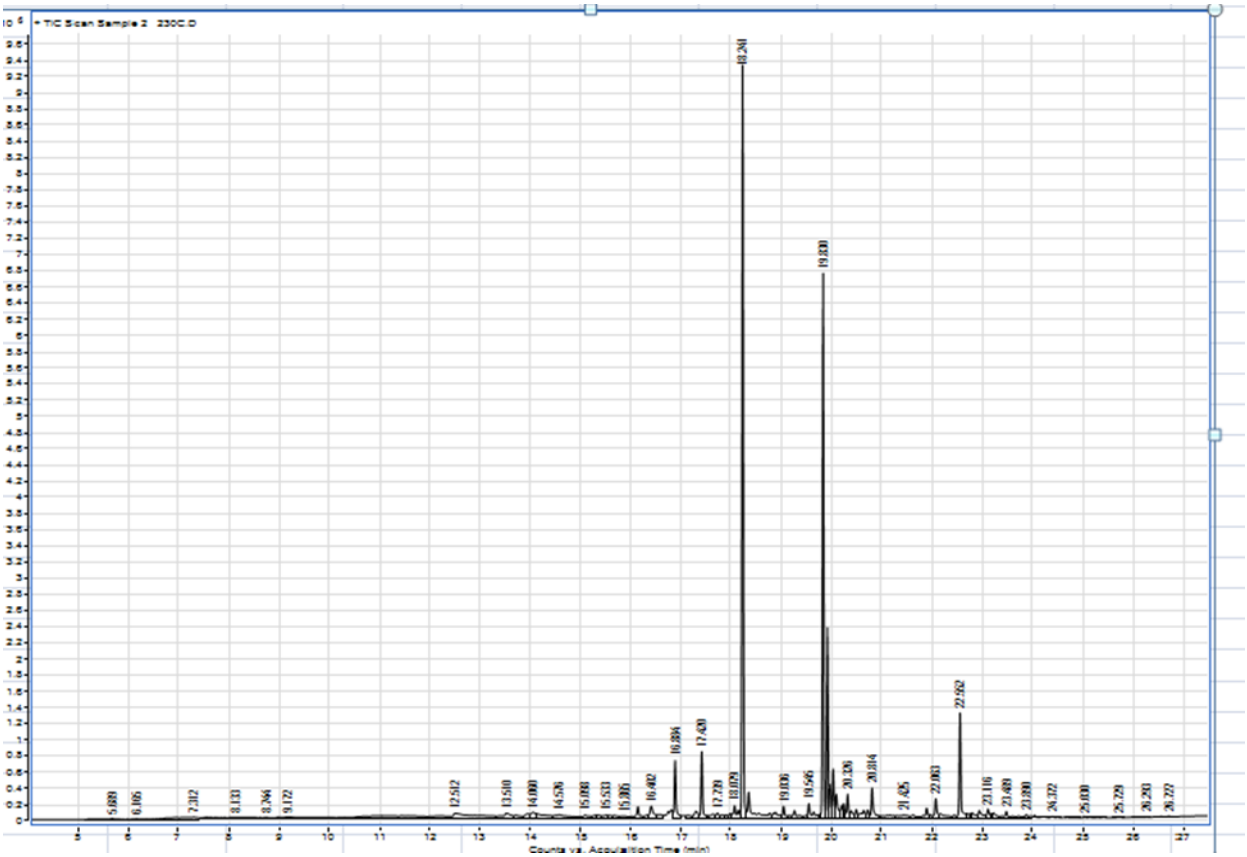

Figure (1) : GC/MS identification of Myrrh essential oil components of hexane extract .

Table(2) :Compound List of hexane extract at 230 oC

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 3, Issue 7, July, 2022

Name	Formula	Base Peak	RT min.	Area	Area%
Cyclohexane, 1- ethenyl 1-methyl-2, 4-bis (1-methylethenyl) , [1S - (1.alpha.,2.beta.,4.beta.)	C15H24	93.1	16.882	1408721	3.242873
Cyclohexane 1-ethenyl-1-methyl-2-,(1-methylethenyl) -4- (1-methylethylidene)	C15H24	121.1	17.419	1476390	3.398647
Benzofuran, 6- ethenyl-4,5,6,7 tetrahydro-3,6 dimethyl -5- isopropeny l-, trans	C15H20O	108.1	18.242	14496680	33.37133
3,5,8a-trimethyl -4,4a 8a,9- tetrahydronaphth [2,3-b] furan	C15H18O	108.1	19.83	11132105	25.62609
(4a S,8aS)-3,8a-Dimethyl 5-methylene-4,4a,5,6,8a 9-hexahydronaphtho,[2,3-b]furan	C15H18O	106.1	19.918	4150743	9.555003
3,5,8 a-trimethyl 4,4a,8a,9-tetrahydronaphtho [2,3-b] furan	C15H18O	108.1	20.033	1159920	2.670134
4,4'-Dimethyl-2,2'-dimethylenebicyclohexyl-3,3'-diene	C16H22	108.1	22.555	2429337	5.592329
Other small ingredients	--	--	--	16.54	100

Discussion

Myrrh oil is used in multidrug pharmaceuticals; In addition to Mirazid, the anti-worming soft gel contains an extract of myrrh refined from oleo resins [6]. C. molmol's water-repellent compounds include hexane myrrh extract and essential oils. They must either be dissolved or emulsified to create stable liquid forms. The water-soluble components of myrrh essential oils were found to be highly bactericidal against S. aureus, P. aeruginosa, and E. coli in this study. Although DMSO is used as an effective emulsifier, it may impair the antibacterial action of myrrh essential oils.

C. myrrha essential oil and related species has been recorded to contain a number of components, most notably terpenoids and sesquiterpenes. Terpenoids have been shown to possess a wide range of biological activities including molluscicide, antihyperglycemic [19], local anesthetic [20], cytotoxic [21] and antimicrobial [22]. In this study, myrrh essential oils showed significant bactericidal activity against gram-positive and gram-negative bacteria which are known to cause many infections of the urinary tract, respiratory tract mucous membranes and skin [23]. These bacterial species rapidly develop resistance against many antibiotics through various mechanisms [24, 25]. Thus, the observed antibacterial activity of essential oils made it particularly useful because microbial resistance against myrrh essential oils has not been reported. Several studies with different extraction methods recorded antibacterial activity of myrrh. In Egypt, Omer and his colleagues found that myrrh ethyl acetate extract inhibited bacterial growth of Proteus mirabilis , Proteus vulgaris and Acinetobacter baylyi [18] and other research team prepared two dosage forms against K. pneumoni, S. aureus and E. coli [26].In Saudi Arabia , Bhattacharjee and Alenezi concluded that myrrh oil has the potential activity that kills bacteria without resistance development [27]. The result of a study was done in Iraq referred to antimicrobial potential of myrrh ethanolic extract against 12 clinical isolates of gram negative and gram positive [28]. In conclusion, the in vitro antimicrobial activity of myrrh hexane extract, which was recorded in this study , along with the results of other studies consistent with the results of our study

ISSN: 2776-1010 Volume 3, Issue 7, July, 2022

confirmed the beneficial effects of myrrh extracts with a good selectivity index (SI > 3 [14] makes it a promising and safe candidate for implying in pharmaceutical products and natural medicine use.

References

1. Haffor AS (2010). Effect of myrrh (Commiphora molmol) on leukocyte levels before and during healing from gastric ulcer or skin injury. J. Immunotoxicol. 7, 68–75
2. Lemenith, Mulgeta, and Demel Teketay (2003). "Frankincense and myrrh resources of Ethiopia: II. Medicinal and industrial uses." SINET: Ethiopian Journal of Science 26, no. 2: 161-172.
3. Hanif, M. A., Nisar, S., Khan, G. S., Mushtaq, Z., & Zubair, M. (2019). Essential oils. In Essential oil research (pp. 3-17). Springer, Cham.
4. Elzayat, E. M., Auda, S. H., Alanazi, F. K., & Al-Agamy, M. H. (2018). Evaluation of wound healing activity of henna, pomegranate and myrrh herbal ointment blend. Saudi pharmaceutical journal, 26(5), 733-738.
5. Lee, K., Lee, J. H., Kim, S. I., Cho, M. H., & Lee, J. (2014). Anti-biofilm, anti-hemolysis, and anti-virulence activities of black pepper, cananga, myrrh oils, and nerolidol against Staphylococcus aureus. Applied microbiology and biotechnology, 98(22), 9447-9457.
6. Ebani, V.V., Bertelloni, F., Najar, B., Nardoni, S., Pistelli, L. and Mancianti, F., 2020. Antimicrobial activity of essential oils against Staphylococcus and Malassezia strains isolated from canine dermatitis. Microorganisms, 8(2), p.252.
7. Happy, Afroza Akter, Ferdoushi Jahan, and Md Abdul Momen. "Essential Oils: Magical Ingredients for Skin Care." Journal of Plant Sciences 9, no. 2 (2021): 54-64.
8. Lemenith, Mulgeta, and Demel Teketay. "Frankincense and myrrh resources of Ethiopia: II. Medicinal and industrial uses." SINET: Ethiopian Journal of Science 26, no. 2 (2003): 161-172.
9. Littman, R. J., Silverstein, J., Goldsmith, D., Coughlin, S., & Mashaly, H. (2021). Eau de Cleopatra: Mendesian Perfume and Tell Timai. Near Eastern Archaeology, 84(3), 216-229.
10. Sofowora, Abayomi, Eyitope Ogunbodede, and Adedeji Onayade(2013). "The role and place of medicinal plants in the strategies for disease prevention." African journal of traditional, complementary and alternative medicines 10.5: 210-229.
11. Laureano, Ana Cristina, Robert A. Schwartz, and Philip J. Cohen (2014). "Facial bacterial infections: folliculate." Clinics in dermatology 32.6: 711-714.
12. Panchaprateep, Ratchathorn, Aline Tanus, and Antonella Tosti (2015). "Clinical, dermoscopic, and Histopathological features of body hair disorders." Journal of the American Academy of Dermatology 72.5: 890-900.
13. Sakamoto, K., Jin, S. P., Goel, S., Jo, J. H., Voisin, B., Kim, D. & Nagao, K. (2021). Disruption of the end peptidase ADAM10-Notch signaling axis leads to skin dysbiosis and innate lymphoid cell-mediated hair follicle destruction. Immunity, 54(10), 2321-2337.
14. Khalil, Noha, Sahar Fikry, and Osama Salama(2020). "Bactericidal activity of Myrrh extracts and two dosage forms against standard bacterial strains and multidrug-resistant clinical isolates with GC/MS profiling." AMB Express 10, no. 1: 1-10.

ISSN: 2776-1010 Volume 3, Issue 7, July, 2022

15. Nazar Muhammad Samein1 Fouad Hussein Kamel (2019). Extraction of compounds from Thyme leaves and their antimicrobial activity. Diyala Journal of Agricultural Sciences (DJAS), 11(1): 18-24.
16. Fouad H. Kamel, Shawnim Abdulla Ismail, Sangar Sabah Sabir, Adnan N. Albidani (2020). Indian Journal of Public Health Research and Development.Vol.11.No.2.page 2481-84.
17. Fouad Hussein Kamel, Sara Ibrahim Othman (2020). Poultry resources multidrug resistance bacteria to human. Indian Journal of Public Health Research and Development.Vol.11.No.2.pp.2124-2127.
18. Omer, Elsayed, et al (2019) . "Plantago squarrosa Murray extracts inhibit the growth of some bacterial triggers of autoimmune diseases: GC–MS analysis of an inhibitory extract." Inflammopharmacology 27.2: 373-385.
19. Agwaya M, Vuzi P, Nandutu A(2016). Hypoglycemic activity of aqueous root bark extract Zanthoxylum chalybeum in alloxan-induced diabetic rats. J Diabetes Res. 2016:5.
20. Tsuchiya H.(2017). Anesthetic agents of plant origin: a review of phytochemicals with anesthetic activity. Molecules.: 10.3390/molecules22081369.
21. Shoaib M, Shah I, Ali N, Adhikari A, Tahir M, Shah S, Ishtiaq S, Khan J, Khan S, Umer M(2017) . Sesquiterpene lactone! a promising antioxidant, anticancer and moderate antinociceptive agent from Artemisia macrocephala jacquem. BMC Complement Altern Med.; 17(1):27.
22. Zengin H, Baysal AH.(2014). Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. Molecules.; 19(11):17773–17798.
23. Alanazi M, Alqahtani F, Aleanizy F.(2018).An evaluation of E. coli in urinary tract infection in emergency department at KAMC in Riyadh, Saudi Arabia: retrospective study. Ann Clin Microbiol Antimicrob.; 17(1):3.
24. Kidd T, Mills G, Sá-Pessoa J, Dumigan A, Frank C, Insua J, Ingram R, Hobley L, Bengoechea J.(2017) A Klebsiella pneumoniae antibiotic resistance mechanism that subdues host defences and promotes virulence. EMBO Mol Med.;9(4):430–447.
25. Zhu N, Kikuzaki H, Sheng S, Sang S, Rafi M, Wang M, Nakatani N, DiPaola R, Rosen R, Ho C. (2001) Furanosesquiterpenoids of Commiphora myrrha. J Nat Prod.;64(11):1460–1462.
26. Khalil N , Fikry S and Salama O. (2020). Bactericidal activity of Myrrh extracts and two dosage forms against standard bacterial strains and multidrug-resistant clinical isolates with GC/MS profling. AMB Expr, 10(21):1-10.
27. Bhattacharjee M and Alenezi T.(2020). Antibiotic in myrrh from Commiphora molmol preferentially kills nongrowing bacteria. Future Sci OA. 6(4).
28. Alasady D. , Mhadei K. and Naser M.(2021). Evaluation of Antimicrobial Activity of Commiphora myrrh against Standard Bacterial Strains and Clinical Isolates with Chemical Analysis Profiling . Journal of Pharmaceutical Research International 33(47A): 714-723.