
Antibacterial Activity of Triterpenoid Compounds

Qutaiba Saleh Essa^{1*}, Suha Maher Abed², Hayder Mudheher Abbas³

^{1*}*the General Directorate of Education for Nineveh, Iraq.*

^{2,3}*Department of Biology, College of Sciences, Tikrit University, Iraq.*

Corresponding Email: ^{1*} aboyahya12323@gmail.com

Received: 04 February 2024

Accepted: 19 April 2024

Published: 04 June 2024

Abstract: *The goal of the study was to ascertain whether particular pathogenic bacteria could be inhibited by triterpenoid substances obtained from Rosmarinus officinalis (rosemary) extracts. The time frame for completing this construction was July 2023–April 2024. At the beginning, 100 specimens were taken from patients who were undergoing treatment at the Burn Cure Center in Nineveh Governorate, Iraq. Routine biochemical testing was used to determine burn infections caused by bacteria, and the Vitek2 compact system was used to confirm the diagnosis. Acinetobacter baumani and Pseudomonas auroginosa were the most common isolates. In order to extract the substance, rosemary (R. officinalis) leaves were air-dried in the shade and then processed into a fine powder using a grinding machine. A Soxhlet device was used to extract 50 g of powdered leaves for six hours at 40–60 °C using 250 ml of 75% petroleum ether. Afterwards, the mixture was dried out at 40 °C in a rotating evaporator. After being weighed, the extract residue was stored until needed. Using gas chromatography/mass spectrometry (GC/MS), the crude extract was examined. Ursolic acid (1.38), oleanolic acid (3.27), and other triterpenoid substances with varying ratios were identified by GC-MS analysis. The antibacterial activity of triterpenoid compounds was assessed at concentrations of 12.5%, 25%, 50%, and 100% using the well-diffusion Kirby-Bauer method. The test was evaluated in comparison to six bacterial strains. The results indicated that Staphylococcus aureus (50%:22mm – 100%:26mm), Staphylococcus haemolyticus (100%:17mm), and Acinetobacter baumani (50%:18mm – 100%:22mm) had the highest inhibitory.*

Keywords: *Rosmarinus Officinalis, Triterpenoid, Antibacterial Activity, GC-MS.*

1. INTRODUCTION

The skin, being the largest organ in the body, is considered to be one of the most vital barriers against external infections (Dąbrowska, A. K., et al., 2018). This organ also plays a role in sensory perception, hormone balancing, temperature and humidity management, and burn injuries that cause skin tissue deterioration and loss of integrity (Dąbrowska, A. K., et al.,

2018). Resolving burn injuries is becoming more challenging, making it one of the most prevalent public health emergencies globally. Burn injuries are caused by electric shock, radiation, fire, strong acids, fuel, and other toxic substances. They can also result from organic tissue injury (Uddin et al., 2018). Because burns remove the skin's outer layer, bacteria can penetrate and infect the injured area (Mesbahi et al., 2021)

Burn injuries and the ensuing infections have been one of the main issues facing medical systems worldwide (Kelly et al., 2022). An estimated 38,000 people in the US sought medical assistance and were admitted to burn centers in 2018 according to reports (Glat et al., 2024). Burn infections continue to be one of the leading causes of death in the modern world, despite the advancements in burn care over the past 50 years (Vinaik et al., 2019). Many studies over the past ten years have shown that 42–65% of burn patients pass away from microbial infections (Vinaik et al., 2019). Furthermore, those with infectious burns have a death rate that is about twice as high as people with non-infectious burns (Medisa Primasari & Budi, 2024). The removal of the skin's protective layer, which impairs immunity and makes the body more vulnerable to infections, is the most detrimental effect of burns (Gour. et al, 2024). Moreover, the more complex interaction of anti-inflammatory signals results in anomalies in the innate and adaptive immune systems (Gour. et al., 2024). Furthermore, tracheal intubation, arterial lines, venous and urinary catheters, and prolonged hospital stays have been associated with an increased risk in patients with burns (Duan et al., 2024). Antibiotic resistance emerged rapidly after the first antibiotic was discovered and is presently one of the largest issues facing the medical community, especially in burn units (Liu et al., 2024). In addition to posing a serious risk of nosocomial infections, including lung, urinary tract, and cellulitis infections, multidrug-resistant microbes are one of the main reasons of death for burn patients. The development of multidrug-resistant illnesses is undoubtedly influenced by both empirical antibiotic therapy and extended hospital stays. Gram-positive bacteria were the most often isolated pathogens in the early days of admitting burn patients, and their antibiotic profile was more sensitive. With time, gram-negative bacteria develop greater resistance. (Hemmati et al, 2023)

Aromatic rosemary is a native of the Mediterranean region, *Rosmarinus officinalis* L. (Labiaceae). Rosemary essential oils (REOs) have been utilized in aromatherapy, preparing food, and medical applications since antiquity (Meziane. et al, 2024), and they are recognized in many nations and listed in official pharmacopeias (Lolas .et al,2024). The antibacterial, insecticidal, anti-inflammatory, and antioxidant qualities of these substances have led to their widespread application in cosmetics, therapy, and sterilizing (González-Minero et al., 2020). Furthermore, these potentially useful organic substances have been applied in novel ways in other fields, including medicine delivery methods (Kalaki Kordkolaei et al., 2020), non-antibiotic feed additives (Sgarro et al., 2024), and innovative packaging techniques (Hosseini et al., 2021). REOs are used as flavorings in food, packaging, and skin care products for a long time in the cosmetics industry.

2. RELATED WORKS

Though much remains unknown about their pharmacology, pentacyclic triterpenoids are among the most important compounds identified in plants. The triterpenoids with oleanane,



ursane, and lupane skeletons—most notably oleanolic acid and ursolic acid—are the most often studied of these secondary plant metabolites. Gudoityte et al., 2021).

In many scientific and technological domains, gas chromatography (GC) is a widely employed method. GC has been important in determining the number of components and their ratios in a combination for more than 50 years. It is uncertain and constrained how these separated and measured molecules' types and chemical structures can be determined, which calls for the use of a spectrophotometer detecting tool. Of them, the mass spectrometric detector is the most commonly used. (MSD), which offers the mass spectrum—or the molecule's "fingerprint" When a high resolution mass spectrometer is used, mass spectral examinations provide the molecular weight, element composition, presence of functional groups, and, in certain cases, the structure and location isomerism of the molecule. (Muhamma, al., 2024)

Mass spectrometry and gas chromatography used to detect different chemicals in an extract is known as gas chromatography/mass spectrometry (GC/MS). (Muhammad et al., 2024). One study goal was to include: Using the GC-Mass method, extract the triterpenoid components from the rosemary plant (*Rosemarinus officinalis*), and investigate the antibacterial activity of the rosemary extract against certain isolates.

3. METHODOLOGY

Materials and Methods:

Collection of Burns Samples:

This construction was completed between July 2023 and April 2024. Initially, patients at the Burn Cure Center in Nineveh Governorate, Iraq, provided one hundred specimens. Based on standard biochemical testing, bacteria isolated from burn infections were identified, and the Vitek2 compact system was used to validate the diagnosis.

Solutions:

1. The McFarland Turbidity Standard

The McFarland No. 0.5 turbidity standard before standardizing the amount of bacterial cells, which should be 1.5×10^8 CFU/ml, the components were well combined.

2. Sigma-Aldrich (Sigma-Aldrich, Darmstadt, Germany) was the source of the analytical grade dimethyl sulfoxide (DMSO).

Plant Samples Collection, Preparing, and Extraction of Essential Oils:

After being gathered from various neighborhood stores in Mosul, the rosemary (*R. officinalis*) leaves were air-dried in the shade and ground into a fine powder using a grinding machine. Using a soxhlet apparatus, 50 g of leaf powder were extracted over the course of six hours at 40–60 °C using 250 ml of 75% petroleum ether. A rotary evaporator operating at 40°C was then used to dry the solution. For subsequent research, 400 mg/ml of rosemary extract was reconstituted in dimethyl sulfoxide (DMSO) (Jai Kumar & Geetha, 2021).

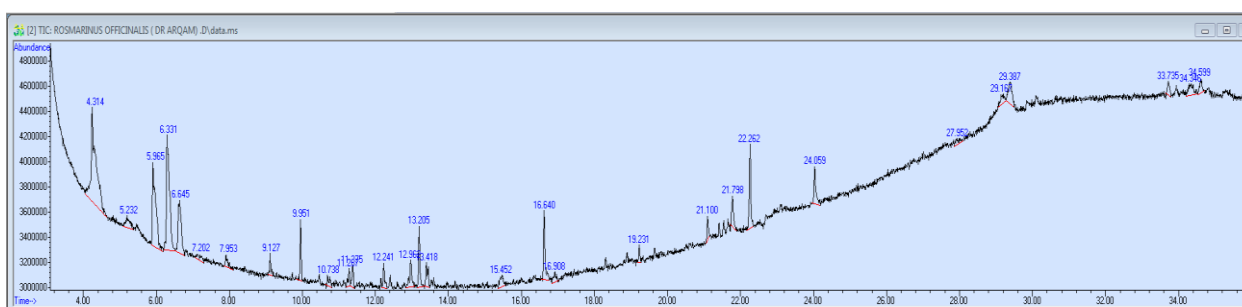
Gas Chromatography/Mass Spectrometry (GC-MS):

The components of *R. officinalis*' extracted essential oils were examined using a Perkin Elmer Clarus 500 gas chromatograph connected to a Perkin Elmer Clarus 560 mass spectrometer. A Perkin Elmer Elite-5 fused-silica capillary column (30 m × 0.25 mm, film thickness 0.25 μm) was employed to separate the components of the EOs. The temperature of the column was set to vary at a rate of 4 °C/min from 50 °C for 5 min to 280 °C. Throughout all of the chromatographic runs, the carrier gas flow rate of helium was maintained at 1 mL/min. 0.2 μL of pure EO was injected in split mode at 250 °C with a splitting ratio of 1:50. A comprehensive scan mode encompassing 50-500 m/z was gathered. By comparing the retention indices of the EOs to those documented in the literature and their MS to reference spectra in the NIST mass spectrometry data center, the chemical components of the EOs were identified.

4. RESULTS AND DISCUSSION

Results:

The identification and quantification of thirty distinct compounds from *R. officinalis* EOs were made possible by the GC-MS analysis results (Figures 1 and 2). These substances, which are triterpenoid compounds, such as ursolic acid (1.38) and oleanolic



Acid (3.27)

Figure (1)

Data Path: D:\MassHunter\GCMS\1\data\

Data File: ROSMARINUS OFFICINALIS

Acq On: 17 Dec 2023 10:20

Operator:

Sample: ROSMARINUS OFFICINALIS.

Misc:

ALS Vial: 1 Sample Multiplier: 1

Search Libraries: C:\GCMS\firmware\NIST11.L

Peak	Retention Time	Area% (Concentrations)	Library/ Id	Reference
26	29.165	1.38	C:\GCMS\ firmware Ursolic acid	128345

27	29.390	3.27	C:/GCMS/ firmware Oleanolic acid	181285
----	--------	------	----------------------------------------	--------

Figure -2

The antibacterial activity:

The antibacterial activity of triterpenoid compounds were assessed against six bacterial strains (at concentrations 12.5%, 25%, 50%, 100%)

strains	12.5 %	25 %	50%	100 %
Acinetobacter baumani	16	16	18	22
Staphylococcus aureus	15	19	22	26
Staphylococcus haemolyticus	R	R	13	17
E.coli	R	R	R	R
Pseudomonas auroginosa	R	R	R	R
Proteus mirabilis	R	R	R	R

Figure (3)

Discussion:

The findings illustrated in Figure 3 indicate that the triterpenoid compounds exhibited greater efficacy against staphylococcus aureus (gram-positive bacteria) in comparison to Acinetobacter baumani (gram-negative bacteria). The structure of these bacterial types' cell walls could be the cause of this. Moreover, efflux pumps, which remove several substances from the periplasm and transport them outside of the cell, strengthen the intrinsic resistance of gram-negative bacteria (Maillard & Pascoe, 2024).

5. CONCLUSION

The results of this study show that the triterpenoid compounds exhibited potent antibacterial properties, suggesting that they could be employed as both natural and pharmaceutical treatments to treat bacterial infections that cause burns.

6. REFERENCES

1. Dąbrowska AK, Spano F, Derler S, Adlhart C, Spencer ND, Rossi RM. The relationship between skin function, barrier properties, and body-dependent factors. *Skin Research and Technology*. 2018 May; 24(2):165-74.
2. Duane TM, Huston JM, Collom M, Beyer A, Parli S, Buckman S, Shapiro M, McDonald A, Diaz J, Tessier JM, Sanders J. Surgical infection society 2020 updated guidelines on the management of complicated skin and soft tissue infections. *Surgical infections*. 2021 May 1; 22(4):383-99.
3. Uddin, M. N., Jabeen, B., Saadat Mehmood, M., & Rizwan, M. (2018). 15. Characterization of isolated bacteria from burn patients and its susceptibility against different antibiotics in district Swat. *Pure and Applied Biology (PAB)*, 7(1), 121-132.



4. Tchakal-Mesbahi A, Abdouni MA, Metref M. Prevalence Of multidrug-resistant bacteria isolated from burn wounds In Algeria. *Annals of burns and fire disasters*. 2021 Jun 6; 34(2):150.
5. Hemmati J, Azizi M, Asghari B, Arabestani MR. Multidrug-resistant pathogens in burn wound, prevention, diagnosis, and therapeutic approaches (Conventional Antimicrobials and Nanoparticles). *Canadian Journal of Infectious Diseases and Medical Microbiology*. 2023 Jul 22; 2023.
6. Meziane H, Zraibi L, Albusayr R, Bitari A, Oussaid A, Hammouti B, Touzani R. *Rosmarinus officinalis* Linn. Unveiling its multifaceted nature in nutrition, diverse applications, and advanced extraction methods. *Journal of Umm Al-Qura University for Applied Sciences*. 2024 Apr 3:1-29.
7. Lolas, A., Molla, A., Georgiou, K., Apostologamvrou, C., Petrotou, A., & Skordas, K. (2024). Effect of Mussel Shells as Soil pH Amendment on the Growth and Productivity of Rosemary (*Rosmarinus officinalis* L.) Cultivation. *Agriculture*, 14(1), 144.
8. Lolas A, Molla A, Georgiou K, Apostologamvrou C, Petrotou A, Skordas K. Effect of Mussel Shells as Soil pH Amendment on the Growth and Productivity of Rosemary (*Rosmarinus officinalis* L.) Cultivation. *Agriculture*. 2024 Jan 18; 14(1):144.
9. González-Minero FJ, Bravo-Díaz L, Ayala-Gómez A. *Rosmarinus officinalis* L. (Rosemary): An ancient plant with uses in personal healthcare and cosmetics. *Cosmetics*. 2020 Oct 3; 7(4):77.
10. Sgarro MF, Maggiolino A, Forte L, Matera R, Bifulco G, Claps S, Natrella G, De Palo P. Effect of dietary administration of red orange and lemon extract on volatile compounds: profile and sensory parameters of lamb meat. *Italian Journal of Animal Science*. 2024 Dec 31; 23(1):101-13.
11. Hosseini F, Miri MA, Najafi M, Soleimanifard S, Aran M. Encapsulation of rosemary essential oil in zein by electrospinning technique. *Journal of Food Science*. 2021 Sep; 86(9):4070-86.
12. Kalaki Kordkolaei S, Kanani MR, Tabefam M, Namazi Sarvestani N, Hamburger M, Moridi Farimani M. Terpenoids and phenolics of *Micromeria persica*. *Natural product research*. 2020 Oct 17; 34(20):2913-8.
13. Al Bayati MH, Cengiz MF, Kitiş YE, Çınar O. Comparison of antimicrobial activities of oregano, lavender, sage, anise and clove extracts obtained by supercritical fluid carbon dioxide extraction and essential oils obtained by hydrodistillation. *Journal of Essential Oil Research*. 2024 Mar 30:1-3.
14. Muhamma SB. Fourier Transform Infra-Red Spectroscopy and Gas Chromatography/Mass Spectrometry (GC/MS) in Herbal Medicine and Pharmaceutical Drugs Analysis. *Journal of Current Medical Research and Opinion*. 2024 Mar 16; 7(03):2165-72.
15. Balbaa MT, Taher HM, Kamal Hamza N, Balbool BA. Inhibition of Glucosyltransferase enzyme of *Streptococcus mutans* by natural products-A Literature Review. *MSA Dental Journal*. 2023 Oct 1; 2(4):99-105.
16. Kelly EJ, Oliver MA, Carney BC, Shupp JW. Infection and burn injury. *European Burn Journal*. 2022 Feb 22; 3(1):165-79.



17. Glat P, Quirk L, Hultman S, Kesey J, Jain A, Griswald J, Fitzgerald N, Wibbenmeyer L, Amani H, Cramer C, Hickerson WL. Establishing Consensus of Best Practice for CEA Use in Treatment of Severe Burns: A US Burn Provider Delphi Study. *Journal of Burn Care & Research*. 2024 Mar 19; irae050.
18. Vinaik R, Barayan D, Shahrokhi S, Jeschke MG. Management and prevention of drug resistant infections in burn patients. *Expert review of anti-infective therapy*. 2019 Aug 3; 17(8):607-19.
19. Primasari M, Budi AS, Hariani L, Kurniati ND, Saputro ID. Risk factors of emerging multidrug resistant *Acinetobacter baumannii* in burn patients at burn Unit of Dr. Soetomo Hospital. *The Medical Journal of Malaysia*. 2024 Mar 1; 79(2):115-8.
20. Gour N, Yong HM, Magesh A, Atakkatan A, Andrade F, Lajoie S, Dong X. A GPCR-neuropeptide axis dampens hyperactive neutrophils by promoting an alternative-like polarization during bacterial infection. *Immunity*. 2024 Feb 13; 57(2):333-48.
21. Duan D, Deng H, Chen Y, Wang Y, Xu W, Hu S, Liu D, Mao Y, Zhang Z, Xu Q, Han C. Associated predictors of prolonged length of stay in patients surviving extensive burns: A large multicenter retrospective study. *Burns*. 2024 Mar 1; 50(2):413-23.
22. Liu GY, Yu D, Fan MM, Zhang X, Jin ZY, Tang C, Liu XF. Antimicrobial resistance crisis: could artificial intelligence be the solution? *Military Medical Research*. 2024 Jan 23; 11(1):7.
23. Maillard JY, Pascoe M. Disinfectants and antiseptics: mechanisms of action and resistance. *Nature Reviews Microbiology*. 2024 Jan; 22(1):4-17.