Journal of Prevention, Diagnosis and Management of Human Diseases
Vol: 04, No. 02, July-Dec 2024
ISSN: 2799-1202, DOI: 10.55529/jpdmhd.42.35.41

Research Paper

Antibacterial activitiy of triterpeniod compounds

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Article Info

Article History:
Received: 27 April 2024
Revised: 06 July 2024
Accepted: 12 July 2024
Published: 28 August 2024

Keywords:
Rosmarinus Officinal
Triterpenoid compounds
Antibacterial Activity
GC-MS Analysis
Multidrug-Resistant Bacteria

ABSTRACT

Background: Burn wound infections represent a critical clinical challenge, with drug-resistant bacteria such as Acinetobacter baumannii and Pseudomonas aeruginosa frequently implicated. Plant-derived triterpenoid compounds, including those from Rosmarinus officinalis (rosemary), have demonstrated potential antimicrobial properties and warrant systematic investigation.
Objective: To evaluate the antibacterial activity of triterpenoid substances extracted from R. officinalis against pathogenic bacteria isolated from burn wound patients.
Methods: This study was conducted from July 2023 to April 2024. One hundred specimens were collected from burn patients at the Burn Care Center, Nineveh Governorate, Iraq. Bacterial identification was performed via routine biochemical tests and confirmed using the Vitek2 compact system. Rosemary leaves were shade-dried, powdered, and subjected to Soxhlet extraction using 250 ml of 75% petroleum ether at 40–60°C for six hours. The extract was concentrated via rotary evaporation at 40°C. Phytochemical profiling was conducted by gas chromatography/mass spectrometry (GC/MS). Antibacterial activity was assessed against six bacterial strains at concentrations of 12.5%, 25%, 50%, and 100% using the well-diffusion Kirby-Bauer method.
Results: A. baumannii and P. aeruginosa were the predominant isolates. GC/MS analysis identified ursolic acid (1.38%) and oleanolic acid (3.27%) among the triterpenoid constituents. The greatest zones of inhibition were recorded for Staphylococcus aureus (22 mm at 50%; 26 mm at 100%), Acinetobacter baumannii (18 mm at 50%; 22 mm at 100%), and Staphylococcus haemolyticus (17 mm at 100%).
Conclusion: Triterpenoid extracts from R. officinalis exhibit concentration-dependent antibacterial activity, with notable efficacy against S. aureus and A. baumannii. These findings support further investigation of rosemary-derived compounds as adjunctive agents in burn infection management.

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1. INTRODUCTION

The skin, being the largest organ in the body, is considered to be one of the most vital barriers against external infections [1]. 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 [1]. Resolving burn injuries is becoming more challenging, making it one of the most prevalent public health emergencies globally [2]. Burn injuries are caused by electric shock, radiation, fire, strong acids, fuel, and other toxic substances. They can also result from organic tissue injury [3]. Because burns remove the skin's outer layer, bacteria can penetrate and infect the injured area [4].

Burn injuries and the ensuing infections have been one of the main issues facing medical systems worldwide [5]. An estimated 38,000 people in the US sought medical assistance and were admitted to burn centers in 2018 according to reports [6]. 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 [7]. Many studies over the past ten years have shown that 42–65% of burn patients pass away from microbial infections [7]. 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 [8]. Moreover, the more complex interaction of anti-inflammatory signals results in anomalies in the innate and adaptive immune systems [8]. Furthermore, tracheal intubation, arterial lines, venous and urinary catheters, and prolonged hospital stays have been associated with an increased risk in patients with burns [9]. 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 [10]. 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 [11].

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 [12], [13] and they are recognized in many nations and listed in official pharmacopeias [14], [15]. The antibacterial, insecticidal, anti-inflammatory, and antioxidant qualities of these substances have led to their widespread application in cosmetics, therapy, and sterilizing [16], [17]. Furthermore, these potentially useful organic substances have been applied in novel ways in other fields, including medicine delivery methods [18], [19] non-antibiotic feed additives [20], and innovative packaging techniques [21]. REOs are used as flavorings in food, packaging, and skin care products for a long time in the cosmetics industry.

2. RELATED WORK

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.

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 [22].

Mass spectrometry and gas chromatography used to detect different chemicals in an extract is known as gas chromatography/mass spectrometry (GC/MS) [22]. One study goal was to include: Using the GC-Mass method, extract the triterpeniod components from the rosemary plant (Rosemarinus officinal is), and investigate the antibacterial activity of the rosemary extract against certain isolates [23].

3. METHODOLOGY

3.1. 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 × 108 CFU/ml, the components were well combined.
2. Sigma-Aldrich (Sigma-Aldrich, Darmstadt, Germany) was the source of the analytical grade dimethyl sulfoxide (DMSO).

3.2. 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).

3.3. Gas Chromatography/Mass Spectrometry (GC-MS)

The components of R. officinal is' 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

4.1. Result

The identification and quantification of thirty distinct compounds from R. officinalis EOs were made possible by the GC-MS analysis results Figure 1. These substances, which are triterpeniod compounds, such as ursolic acid (1.38) and oleanol Acid (3.27).

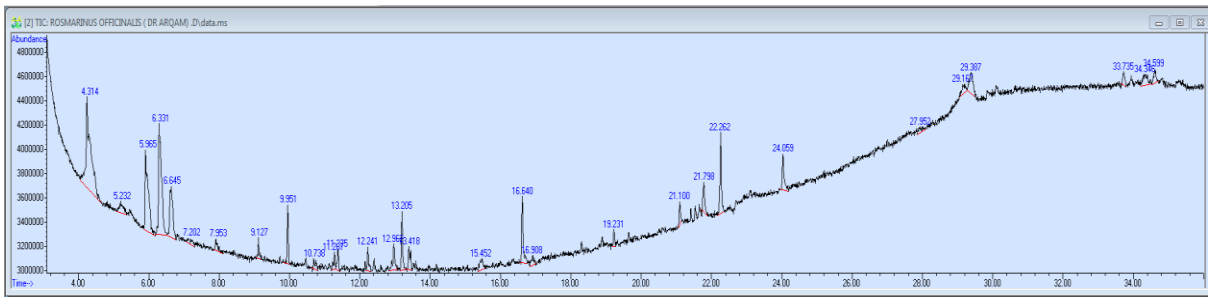

Figure 1. Total Ion Chromatogram (TIC) of GC/MS Analysis of Rosmarinus officinalis Crude Triterpenoid Extract

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

Table 1. GC/MS Analysis Results of Triterpenoid Compounds Identified in Rosmarinus officinalis Crude Extract

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

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

Table 2. Inhibition Zone Diameters (mm) of Rosmarinus officinalis Triterpenoid Extract Against Bacterial Strains at Various Concentrations Using the Well-Diffusion Method

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
Pseudomomonas auroginosa	R	R	R	R
Proteus mirabilis	R	R	R	R

4.2. Discussion

The findings illustrated indicate that the triterpeniod compounds exhibited greater efficacy against staphylococcus aureus (gram-positive bacteria) in comparison to Acinetobacer 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.

5. CONCLUSION

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

Acknowledgments

The authors have no specific acknowledgments to make for this research.

Funding Information

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Qutaiba Saleh Essa	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	
Suha Maher Abed		✓		✓		✓		✓	✓			✓		✓
Hayder Mudheher Abbas	✓	✓		✓	✓		✓			✓		✓	✓	

C: **C**onceptualization
M: **M**ethodology
So: **So**ftware
Va: **Va**lidation
Fo: **Fo**rmal analysis
I: **I**nvestigation
R: **R**esources
D: **D**ata Curation
O: Writing- **O**riginal Draft
E: Writing- Review& **E**diting
Vi: **Vi**sualization
Su: **Su**pervision
P: **P**roject administration
Fu: **Fu**nding acquisition

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Informed Consent

All participants were informed about the purpose of the study, and their voluntary consent was obtained prior to data collection.

Ethical Approval

The study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki and approved by the relevant institutional authorities.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to Cite: Qutaiba Saleh Essa, Suha Maher Abed, & Hayder Mudheher Abbas. (2024). Antibacterial Activitiy of Triterpeniod Compounds. Journal of Prevention, Diagnosis and Management of Human Diseases, 4(2), 35–41. https://doi.org/10.55529/jpdmhd.44.35.41

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