

*Microbial Science Archives* (ISSN: 2583-1666)

An International Research Journal

Journal homepage: [www.microbial.sciencearchives.org](http://www.microbial.sciencearchives.org)

<https://doi.org/10.47587/MSA.2023.3104>



Research Article

## Detection of fatty acids in the cell membrane of *Escherichia hermanii* from different clinical samples in Mosul city, Iraq

Samaa Mones Saeed  and Aws Ibrahim Sulaiman 

Department of Biology, College of Science, University of Mosul, Mosul, Iraq

Received: Feb 2, 2023/ Revised: Mar 5, 2023/Accepted: Mar 7, 2023

(✉) Corresponding Author: [samaalhamdany@gmail.com](mailto:samaalhamdany@gmail.com)

### Abstract

Single dimension gas chromatography (GC) is frequently employed in conjunction with mass spectrometry for the analysis of a wide range of lipid samples from a variety of sources (MS). The result of the analysis of membrane lipid of *E. hermanii* by this technique is that most of the lipids are (Ricinoleic.acid:12420143, Glycerin:6814721 ,2 ,4 Do decadienal, E, E:5915936, Chloromethyl 6-chloroundecanoate:4761461, Ethyl 5-methylhexanonate:3468461, Glycerin:3272473, propane,2-fluoro-2-methyl :3146268, p-Dioxane-2,3-diol:2993346, N, N-Dimethyl-O-(1-methyl-butyl)-hydroxyl:2378662,1-5-Ethyl-tetrahydrofuran-2-yl-3,3-dimethyl:2305938) respectively.

**Keywords:** *Escherichia hermanii*, Fatty acid, cell membrane GC-MS analysis

### Introduction

*Escherichia hermanii* was first detected in 1982. It is a new species within the genus *Escherichia* based on information related to DNA and phenotypic characteristics, it is a gram-negative bacterium, facultatively anaerobic, rod-shaped, do not differ from opportunistic bacteria in that they cause various diseases in immunocompromised persons, such as cancer patients, AIDS and autoimmune diseases, some of them product carbapenems enzyme (Brenner et al., 1982; Rice et al., 1992).

This bacterium was isolated from various clinical sources such as wounds, burns, feces, and urine. in some cases, it was the main cause of nephritis, urinary tract infection, sepsis and infection, and the bloodstream associated with the catheter (Poulou et al., 2008; Kaewpoowat et al., 2013; Tong et al., 2014).

Gas chromatography separates mixture components, while mass spectrometry determines their chemical identities. Gas chromatographic methods, especially GC-MS and GC-FID, are critical chemical instruments for testing volatile

medications because they can separate a complex mixture of volatile organic molecules. Gas chromatography (GC) is a sensitive technology that accurately and reproducibly quantifies the fatty acid content of cells, tissues, and plasma/serum. GC-MS profiles can be difficult to evaluate visually for categorization or discrimination. Thus, chemometric methods help evaluate data and provide probability reasoning about the conclusion (Ghazi et al., 2022 and Savych et al., 2022).

This study aimed to identify types of fatty acids present in the cell membrane of *E. hermanii*

### Material and methods

*E. hermanii* isolated from clinical sources after being identified by vitek 2 and molecular we prepare the sample for GC-Mass by (Sasser, 1990)

### Harvesting

40mg of bacterial cells was collected using loop from the 3/4 of culture plate.

### Saponification

1ml of reagent 1 is applied to each cultured tube and tubes were sealed and vortexed. The tubes were put in water at 100°C for 5 min. Tubes were vortexed strongly for 5-10 sec and returned to the water bath for a 30 min heating process.

### Methylation

Two milliliters of Reagent 2 are poured into the uncapped, chilled tubes. There is a short vortex created at the end of the tubes and then they are sealed. The tubes were heated at 80±1°C for 10±1 min after being subjected to a vortex. (Time and temperature are very important for this stage.).

### Extraction

Slow rotation using a clinical tumble machine for around 10 minutes. Pipetting out the aqueous (lower) phase from the tubes and disposing of it is the first step once the caps have been removed.

### Base Wash

3ml of reagent 4 was added in tubes to remain organic phase. The tubes were closed and tumbled for 5 min. After uncapping, around two-thirds of the organic phase is pipetted into a GC vial. The tubes closed for analysis. To separate fatty acids from lipids, four reagents are required.

### Reagent 1

Saponification was done using 45gm of NaOH, 150 ml of methanol, and 150 ml of distilled water. The use of an auto pipet for dispensing ensures reproducibility and permits a high number of assays each day.

### Reagent 2

For the methylation reaction, it will need 325ml of certified 6.0N HCl and 275ml of methyl alcohol. As a result, the solution's pH falls below 1.5, leading to fatty acid methylation (hence the higher volatility in a partly polar column). The methyl ester of the fatty acid is not very water soluble at the moment.

### Reagent 3

Extraction 200ml hexane and 200ml MTBE (100 methanol and 100 butanol) (Originally, it was supposed to be isobutene, but because it was not available, we modified it and used butanol instead with methanol to give us MTBE, and it did succeed). This extracts the fatty acid methyl esters in the organic phase by gas chromatography.

### Reagent 4

10.8gm of NaOH dissolved in 900ml of distilled water. A column can complete more than 10,000 analyses prior it is requiring repair. Iraq / University of Samarra / College of Applied Sciences, Central Laboratory performed the analysis using a Shimadzu instrument.

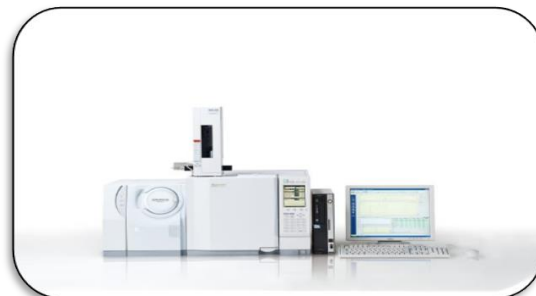


Fig. 1 GC-mass devise Shimadzu (GCMS -QP2010 PLUS)

### Results and discussion

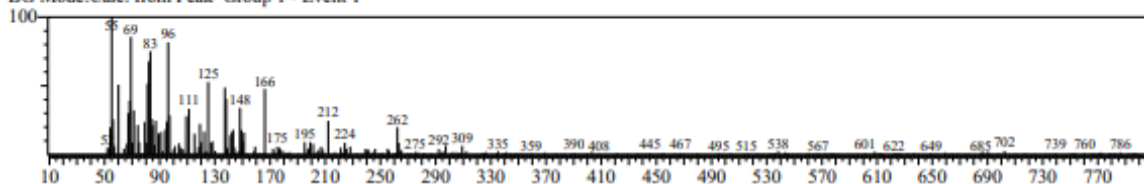
The result of the fatty acids analysis of this bacteria showed that they possessed a large number of fatty acids (Fig. 2), and that most of the lipids are (Ricinoleic.acid:12420143, Glycerin:6814721,2,4 Do decadienal, E, E:5915936, Chloromethyl 6-chloroundecanoate:4761461, Ethyl 5-methylhexanonate:3468461, Glycerin:3272473, propane,2-fluoro-2-methyl:3146268, p-Dioxane-2, 3- diol: 2993346, N, N- Dimethyl - O - (1-methyl-butyl)- hydroxy 1:2378662, 1-5-Ethyl-tetrahydrofuran-2-yl-3,3-dimethyl:2305938). and the concentration of the top ten fatty acids were the following in (Table 1).

Table 1. Top ten fatty acids with their concentrations

| Name of fatty acid                                | Area (concentration) |
|---|----------------------|
| 1. Ricinoleic acid                                | 12420143             |
| 2. Glycerin                                       | 6814721              |
| 3. 2,4Dodecadienal, (E, E)                        | 5915936              |
| 4.Chloromethyl6-chloroundecanoate                 | 4761461              |
| 5. Ethyl 5-methylhexanonate                       | 3468461              |
| 6. Glycerin                                       | 3272473              |
| 7. propane,2-fluoro-2-methyl                      | 3146268              |
| 8. p-Dioxane-2,3-diol                             | 2993346              |
| 9.N, N-Dimethyl-O-(1-methyl-butyl)-hydroxyl       | 2378662              |
| 10.1-(5-Ethyl-tetra-hydrofuran-2-yl)-3,3-dimethyl | 2305938              |

<< Target >>

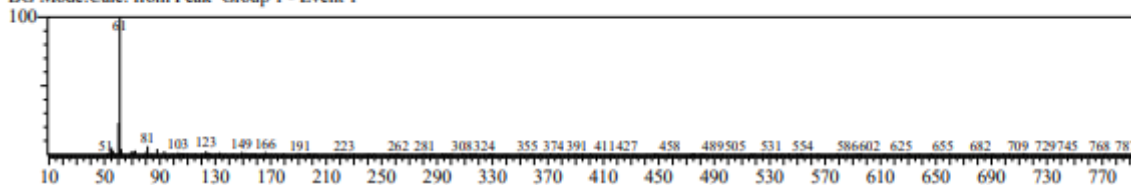
Line#:63 R.Time:10.115(Scan#:1424) MassPeaks:320  
 RawMode:Averaged 10.110-10.120(1423-1425) BasePeak:55.15(1446)  
 BG Mode:Calc. from Peak Group 1 - Event 1



Ricinoleic acid

<< Target >>

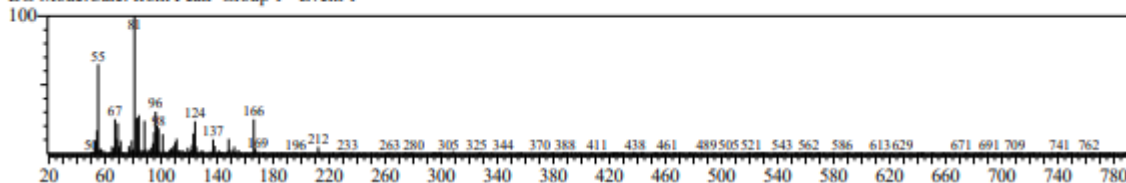
Line#:38 R.Time:5.570(Scan#:515) MassPeaks:366  
 RawMode:Averaged 5.565-5.575(514-516) BasePeak:61.10(7897)  
 BG Mode:Calc. from Peak Group 1 - Event 1



Glycerin

<< Target >>

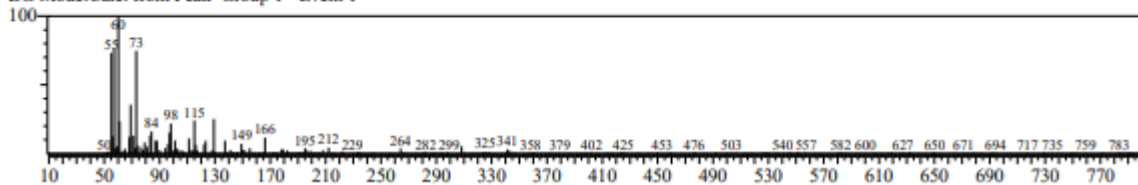
Line#:47 R.Time:7.510(Scan#:903) MassPeaks:449  
 RawMode:Averaged 7.505-7.515(902-904) BasePeak:81.10(22146)  
 BG Mode:Calc. from Peak Group 1 - Event 1



2,4Dodecadienal,(E,E)

<< Target >>

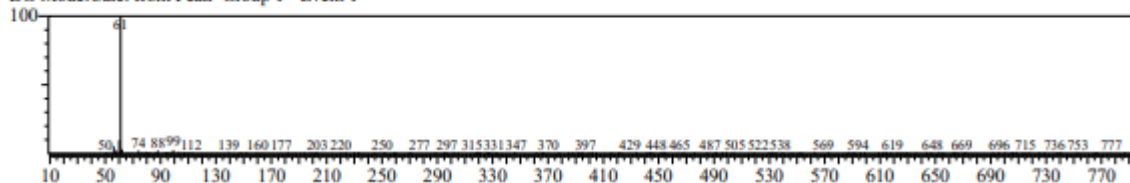
Line#:46 R.Time:7.310(Scan#:863) MassPeaks:385  
 RawMode:Averaged 7.305-7.315(862-864) BasePeak:60.10(4856)  
 BG Mode:Calc. from Peak Group 1 - Event 1



Chloromethyl6-chloroundecanoate

<< Target >>

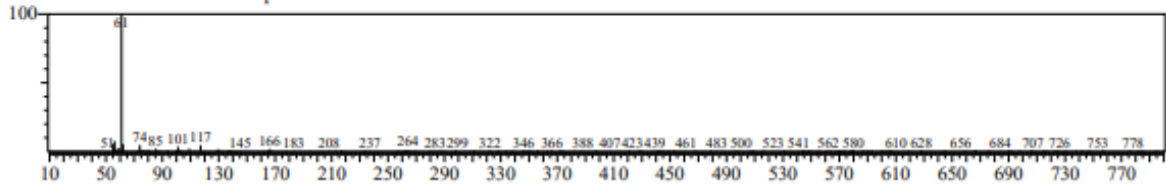
Line#:34 R.Time:5.350(Scan#:471) MassPeaks:365  
 RawMode:Averaged 5.345-5.355(470-472) BasePeak:61.10(12744)  
 BG Mode:Calc. from Peak Group 1 - Event 1



Ethyl 5-methylhexanoate

<< Target >>

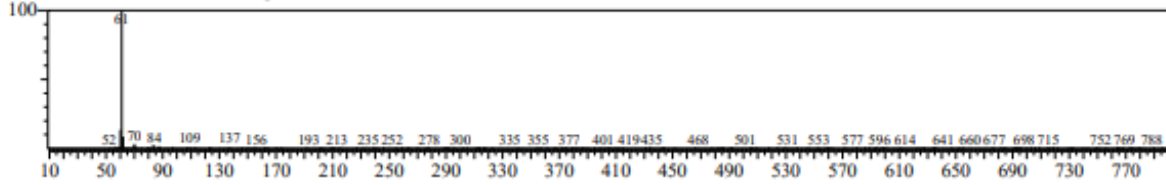
Line#:24 R.Time:4.810(Scan#:363) MassPeaks:337  
 RawMode:Averaged 4.805-4.815(362-364) BasePeak:61.10(11511)  
 BG Mode:Calc. from Peak Group 1 - Event 1



Glycerin

<< Target >>

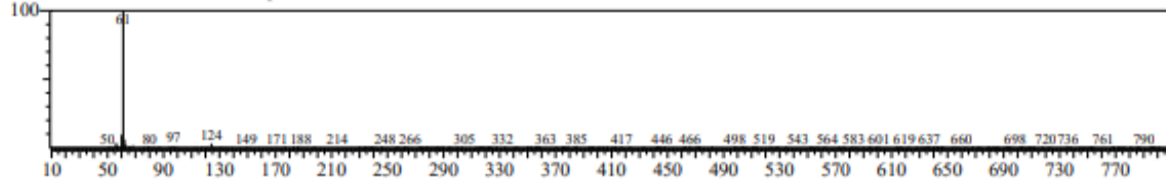
Line#:30 R.Time:5.145(Scan#:430) MassPeaks:373  
 RawMode:Averaged 5.140-5.150(429-431) BasePeak:61.10(16375)  
 BG Mode:Calc. from Peak Group 1 - Event 1



propane,2-fluoro-2-met

<< Target >>

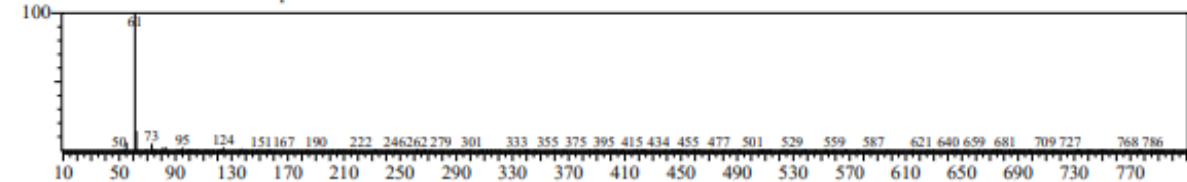
Line#:36 R.Time:5.455(Scan#:492) MassPeaks:340  
 RawMode:Averaged 5.450-5.460(491-493) BasePeak:61.10(21430)  
 BG Mode:Calc. from Peak Group 1 - Event 1



p-Dioxane-2,3-diol

<< Target >>

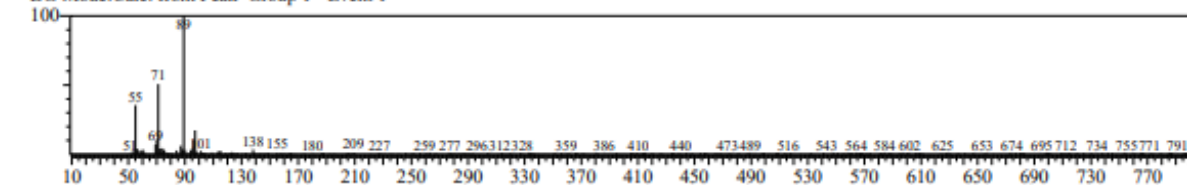
Line#:37 R.Time:5.525(Scan#:506) MassPeaks:344  
 RawMode:Averaged 5.520-5.530(505-507) BasePeak:61.10(12009)  
 BG Mode:Calc. from Peak Group 1 - Event 1



N, N-Dimethyl-O-(1-methyl-butyl)-hydroxyl

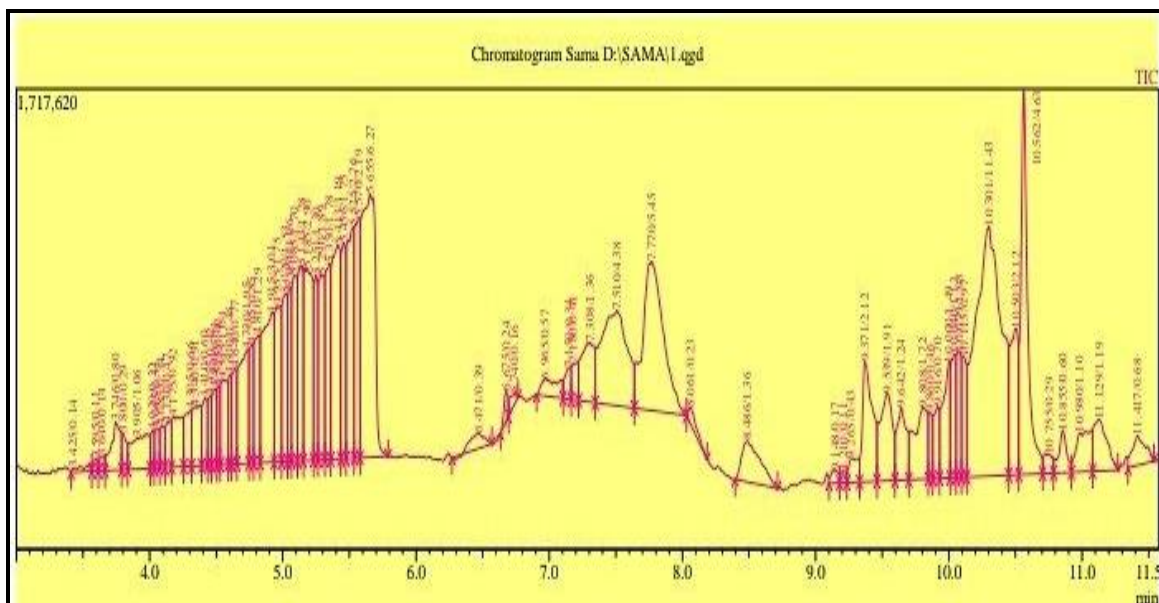
<< Target >>

Line#:53 R.Time:9.265(Scan#:1254) MassPeaks:425  
 RawMode:Averaged 9.260-9.270(1253-1255) BasePeak:89.10(12223)  
 BG Mode:Calc. from Peak Group 1 - Event 1



1-(5-Ethyl-tetra hydrofuran-2-yl)-3,3-dimethyl

Fig. 2 Analysis of the top ten fatty acids with their concentrations



**Fig. 3 Diagram of GC-Mass Analysis**

In contrast to many phenotypic characteristics, bacterial fatty acids are genetically considerably conserved due to their essential involvement in cell structure and function, according to Dawyndt and colleagues (2006). Since the development of GC analysis fatty acids in cells, various taxonomic investigations have made extensive use of this method. Numerous scientific articles have employed fatty acids with (9- 20) carbons to classify bacterial genera and species, including non-fermentative Gram-negative microbes. The identification of microbes based on their fatty acid component has been a standard in polyphasic taxonomy for more than 10 years, as a result of the various developments in automated calibration and interpretation of chromatographic profiles.

### Conclusions

It is concluded from this study that the highest fatty acid present in the cell membrane of *E.hermannii* bacteria is Ricinolic acid with a concentration of (12420143), which can be known through the discovery of materials capable of destroying this fatty acid as one of the means of eliminating this bacteria

### Ethics statement

We obtained the Ethical approval letter for our research from the ministry of health and environment / Ninawa Health Department Location with number (44816) on (14 /12/ 2021).

### Conflict of Interest

The author hereby declares no conflict of interest.

### Consent for publication

The author declares that the work has consent for publication.

### Funding support

The author declares that they have no funding support for this study.

### Acknowledgment

The researchers convey their gratitude and thanks to the University of Mosul/College of Science/Department of Biology for their assistance with this study.

### References

- Brenner, D., Davis, B., Steigerwalt, A., Riddle, C., McWhorter, A., Allen, S., Farmer III, J., Saitoh, Y. and Fanning, G. (1982). Atypical bio groups of 617 *Escherichia coli* found in clinical specimens and description of *Escherichia hermannii*. nov." *Journal of clinical microbiology* 15(4): 703-713.
- Dawyndt, P., Vancanneyt, M., Snauwaert, C., De Baets, B., De Meyer, H. and Swings, J. (2006). Mining fatty acid databases for detection of novel compounds in aerobic bacteria. *Journal of microbiological methods*. 66(3): 410-433.
- Ghazi, M. G. M., Lee, L. C., Sino, H. and Halim, M. I. A. (2022). Review of Contemporary Chemometric Strategies Applied on preparing GC-MS data in forensic analysis. *Microchemical Journal*. 107732.
- Kaewpoowat, Q., Permpalung, N. and Sentochnik, D. (2013). Emerging *Escherichia* pathogen. *J Clin Microbiol* 51(8):2785-2786.
- Poulou, A., Dimitroulia, E., Markou, F. and Tsakris A. (2008) *Escherichia hermannii* as the sole isolate from a patient with purulent conjunctivitis. *Journal of Clinical Microbiology*. 46(11):3848-49.
- Rice, E. W., Johnson, C. H., Wild, D. K. and Reasoner, D. J. (1992). Survival of *Escherichia coli* O157: H7 in drinking water associated with a waterborne disease outbreak of hemorrhagic colitis. *Letters in applied microbiology*, 15(2), 38-40.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids.

Savych, A., Marchyshyn, S., Mosula, L., Bilyk, O., Humeniuk, I. and Davidenko, A. (2022). Analysis of amino acids content in the plant components of the antidiabetic herbal mixture by GC-MS. *Pharmacia*. 69, 69.

Tong, Y. Q., Xin. B. and Sun, S. Q. (2014) Pyelonephritis Caused Solely by *Escherichia hermanii*. *Jundishapur Journal of Microbiology*.7(5):18138.

**How to cite this article**

Saeed, S. M. and Sulaiman, A. I. (2023). Detection of fatty acids in the cell membrane of *Escherichia hermanii* from different clinical samples in mosul city, Iraq. *Microbial Science Archives*, Vol. 3(1), 18-23. <https://doi.org/10.47587/MSA.2023.3104>

This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/)



**Publisher's Note:** MD International Publishing stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.