المجلة العراقية للبحوث الإنسانية والإجتماعية والعلمية
Iraqi Journal of Humanitarian, Social and Scientific Research
Print ISSN 2710-0952 Electronic ISSN 2790-1254
العـــدد 17 أيـار 2025
No.17 May 2025

تحليل GC-MS للمركبات النشطة بيولوجيًا من مستخلصات الإيثانول البكتيرية ذات الخصائص المضادة للبكتيريا الشبيهة بالسيفيكسيم

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الملخص

تم استخدام تقنية كروماتوغرافيا الغاز – مطياف الكتلة (GC-MS) لتحليل المستخلص الإيثانولي لعزلات بكتيرية يُعتقد أنها تنتج موادًا ذات نشاط مضاد حيوي شبيهة بالسيفيكسيم .كشف التحليل عن وجود عدة مركبات حيوية فعالة، مثل المركبات الحلقية غير المتجانسة، ومشتقات الأحماض الأمينية، وأنواع مختلفة من النيوكليوزيدات .ومن الجدير بالذكر أنه تم التعرف على مشتقات البيرولو[1,2-a]بيرازين-1,4-ديون، ومركبات قائمة على الثيوفين، والتي ترتبط هيكليًا بالمضادات الحيوية من نوع β-lactam مثل السيفيكسيم . تدعم هذه النتائج الفرضية القائلة بأن السلالات البكتيرية تمتلك القدرة على تصنيع مستقلبات مشابهة للسيفيكسيم .كما توفر هذه الدراسة فهمًا أعمق للمسارات الأيضية التي تساهم في إنتاج المضادات الحيوية لدى السلالات البكتيرية المعزولة من التربة.

الكلمات المفتاحية :- عينة تربة، ميكروبات، سيفيكسيم، GC-MS

GC-MS Analysis of Bioactive Compounds from Bacterial Ethanol Extracts with Cefixime-Like Antibacterial Properties

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Abstract:- Gas chromatography–mass spectrometry (GC-MS) was utilized to examine the ethanol extract of bacterial isolates believed to generatecefixime-antimicrobial substances. The evaluation uncovered several bioactive elements, such as heterocyclic substances, amino acid derivatives, and nucleoside variants. Significantly, pyrrolo[1,2-a]pyrazine-1,4-dione derivatives and thiophene-based compounds, structurally related to β-lactam antibiotics such as cefixime, were recognized. These results bolster the theory that the bacterial strains possess the ability to synthesize cefixime-like metabolites. The research additionally offers understanding of the metabolic routes that support antibiotic synthesis in bacterial strains obtained from soil.

keyword:-soil specimen , microbes , Cefixime , GC-MS

Introduction:

The worldwide increase in antimicrobial resistance poses a serious danger to public health, highlighting the pressing requirement for new antibiotic discovery and

advancement (Bush, 2018). Microorganisms residing in the soil, especially actinomycetesand specific Gram-positive bacteria, are recognized for generating a wide variety of bioactive secondary metabolites that act as abundant resources for pharmaceutical lead compounds (Takahashi &Omura, 2003). Among these, cephalosporins—β-lactam antibiotics—have continued to be a fundamental element in the management of bacterial infections because of their wide-ranging effectiveness and resilience against numerous β-lactamases (Drawz & Bonomo, 2010). Cefixime, a third-generation cephalosporin, is especially potent against Gram-negative bacteria and is commonly utilized in medical environments for treating urinary, respiratory, and gastrointestinal infections (Bryskier, 2005).

Structurally, cefiximeconsists of a β-lactam ring connected to adihydrothiazine ring, a vinyl group, and a thiophenemoiety, which adds to its improved antibacterial characteristics and resilience (Bush, 2018). These structural attributes function as biomarkersfor identifying cefixime-compounds similar to those found in natural extracts using sophisticated analytical methods.In the past few years, gas chromatography–mass spectrometry (GC-MS) has emerged as an essential instrument for detecting volatile and semi-volatile metabolites in intricate biological specimens (Elshafieet al., 2019). The integration of gas chromatographic separation with mass spectral identification facilitates accurate detection of cefixime-associated scaffolds such as thiophenes, pyrazines, and derivatives of amino acids (Walsh &Wencewicz, 2013).

To detect different chemicals within a test sample, analysts employ a method known as gas chromatography-mass spectrometry (GC-MS), which merges the benefits of mass spectry and gas chromatography. (Sparkman et al.,2011) . Uses of GC-MS encompass identifying narcotics, studying fires, analyzing environmental conditions, researching explosives, and recognizing unknown substances, including material samples collected from Mars during probe missions since the 1970s. GC-MS can also be utilized to detect chemicals on individuals or in baggage.utilisedin airport security. In specimens that were once thought to have broken down beyond recognition, it can also identify trace elements. It facilitates the analysis and discovery of materials in extremely low amounts, akin to liquidchromatographymass spectrometry (Jones,2019).

This research intends to explore the chemical makeup of ethanol extracts obtained from bacterial isolates in soil through GC-MS. The objective is to pinpoint possiblecefixime-compounds derived from structural resemblance and documented biological effects, thus aiding in the identification of new antimicrobialagents (Süssmuth & Mainz, 2017).

Materials and methods: -

Isolated of bacteria:

From 50 soil sample the Bacterial strains were sourced from soil samples through serial dilution and plating methods on nutrient agar. The colonies were re-cultured and incubated in aerobic settings to boost metabolite synthesis. Ethanol extraction involved immersing the bacterial biomass in 95% ethanol for 48 hours. The extract was filtered and concentrated under diminished pressure.

Gas chromatography–mass spectrometry (GC/MS).

Mass spectrometer evaluation and gas chromatography–mass spectrometry (GC/MS) GC/MS examination of the ethyl acetate extract (EAE) was carried out using a Shimadzu GC/MS (GC-17A) equipped with a ZB-1 MS fused silica capillary column (30 m × 0.25 mmi.d., film thickness 0.25 m) because of its exceptional antibacterial efficacy. A 70 eV ionization energy was used in an electron ionization system for GC/MS analysis. At a constant flow rate of 1 milliliter per minute, helium functioned as the carrier gas. The MS transfer line and injector temperatures were set to 320°C and 260°C, respectively.

The temperature of the oven was set to grow at a rate of 3°C per minute from 60°C to 320°C, then to remain isothermal for 11 minutes before increasing at a rate of 10°C per minute to 320°C. In the splitless arrangement, 1.0 liters of diluted samples (1/100, v/v in methanol) were manually injected. By contrasting each component's average peak area with the total areas, the relative fraction of each component was ascertained. mass spectrometer: Shimadzu GC/MS (GC-17A) equipment running at 70 eV; 1.5 s scan time; 40–300 amu mass range. ChemStation was the software used to handle chromatograms and mass spectra.

Result and discussion:

Isolation of bacteria

This research demonstrated that the most prevalent bacteria extracted from soil was*Ralstonia mannitolilytica*this research was inconsistent with studyLugauskaset al. (2005), who discovered that Bacillus species are the predominant bacteria in soils tainted with lead. The identification and prevalence of Bacillus subtilis in this research may not be unexpected since, in addition to their capability to generate spores, they are native to the soil ecosystem and are recognized for their persistence in such settings (Atlas and Bartha, 2007).

But the study was agreement with study (Mahala et al.,2024) showed that the *Ralstonia mannitolilytica*is a newly recognized aerobic gram-negative bacterium responsible for infections in immunocompromised individuals. It has been found in soil, water, the human body, and on plant surfaces; however, this is the initial report of*Ralstonia mannitolilytica*detached from the larvae of Brahmina coriacea in the world. The study was agreement with study (Cycoń et al.,2011) showed that Strains of *Burkholderia cepacia* (DS-1), *Pseudomonas* sp. (DS-2) and *Sphingomonas paucimobilis*(DS-3) extracted from soil that had been treated with 2,4-D earlier were able to break down this herbicide in MSM and utilize it as their sole carbon and energy source. As shown bythe information gathered from chemical evaluations suggests that strains DS-1 and DS-2 might also have the capability to break down 2,4-DCP.

Table (1) Identification of bacteria by Vitek 2 system

No.	Symbol	The identification by Vitek 2	Cefixime concentration (mg/L)
1	S 1	*Ralstonia mannitolilytica*	30.816
2	S 5	*Sphingomonas paucimobiltis*	35.594
3	S 6	*Ralstonia mannitolilytica*	41.722
4	S 7	Non or low reactive biopattern	32.095
	***P* value**		**0.001289**
	LSD		**2.597127**

GC-MS Examination of Bacterial Ethanol Extract

Gas chromatography–mass spectrometry (GC-MS) examination was performed on the ethanol extract of bacterial isolates believed to be producing cefixime-like antibacterial substances. The examination disclosed 25 chromatographic peaks, with each peak linked to one or several bioactive elements. Table (4-7) consolidates the retention times (RT), peak areas (%), and the recognized compounds.

Table (1) Significant Compounds Detected by GC-MS in the Ethanol Extract

Peak RT (min)	Area (%)	Identified Compounds	Activity
1	4.424 6.42	2-Nonanol 2-Heptanol	Antimicrobial, Solvent
5	7.398 6.61	Glycyl-D-threonine Oxirane derivatives	Antibacterial, Antiviral
6	7.587 13.30	2-Methoxythiophene, Thiophene 1-Propene-1,1'-thiobis	Antibacterial, Anti-

			inflammatory
8	9.641 2.07	Alanine N-methyl-n-propoxycarbonyl-butyl ester	Antimicrobial peptide derivative
13	15.524 6.68	N-(3-Methylaminopropyl)-N-methylformamide Cyanoacetylurea	DNA synthesis inhibitor bioactive amide
15	16.776 9.05	l-Valine l-Norvaline derivatives	β-lactam analogs
16	17.226 5.80	Pyrrolo[1,2-a]pyrazine-1 4-dione derivatives	Broad-spectrum antimicrobial
18	18.218 11.30	Pyrrolo[1,2-a]pyrazine-1 4-dione	Cefixime-similar core (pyrazine)
19	18.446 10.57	Hexahydro-pyrazinone derivatives	Antibiotic precursor analogs
23	23.005 2.43	Ergotaman-3’ Pyrrolo[1,2-a]pyrazine	Antibacterial CNS-active
24	23.377 1.20	Cytidine derivatives	Nucleoside analog Antiviral
Other Peaks Various	<1% - 5%	Hydrocarbons amino acid esters siloxanes	Potential co-metabolites

Representative GC-MS Chromatogram

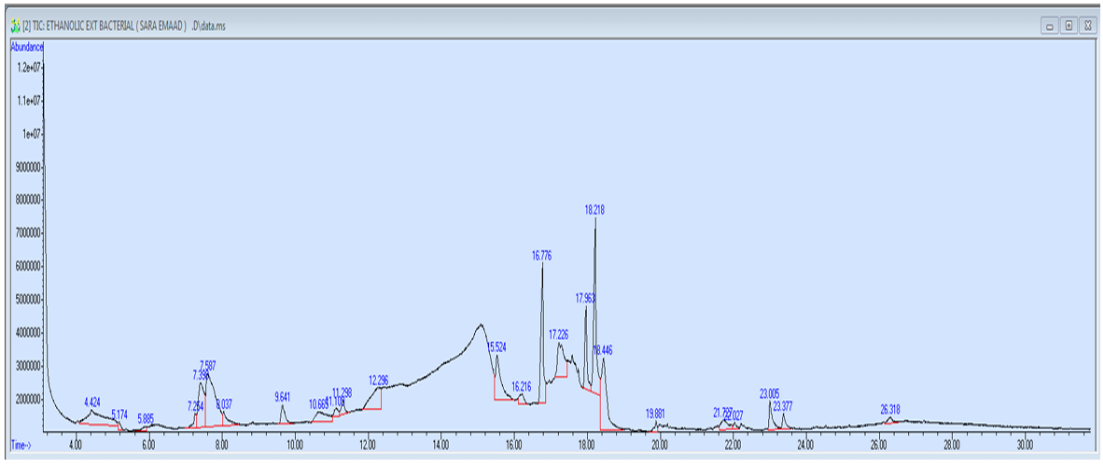

Figure 1 .GC-MS Total Ion Chromatogram (TIC) of ethanol extract from bacterial isolate.

Diversity of Compounds Detected

GC-MS examination revealed the existence of a diverse range of bioactive and structurally important compounds, many of which are recognized for their antibacterial characteristics. The detection of heterocyclic compounds, such aspyrrolo[1,2-a]pyrazine-1,4-dione, along with peptidyl derivatives, reinforces the

theory that the isolate has the capability to synthesizecefixime-like or β-lactam-like molecules.

Numerous compounds deserve to be emphasized because of their significance to β-lactam antibiotics:

Pyrrolo[1,2-a]pyrazine-1,4-dione (RT: 17.226, 18.218, 23.005) is structurally connected to pyrazine frameworks present in cephalosporins and penems. This category demonstrates recognized antibacterial properties and structural resemblance to the D-Ala-D-Ala dipeptide, which is the binding target for β-lactam antibiotics (Drawz and Bonomo, 2010 , Bush , 2018).

Thiophenederivatives (RT: 7.587) have been incorporated into numerous synthetic alterations of cephalosporins, including cefixime, owing to their capacity to improve spectrum and infiltration into Gram-negative bacteria (Bryskier , 2005).

Valine/NorvalineEsters (RT: 16.776) are typical of the side chains found in semi-synthetic antibiotics, indicating that the strain responsible has the biosynthetic capabilities to produce bioactive peptide derivatives (Walsh and Wencewicz,2013).

Evidence for Cefixime-like Compound Production

Cefiximecontains a β-lactam ring, a carboxyl group, a vinyl group, and a thiophenemoiety. Although GC-MS fails to identify intact β-lactam rings because they become unstable during fragmentation, it can detect precursors or related heterocycles (like pyrazines andthiophenes) firmly backs the biosynthetic ability for cephalosporin-like compounds (Elshafie et al.,2019 ; Takahashi and Omura,2003).

Further, the detection of peptide-like structures such as Glycyl-D-threonine and N-substituted amino acid esters indicate active pathways for peptide synthesis, likely engaging non-ribosomal peptidesynthetases(NRPS), a typical pathway for the production of antibiotics in bacteria (Süssmuth and Mainz,2017).

Table (2)Biological Relevance of Detected Compounds

Compound Type	Relevance
Pyrrolo[1,2-a]pyrazines	Antibacterial, β-lactam mimics) Bush , 2018 , Elshafie *et al*.,2019)
Amino acid esters (Valine/Leucine)	Cephalosporin precursors (Walsh and Wencewicz, 2013; Takahashi and Omura, 2003)

Thiophenes	Enhancers of antibiotic spectrum (Bryskier,2005)
Cyanoacetylurea	DNA replication inhibitor (Dandapani and Marcaurelle,2010)
Nucleoside analogs (Cytidine)	Antiviral and supportive co-metabolites (De Clercq,2009)

These findings collectively suggest that the bacterial isolate possesses a viable secondary metabolite pathway capable of producing bioactive compounds that share structural and functional similarities withcefixime, possibly contributing to its antibacterial effects.

Conclusion:

The GC-MS examination of the ethanol extract from the investigated bacterial isolate shows a diverse array of heterocyclic, peptidyl, and sulfur-containing substances, many of which exhibit structural similarities to recognized β-lactam antibiotics such ascefixime.

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