Current Clinical and Medical Education

Received 22 May 2024 | Revised 24 May 2024 | Accepted 25 Jun 2024 | Published Online 27Jun 2024

Published By: Vision Publisher

ISSN 2942-0776

CCME 02 (6), 48-59

Bioactive Chemical Compounds Released from Haemophilus influenzae by Using Gas Chromatography Mass Spectrometer GC-MS and Investigation Effect of Citrullus colocynthis, Urtica dioica and Foeniculum vulgare as Antibacterial Activity

Abeer Fauzi Murad Al-Rubaye†[1], Bashar Oda Jawad [2]

[1]Department of Biology, College of Science for Women, University of Babylon, Iraq

[2]Hammurabi College of Medicine, University of Babylon, Iraq

Abstract: Background : Various VOCs coming from or absorbed by the Haemophilus influenzae cultures were identified using the GC-MS after the headspace sampling was done with the help of multibed sorption tubes. During bacteria cultivation, sampling was done at various time intervals to analyze the changes that occurred in VOC metabolism. The purpose of our study: Studying the bioactive volatiles produced by Haemophilus influenzae and Examining the antibacterial potential of the medicinal plants Citrullus colocynthis and Urtica dioica with Foeniculum vulgare.

Materials and Methods: The Fresh of Citrullus colocynthis, Urtica dioica, and Foeniculum vulgare used in this present study was bought form a local market in Hilla city /Iraq. Each plant sample was ground in a mortar approximately weighing one hundred grams. Each plant powder 10g was extracted with ethanol extract of 100ml the solution was filtered and evaporated. The examination itself was performed using a GC–MS approach, and an Agilent 789 A instrument was used. The extracts were prepared and the sterile blotting paper disc (5 mm) was soaked in the diluted extract at two final concentrations; 50 µL and100 µL per disc.

Results : GC-MS analysis of Haemophilus influenzae found thirteen bioactive compounds. Investigation effect of ethanolic extract of Citrullus colocynthis, Urtica dioica and Foeniculum vulgare comparison with Rifambin and Cefotoxime (standard antibiotics) as antibacterial activity against Haemophilus influenzae recorded 16.29±0.06, 10.89±0.03, and 12.36±0.03 respectively for Citrullus colocynthis while recorded 15.09±0.05, 13.08±0.04, and 09.73±0.03 respectively for Urtica dioica. Investigation effect of ethanolic extract of Foeniculum vulgare recorded 14.73±0.05, 10.94±0.03, and 09.64±0.04 respectively. Citrullus colocynthis 16.29±0.06 mm was very highly active against Haemophilus influenzae.

Keywords: Haemophilus influenzae, GC-MS, Citrullus colocynthis, Urtica dioica, Foeniculum vulgare, Antibacterial activity.

Corresponding Author: Abeer Fauzi Murad Al-Rubaye †, Department of Biology, College of Science for Women, University of Babylon, Iraq

Supplementary information The online version of this article (https://doi.org/xx.xxx/xxx.xx) contains supplementary material,which is available to autho-rized users.

Introduction

The Gram-negative, pleomorphic, anaerobic pathogen *Haemophilus influenzae* (NTHi) can be found in two forms: These two categories are organized based on the presence of a capsule around the body which, if present, is usually thick. Six serotypes are reported and these are a, b, c ,d, e, and f among which serotype b is well known to cause invasive diseases [1]. Surprisingly, H. influenzae is amongst the main causes of childhood community acquired respiratory tract infections but normal healthy individuals harbor the pathogen in their naso- and oropharynx primarily the reasons why people develop immunity to the pathogen [3, 4]. Disease can occur if the germs follow the airway into the lower respiratory tract, although carriers rarely experience symptoms [4]. Proof of this is the observations of various studies making it clear that changes in the mere composition of pathways or changes in enzymes can mean the difference between high and low virulence in bacterial species such as Mycobacterium species, Legionella pneumophila, and Escherichia coli, among others. For example, whereas M. bovis deficient in phosphenolpyruvate (PEP) carboxykinase which is responsible for the conversion of oxaloacetate to PEP, appeared to be attenuated in mice . Mycobacterium TB and Escherichia coli both use glyoxylate cycle and the TCA cycle enzymes namely isocitrate lyase and succinate dehydrogenase to adapt and thrive [6,7]. Each of these grim propositions has shaped this microorganism allowing hitherto no experimental study of metabolic aspects which permits H. influenzae to dwell in human beings. In earlier studies it was estimated that at least two third of the proteins in H. influenzae had homologs in E. coli and due to gene duplications the major part of the genome in H. influenzae was deleted in order to reduce the number complementarily gene-duplicated gene pairs or paralogs. Since the autocartes are an assemblage of proteins related to the cell wall of the H. influenzae bacterium, they are involved in adhesion and colony formation. Hap*k* autotransporters in the cell wall merge with undisclosed receptors in the epithelium, which enables *H. influenzae* to attach itself to mucus secreting linings or non-ciliated epithelial cells, as indicated earlier [9]. In addition to this, bacterial microcolonies are formed with the help of the Hap*s* autotransporters. They probably form microcolonies that build some biofilms in the body, although some biofilms which cause infection on the middle ear or lungs, for instance, may be formed by these.

Peptidoglycan synthesis is expressed by penicillin binding proteins (PBPs) PBPs are enzymes which catalyse the metabolism of peptidoglycans. They can be involved in essential functions that centrally are needed to construct the cell wall and to modify it. Penicillin and other beta-lactam antibiotics contain an β-lactam ring that it can interact and covalently bind to PBPs and abolish these targets which is why they are called [10]. Some of the H. influenzae isolates possesses mutated PBPs, which are only resistant to antibiotics that produce beta-lactamase that hydrolyse the beta-lactam rings. This resistance is likely to be due to the N526K mutation on the one hand or an R517H alteration on the other hand and a third mutation which is still unidentified up to the present time. The R517H change cannot generate resistance independently as it failed to help the bacteria achieve lesser affinity towards penicillin. When competitively with beta-lactamase was discovered in the 1970s, cephalosporins become the drug of choice with severe H. influenzae infection displacing ampicillin. Nonetheless, resistance has occurred due to alteration to the transpeptidase domain of the cephalospororin, penicillin binding protein 3 (PBP3) [11]. In the early years, different strains of H. influenzae were considered to be encapsulated or non-encapsulated depending upon whether or not, these bacteria had an outer layer coat or a capsule. Various classes of encapsulated strains were however, further classified based on the immune reaction provoked by their specific polysaccharide capsular materials. Despite their name, there are actually only six known types of encapsulated H. influenzae: A, b, c, d, e, and f [12]. Of the division os *H. influenzae*, the most commonly seen one is H. influenzae type b which is encapsulated and has PRP that is mainly prevalent in children. Essentially, kinds d and c have not been documented with much frequency although types a, e, and f have only occurred a few times. It is evident that from the aforesaid discussion about the encapsulated and unencapsulated groups of bacterial strains that the genetic variation seen in the latter is greater [13, 14]. Although all *H. influenzae* isolates can now be marketed with MLSA and other molecular methods, unencapsulated strains continue to be called nontypeable (NTHi) because of the lack of capsular serogroups. Most NTHi strains are believed to be part of the normal flora or phthal genetically or acquired in humans mainly in the genitourinary system, conjunctiva and respiratory tract. If the respiratory chain is available, then the general result of metabolism by H. influenzae will still

be simple chain carboxylic acids no matter how much oxygen is available for usage. As a result, the regulatory mechanisms in H. influenzae may be quite unique and distinct from those in other bacteria capable of the aerobic respiration if a complete TCA cycle is present like in *E. coli*. This "acetate switch" described for *E. coli* is most likely unrelated to the mechanisms that are specific to the regulation of *H. influenzae* to switch from acetate to formate synthesis, this could probably be due to a shift in the major pathway of pyruvate metabolism [15]. The respiratory chain of H. influenzae is quite peculiar because it contains the components that are not generally associated with the energy conservation by means of the proton gradient (e.g., NdhA, Nqr, Nap, TorZ), which might be a consequence of the adaptation to the host condition. It might be worth considering the possibility that H. influenzae has the pathways similar to the differential regulation of the composition and the respiratory chain compared to the pathways involved in energy production associated with virulence in *E. coli* urinary tract infection as mentioned earlier [16]. It has been reported that an incompletely functional TCA cycle may promote the ability of survival in macrophages in case of Salmonella enterica sv typhimurium [17]. As for other effects, similar impacts may be applicable for H. influenzae, yet, the requirement for a further analysis in this regard still remains. The metabolic organization described is quite atypical of the type seen in other organisms, and it exhibits a low level of energy conservation. In this regard, it is critical to mention that with systemic sickness mouse model, pathogenicity of H. influenzae serotype b is considered to be reduced if either pyruvate dehydrogenase (aceEF) or α-ketoglutarate dehydrogenase (sucAB) have been omitted [18, 19]. The objective of this research was to investigate the anti-bacterial efficacy of the medicinal plants: Citrullus colocynthis, Urtica dioica and Foenicum vulgare; as well as isolate and characterize the bioactive volatile compounds synthesized by Haemophilus influenzae.

Materials and Methods

Haemophilus influenzae Isolation and Identification

youngsters under the age of five who were clinically healthy (i.e., not sick with a respiratory infection or taken antibiotics within the last three weeks) were studied at Babylon's Women's and Children's Hospital. Nasopharyngeal swabs were taken from these youngsters. After inserting the swab horizontally towards the nasopharynx and straight back (not upwards), the researcher would rotate the swab up to five times before holding it in place for five to ten seconds to gather sample material. A drop was used to inoculate a chocolate agar plate with bacitracin (20,000 U/L of medium), after which the sample of nasopharyngeal swab was vortexed. The plate was then incubated at 37°C with carbon dioxide. Isolation of Haemophilus influenza is facilitated by bacitracin, which inhibits the growth of numerous other normal floras. Conventional laboratory procedures, including Gram's stain, oxidase test, and colony morphology, validated each isolate.

Gathering relevant information

We sourced our fresh Citrullus colocynthis, Urtica dioica, and Foeniculum vulgare from a neighbourhood market in Hilla city, Iraq. In a mortar, approximately 100 g of every plant was crushed. Prior to filtration and evaporation, precisely 10 g of plant powder from each species was steeped in 100 ml of ethanol extract [20, 21.[

Using gas chromatography and mass spectrometry (GC-MS), we analysed the spectra of bioactive chemicals found in Haemophilus influenzae bacteria.

A 789 by Agilent The test, which used a GC-MS methodology, was carried out by means of a device. A DB-5MS column, bought in Folsom, California, from J&W Scientific, was utilised for the gas chromatography. For this column, the following dimensions were taken: The film has an internal diameter of 0.25 mm, a diameter of 30 m, and a thickness of 0.25 µm. The furnace temperature remained constant throughout the process, in contrast to the prior experiment. We used helium as the carrier gas and maintained a constant flow rate of one millilitre per minute. The gas chromatography (GC) column's effluent was heated to 250 degrees Celsius and then injected directly into the mass spectrometer's (MS) source through a transfer line. During the ionisation process, a voltage of 70 electron volts (eV) was used, and the ion source temperature was kept at 230 degrees Celsius (°C). The measuring range encompassed 41 different atomic mass units (amu), with the maximum value reaching 450.

Bactericidal action against Haemophilus influenzae with the use of three therapeutic herbs: Foeniculum vulgare, Urtica dioica, and Citrullus colocynthis

Two distinct final concentrations (50 µl and 100 µl/disc) of the diluted extract were added to the sterile blotting paper disc (5 mm). Then, the extracts were made. In order to eliminate any excess solvent, the produced discs [21] were dried in a controlled environment (at 37oC overnight) before being utilised in the study.

Data analysis using statistical methods

Data analysis was performed using a number of statistical algorithms on information retrieved from an SPSS (Version 11.6) database. Calculating the average and running an ANOVA were stages involved in these processes.

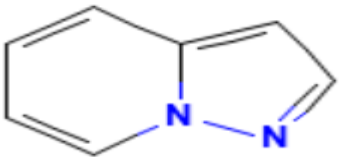
Results and Discussion

The identified chemicals were represented by thirteen peaks in the GC-MS chromatogram. Compounds are : Ethylsulfonylpropanone, Pyrazolo[1.5-a]pyridine , 3-methyl-2-phenyl, 3-Methyl-2-phenylbutanal, 1-Benzylindole-3-carboxylic acid, L-proline, 1-cyclohexanecarbonylpiperidin-4-amine, 2,4-dimethyl-4-phenyloxolane, mono-Methyl isophthalate, 3-(benzyloxymethyl)cyclobutanone, 1-(4-methoxyphenoxy)propan-2-amine, and 2-cyclopropylpropan-2-amine. Bacteria that cause illness can secrete a wide range of volatile compounds. The most significant sepsis pathogens were the subjects of 31 publications detailing their VOC generation in our systematic review. Nevertheless, just a tiny percentage of the metabolites are manufactured solely by the relevant bacterial species. Notably, overall results were contradictory since some investigations failed to reproduce earlier trials' findings. Possible explanations for the high number of research yielding conflicting outcomes include four factors. To begin, different research did not always use the same bacterial subtype. Phage forms of SA affected volatile organic molecules in the headspace, according to one study [22, 23]. The efficiency of enzymes in a certain metabolic pathway may vary across subtypes due to genetic variation. However, phenotyping within bacterial species may benefit from these differences. Nonetheless, this may limit the usefulness of volatile biomarkers in clinical settings for identifying strains [24-27]. The second complicating factor is the growing medium, which provides the components for the volatile organic compounds (VOCs) that are formed [28, 29]. The third element is that measurements were taken at various stages of the bacterial growth course. Metabolite depletion and growth phase (log or stationary) affect headspace metabolites, according to multiple investigations on this issue. Finally, most of the included investigations looked at reference strain cultures. On the other hand, there were studies that looked at clinical samples, which had higher levels of within-class variation [30]. In comparison to reference strain cultures grown in a lab, patient samples lack specificity and differ in the following ways: factors such as conjugate fluid units (CFUs), stage of growth, host reaction, viscosity, complicating medical conditions, and drugs (such as antibiotics [31]).

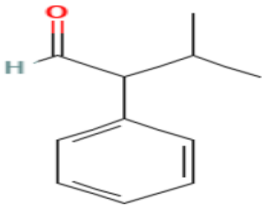
Table 1. GC-MS analysis of *Haemophilus influenzae*.

Compounds	Molecular Formula	Molecular Weight
Ethylsulfonylpropanone	$C_5H_{10}O_3S$	150.20 g/mol
Pyrazolo[1.5-a]pyridine , 3-methyl-2-phenyl	$C_7H_6N_2$	118.14 g/mol
3-Methyl-2-phenylbutanal	$C_{11}H_{14}O$	162.23 g/mol
1-Benzylindole-3-carboxylic acid	$C_{16}H_{13}NO_2$	251.28 g/mol
L-proline	$C_5H_9NO_2$	115.13 g/mol
1-cyclohexanecarbonylpiperidin-4-amine	$C_{12}H_{22}N_2O$	210.32 g/mol
2,4-dimethyl-4-phenyloxolane	$C_{12}H_{16}O$	176.25 g/mol
mono-Methyl isophthalate	$C_9H_8O_4$	180.16 g/mol
3-(benzyloxymethyl)cyclobutanone	$C_{12}H_{14}O_2$	190.24 g/mol
1-(4-methoxyphenoxy)propan-2-amine	$C_{10}H_{15}NO_2$	181.23 g/mol
2-cyclopropylpropan-2-amine	$C_6H_{13}N$	99.17 g/mol

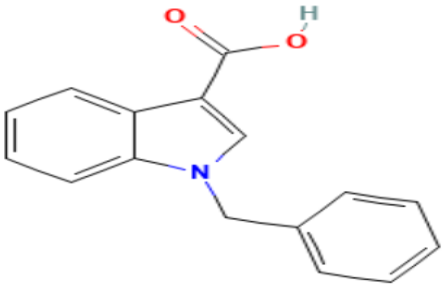
Pyrazolo[1.5-a]pyridine , 3-methyl-2-phenyl



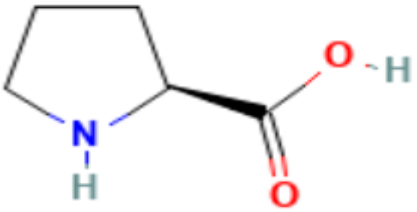
3-Methyl-2-phenylbutanal



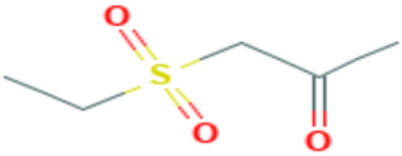
1-Benzylindole-3-carboxylic acid



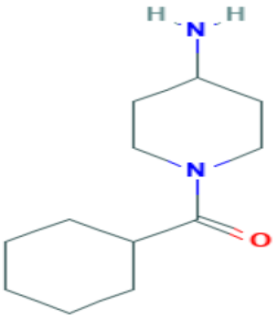
L-proline



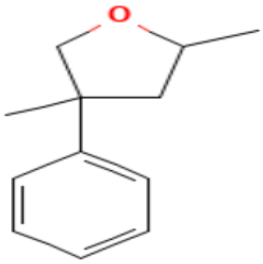
Ethylsulfonylpropanone



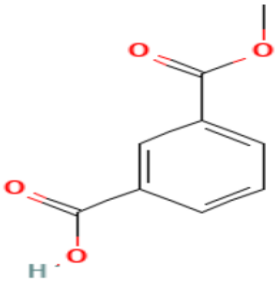
1-cyclohexanecarbonylpiperidin-4-amine



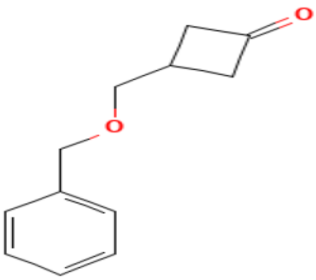
2,4-dimethyl-4-phenyloxolane



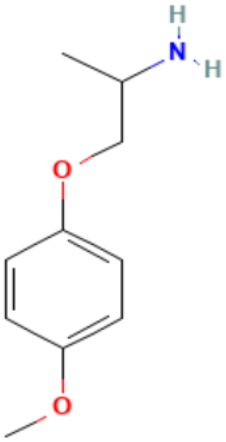
mono-Methyl isophthalate



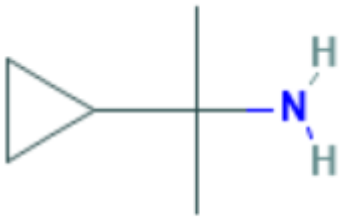
3-(benzyloxymethyl)cyclobutanone



1-(4-methoxyphenoxy)propan-2-amine



2-cyclopropylpropan-2-amine



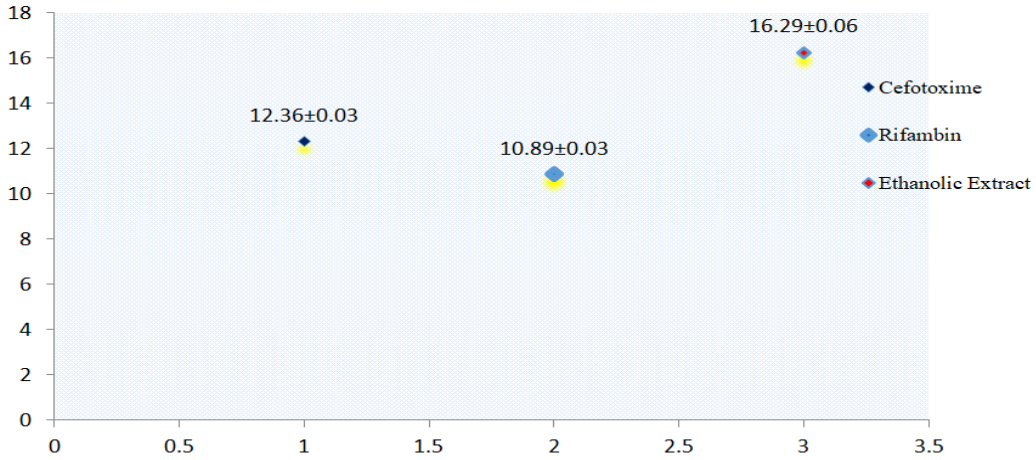


Figure 1. Investigation effect of ethanolic extract of *Citrullus colocynthis* comparison with Rifambin and Cefotoxime (standard antibiotics) as antibacterial activity against *Haemophilus influenzae*

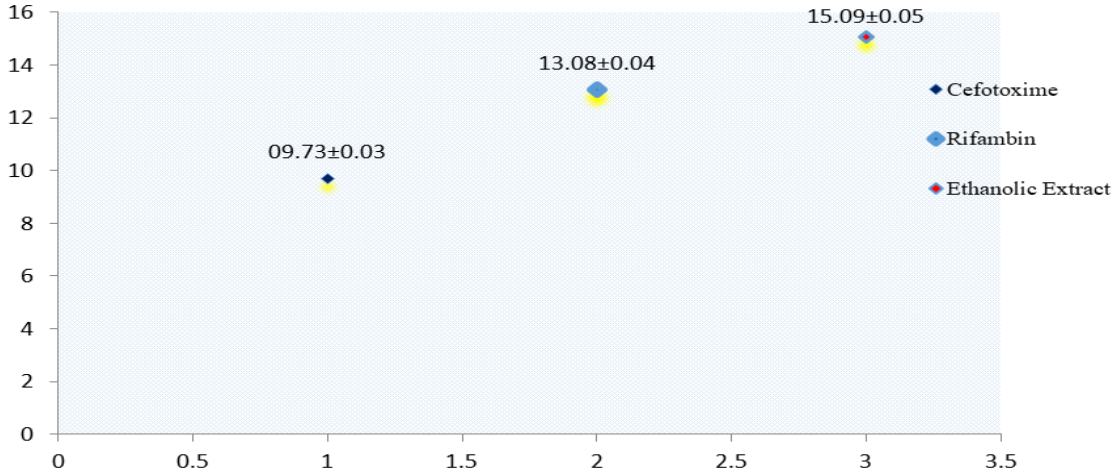


Figure 2. Investigation effect of ethanolic extract of *Urtica dioica* comparison with Rifambin and Cefotoxime (standard antibiotics) as antibacterial activity against *Haemophilus influenzae*

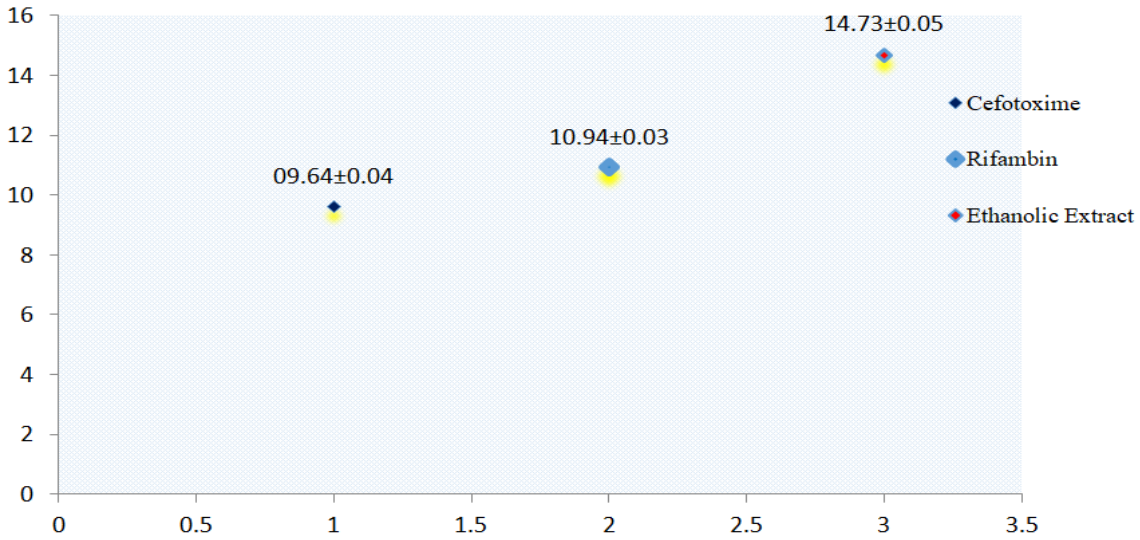


Figure 3. Investigation effect of ethanolic extract of *Foeniculum vulgare* comparison with Rifambin and Cefotoxime (standard antibiotics) as antibacterial activity against *Haemophilus influenzae*

In Figure 1, the antibacterial activity against Haemophilus influenzae was measured at 16.29±0.06, 10.89±0.03, and 12.36±0.03 for Citrullus colocynthis, Urtica dioica, and Foeniculum vulgare, respectively, in comparison to Rifambin and Cefotoxime, two standard antibiotics. For Urtica dioica, the results were 15.09±0.05, 13.08±0.04, and 09.73±0.03 respectively Figure 2. The effects of the ethanolic extract of Foeniculum vulgare on the investigation were recorded as 14.73±0.05, 10.94±0.03, and 09.64±0.04 in Figure 3, consecutively. Against Haemophilus influenzae, Citrullus colocynthis, which measured 16.29±0.06 mm, exhibited extremely strong activity .

In addition to its role in asthma, COPD, and otitis media, it is involved in a wide variety of acute and chronic illnesses. These infections can happen because H. influenzae can live in certain places in humans, like the middle ear, nasopharynx, and the lungs. Each of these niches has its own unique environment in terms of pH, carbon metabolite availability, and oxygen levels. The middle ear, in contrast to the mainly aerobic lung, is nearly entirely anaerobic. Since H. influenzae, like other bacterial pathogens, experiences a wide range of conditions in the human host, it seems to reason that the bacteria should have a metabolic pathway repertoire that allows them to thrive .

In terms of the primary objective of biomarker research, which is to provide evidence of the lack of a bacterial infection, a number of biomarkers are suitable for clinical inquiry. Without the host, no bacterium can create isopentanol, formaldehyde, methyl mercaptan, or trimethylamine. Although they are present in high concentrations in animal breath, the sensitive potential markers ethanol and isoprene are also present. A group of volatile biomarkers that are likely to be produced by many distinct pathogens should be studied if the goal of the study is to rule out bacterial infection as a possible differential diagnosis. There may be a strong negative predictive value if none of these potential markers are found. It is possible to use the following volatile organic compounds (VOCs) to identify individual strains: 2-methyl-butanal and isovaleric acid are components of SA; 1-undecene, 2,4-dimethyl-1-heptane, 2-butanone, 4-methyl-quinazoline, hydrogen cyanide, and methyl thiocyanide are components of PA; and methanol, pentanol, ethyl acetate, and indole are components of EC. So far, the literature has not yielded any potential biomarkers for SP, EF, or KP. A mixture of volatile organic molecules is suggested for the purpose of pathogen species identification. A number of experiments utilising electronic nose technology and a recent article by Thorn demonstrate the benefits of this method [32, 33]. The reporting of diagnostic accuracy (sensitivity and specificity) must adhere to the STARD standards; however, only one study in this analysis does so. The infecting/colonizing bacteria, the growing bacteria, and the resistant bacteria should be the primary foci of pathogen phenotyping. It is worth noting that indole can be utilised to distinguish between clinically significant phenotypes within the same bacterial strain; this is because it is a biomarker for biofilm formation in EC. Two, a rat model showed that tiny chemical molecules containing volatile sulphur dioxide could trigger inflammation; this suggests that these

compounds could be a signal for pathogenicity. Finally, it is possible to track the therapeutic response as adding antibiotics to the culture medium at concentrations higher than the minimum inhibitory concentration (MIC) reduces the formation of multiple volatile organic compound (VOC) types. The VOC concentrations were reduced by antibiotic delivery below the MIC, however only to a lesser degree, indicating a dosage dependency [34, 35]. None of the articles that were considered discussed how bacterial resistance affects volatile organic compounds. A recent study utilising colorimetric electronic nose technology distinguishing methicillin-resistant SA from methicillin-sensitive SA and vancomycin-resistant EF from vancomycin-sensitive EF, however, takes the initial steps in this regard.

There are a number of things to think about before using volatile organic molecules in vivo to diagnose sepsis. Firstly, it's possible that the metabolites produced differ significantly between in vitro growth media and the growth medium inside the host. The second step is for the host to react inflammatoryly to the bacterium, which can cause metabolic changes. Future research should focus on distinguishing between viral and noninfectious inflammatory responses, as this inflammatory response might modify human metabolism on its own. Lastly, VOCs can be ingested or found in the environment. Lastly, even under ideal circumstances, every part of the body, including the lungs, is home to its own distinct microbiome. The metabolites produced by these house bacteria may be identical to one another, which could cause a VOC-based diagnostic test to fail. One possible function of inflammation-altered volatile organic compounds (VOCs) in this context is to aid in the differentiation of colonising bacteria from harmful ones .It is ideal to identify invading germs as soon as feasible in severely sick patients; this allows for rapid identification of morbific pathogen strains and knowledge of antibiotic susceptibility prior to the start of new antimicrobial treatment. Volatile organic compounds (VOCs) that are unique to bacteria are produced as a byproduct of their unique metabolism; these molecules could have diagnostic value. Noninvasive monitoring of volatile metabolites is possible through direct investigation of exhaled air. The six most common and harmful bacteria in sepsis—Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli—gave rise to a wide variety of volatile organic compounds (VOCs). When diagnosing patients in critical care, these VOCs could serve as biological markers. The results of a comprehensive literature search came up with 31 papers. As previously mentioned, all six of the target microorganisms are capable of producing the aforementioned compounds [37, 38]. Biological markers for the presence of these diseases could be these volatile organic compounds (VOCs), since humans do not make them. Staphylococcus aureus used isovaleric acid and 2-methyl-butanal as volatile biomarkers; Pseudomonas aeruginosa used 1-undecene, 2,4-dimethyl-1-heptane, 2-butanone, 4-methyl-quinazoline, hydrogen cyanide, and methyl thiocyanide; and Escherichia coli used methanol, pentanol, ethyl acetate, and indole as volatile biomarkers. It is worth mentioning that certain variables, such as the culture media employed, the stage of bacterial growth, and genetic diversity among strains, were not taken into account when calculating VOC production.

Conclusion

Last but not least, a number of volatile biomarkers have shown great promise as indicators of no infection: • The chemical components of the bacterial species were identified and quantified using GC-MS. Consequently, it is necessary to conduct focused research in order to discover prospective sets of volatile biomarkers and assess the diagnostic efficacy of these indicators in patients with severe illness. The antibacterial activity of the aqueous and ethanol extract of Citrullus colocynthis on numerous pathogenic bacterial strains highlights the plant's potential as a source of novel antibacterial chemicals. Haemophilus influenzae was significantly inhibited by Citrullus colocynthis, which had a diameter of 16.29±0.06 mm.

References

1. D. Fontanals, R. Bou, I. Pons et al., “Prevalence of Haemophilus influenzae carriers in the Catalan preschool population,” European Journal of Clinical Microbiology & Infectious Diseases, vol. 19, no. 4, pp. 301–304, 2000.

2. H. Zhu, A. Wang, J. Tong et al., “Nasopharyngeal carriage and antimicrobial susceptibility of Haemophilus influenzae among children younger than 5 years of age in Beijing, China,” BMC Microbiology, vol. 15, no. 1, p. 6, 2015.
3. G. A. Mackenzie, A. J. Leach, J. R. Carapetis, J. Fisher, and P. S. Morris, “Epidemiology of nasopharyngeal carriage of respiratory bacterial pathogens in children and adults: crosssectional surveys in a population with high rates of pneumococcal disease,” BMC Infectious Diseases, vol. 10, no. 1, p. 304, 2010.
4. O. Abdullahi, J. Nyiro, P. Lewa, M. Slack, and J. A. G. Scott, “+e descriptive epidemiology of Streptococcus pneumoniae and Haemophilus influenzae nasopharyngeal carriage in children and adults in Kilifi district, Kenya,” Pediatric Infectious Disease Journal, vol. 27, no. 1, pp. 59–64, 2008.
5. D. Gkentzi, M. P. Slack, and S. N. Ladhani, “+e burden of nonencapsulated Haemophilus influenzae in children and potential for prevention,” Current Opinion in Infectious Diseases, vol. 25, no. 3, pp. 266–272, 2012.
6. C. T. Lim, N. Parasakthi, and S. D. Puthucheary, “Neonatal meningitis due to non-encapsulated Haemophilus influenzae in a set of twins—a case report,” Singapore Medical Journal, vol. 35, no. 1, pp. 104-105, 1994.
7. S. J. Moghaddam, “Nontypeable Haemophilus influenzae in chronic obstructive pulmonary disease and lung cancer,” International Journal of Chronic Obstructive Pulmonary Disease, vol. 6, pp. 113–123, 2011.
8. T. J. Falla, D. W. M. Crook, W. A. G. Kraak et al., “Populationbased study of non-typable Haemophilus influenzae invasive disease in children and neonates,” 1e Lancet, vol. 341, no. 8849, pp. 851–854, 1993. [13] WHO, Haemophilus influenzae Type B (HiB), WHO fact sheet no. 294, WHO, Geneva, Switzerland, 2005.
9. A. Wang, S. Yu, K. Yao et al., “Antimicrobial susceptibility of Haemophilus influenzae strains and antibiotics usage patterns in pediatric outpatients: results from a children’s hospital in China (2000–2004),” Pediatric Pulmonology, vol. 43, no. 5, pp. 457–462, 2008.
10. Harrington, J. C., Wong, S. M. S., Rosadini, C. V., Garifulin, O., Boyartchuk, V., and Akerley, B. J. (2009). Resistance of Haemophilus influenzae to reactive nitrogen donors and gamma interferon-stimulated macrophages requires the formatedependent nitrite reductase regulator-activated ytfE Gene. Infect. Immun. 77, 1945–1958.
11. Herbert, M., Kraiss, A., Hilpert, A.-K., Schlör, S., and Reidl, J. (2003). Aerobic growth deficient Haemophilus influenzae mutants are non-virulent: implications on metabolism. Int. J. Med. Microbiol. 293, 145–152.
12. Jones, R. W., and Garland, P. B. (1977). Sites and specificity of reaction of bipyridylium compounds with anaerobic respiratory enzymes of Escherichia coli - effects of permeability barriers imposed by the cytoplasmic membrane. Biochem. J. 164, 199–211.
13. Kappler, U., Sly, L. I., and Mcewan, A. G. (2005). Respiratory gene clusters of Metallosphaera sedula - differential expression and transcriptional organization. Microbiology 151, 35–43. doi: 10.1099/mic.0.27515-0
14. Moghaddam, S. J., Ochoa, C. E., Sethi, S., and Dickey, B. F. (2011). Nontypeable Haemophilus influenzae in chronic obstructive pulmonary disease and lung cancer. Int. J. Chronic Obstruct. Pulmon. Dis. 6, 113–123.
15. Papin, J. A., Price, N. D., Edwards, J. S., and Palsson, B. O. (2002). The genome-scale metabolic extreme pathway structure in Haemophilus influenzae shows significant network redundancy. J. Theor. Biol. 215, 67–82.
16. Raghunathan, A., Price, N. D., Galperin, M. Y., Makarova, K. S., Purvine, S., Picone, A. F., et al. (2004). In silico metabolic model and protein expression of Haemophilus influenzae strain Rd KW20 in Rich Medium. OMICS 8, 25–41.

17. Schirra, H. J., Anderson, C. G., Wilson, W. J., Kerr, L., Craik, D. J., Waters, M. J., et al. (2008). Altered metabolism of growth hormone receptor mutant mice: a combined NMR metabonomics and microarray study. PLoS ONE 3:e2764.

18. Tatusov, R. L., Mushegian, A. R., Bork, P., Brown, N. P., Hayes, W. S., Borodovsky, M., et al. (1996). Metabolism and evolution of Haemophilus influenzae deduced from a whole-genome comparison with Escherichia coli. Curr. Biol. 6, 279–291.

19. Wolfe, A. J. (2005). The acetate switch. Microbiol. Mol. Biol. Rev. 69, 12–50.

20. Al-Hwaiti, M.S.; Alsbou, E.M.; Abu Sheikha, G.; Bakchiche, B.; Pham, T.H.; Thomas, R.H.; Bardaweel, S.K. Evaluation of the anticancer activity and fatty acids composition of "Handal"(Citrullus colocynthis L.) seed oil, a desert plant from south Jordan. Food Sci. Nutr. 2021, 9, 282–289.

21. Joshi, G.; Kaur, J.; Sharma, P.; Kaur, G.; Bhandari, Y.; Kumar, R.; Singh, S. P53-mediated anticancer activity of Citrullus colocynthis extracts. Nat. Prod. J. 2019, 9, 303–311.

22. Scott-Thomas AJ, Syhre M, Pattemore PK, Epton M, Laing R, et al. (2010) 2- aminoacetophenone as a potential breath biomarker for Pseudomonas aeruginosa in the cystic fibrosis lung. BMC Pulm Med 10: 56.

23. Di Martino P, Fursy R, Bret L, Sundararaju B, Phillips RS (2003) Indole can act as an extracellular signal to regulate biofilm formation of Escherichia coli and other indole-producing bacteria. Can J Microbiol 49: 443–449.

24. Hu M, Zhang C, Mu Y, Shen Q, Feng Y (2010) Indole affects biofilm formation in bacteria. Indian J Microbiol 50: 362–368.

25. Brooks JB (1977) Detection of bacterial metabolites in spent culture media and body fluids by electron capture gas-liquid chromatography. Adv Chromatogr 15: 1–31.

26. Anema PJ, Kooiman WJ, Geers JM (1973) Volatile acid production by Clostridium sporogenes under controlled culture conditions. J Appl Bacteriol 36: 683–687.

27. Hiele M, Ghoos Y, Rutgeerts P, Vantrappen G, Schoorens D (1991) Influence of nutritional substrates on the formation of volatiles by the fecal flora. Gastroenterology 100: 1597–1602.

28. Scotter JM, Langford VS, Wilson PF, McEwan MJ, Chambers ST (2005) Realtime detection of common microbial volatile organic compounds from medically important fungi by selected ion flow tube-mass spectrometry (SIFT-MS). J Microbiol Methods 63: 127–134.

29. Filipiak W, Sponring A, Bauer M, Filipiak A, Ager C, et al. (2012) Molecular analysis of volatile metabolites released specifically by Staphylococcus aureus and Pseudomonas aeruginosa. BMC Microbiol 12: 113.

30. Dolch ME, Hornuss C, Klocke C, Praun S, Villinger J, et al. (2012) Volatile compound profiling for the identification of gram-negative bacteria by ion molecule reaction–mass spectrometry. J Appl Microbiol 113: 1097–1105.

31. Allardyce RA, Langford VS, Hill AL, Murdoch DR (2006) Detection of volatile metabolites produced by bacterial growth in blood culture media by selected ion flow tube mass spectrometry (SIFT-MS). J Microbiol Methods 65: 361–365.

32. Julak J, Stranska E, Rosova V, Geppert H, Spanel P, et al. (2006) Bronchoalveolar lavage examined by solid phase microextraction, gas chromatography–mass spectrometry and selected ion flow tube mass spectrometry. J Microbiol Methods 65: 76–86.

33. Preti G, Thaler E, Hanson CW, Troy M, Eades J, et al. (2009) Volatile compounds characteristic of sinus-related bacteria and infected sinus mucus: analysis by solid-phase microextraction and gas chromatography–mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 877: 2011–2018.

34. Fend R, Kolk AH, Bessant C, Buijtels P, Klatser PR, et al. (2006) Prospects for clinical application of electronic-nose technology to early detection of Mycobacterium tuberculosis in culture and sputum. J Clin Microbiol 44: 2039–2045.

35. Carey JR, Suslick KS, Hulkower KI, Imlay JA, Imlay KRC, et al. (2011) Rapid identification of bacteria with a disposable colorimetric sensing array. J Am Chem Soc 133: 7571–7576.

36. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, et al. (2003) Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. BMJ 326: 41–44.

37. Savelev SU, Perry JD, Bourke SJ, Jary H, Taylor R, et al. (2011) Volatile biomarkers of Pseudomonas aeruginosa in cystic fibrosis and noncystic fibrosis bronchiectasis. Lett Appl Microbiol 52: 610–613.

38. Scholpp J, Schubert JK, Miekisch W, Geiger K (2002) Breath markers and soluble lipid peroxidation markers in critically ill patients. Clin Chem Lab Med 40: 587–594.