Journal of Current Medical Research and Opinion
Received 21-01-2024| Revised 23-01-2024| Accepted 19-02-2024| Published Online 20-02-2024

DOI: https://doi.org/10.52845/CMRO/2024/7-2-10 ISSN (O) 2589-8779 | (P) 2589-8760
CMRO 07 (02), 2127-2134 (2024)

Original Research

Analysis of Fermentation Product of Bioactive Compounds of *Escherichia coli* Isolated from UTI and Evaluation Effect of Four Tradition Medicinal Plant as Antimicrobial Activity

Rabab J.H. Al Hasseny [1], Abbas K. Al-Mansoori [2], Ahmed Obaid Hossain [3]

[1]Medical Microbiology, Department of Health Food and Nutrition, College of Food Science, Al-Qasim Green University, Iraq.
[2]Department of Genetic Engineering, College of Biotechnology, Al-Qasim Green University, Iraq.
[3]Department of Medical Biotechnology, College of Biotechnology, Al-Qasim Green University, Iraq.

Abstract:

The purpose of this study was to examine four plant extracts for their antibacterial activity against Escherichia coli in vitro and to examine the bioactive chemical products of this bacteria.

Method: Gas chromatography-mass spectrometry (GC-MS) methods were used to investigate the bioactive chemical components, also called secondary metabolites. Afterwards, the antibacterial activity of the Escherichia coli methanolic extract was evaluated in a laboratory setting.

Results: The following were found in the Escherichia coli GC-MS analysis: The compounds listed include 1,2-Epithio-3-hexanol, Oxa-4-azacyclohexane, 1-DL-Lysine monohydrochloride, 2-Methoxy-5-methylpyridine, 5-Methylquinoline, Methyl oleate hydroperoxide, methyl octadeca-9,12,15-trienoate, N-benzylhexadecanamide, Dimethylacetamide, 2-amino-N-ethyl-N-methylacetamide, methyl (Z)-18-hydroperoxyoctadec-9-enoate, Methyl hexadecanoate, n-Butyl oleate, Methyl 16-methylheptadecanoate, 2-Amino-N-methylacetamide. Urtica dioica (Crude) (26.09±0.21) was very highly active against Escherichia coli

Keywords: *Escherichia coli*, Secondary metabolites, Antibacterial, GC/MS.

Introduction:

Inflammation of the kidneys brings in symptoms like painful and frequent urination, which collectively make up urinary tract infections (UTIs), the most common infectious disease in the world. About 150 million cases of UTI occur each year, and they are associated with substantial morbidity and death [1, 2]. The urinary tract pathogen, Escherichia coli, is the most common infectious agent directly responsible for urinary tract infections (UTIs). This pathogenic concept contains four basic steps: internal local mucosal adhesion/colonization, rapid evasion of host resistance, and host termination [2, 3]. In a serious and persistent attempt to create an effective and distinct pharmaceutical treatment targeting urinary tract infections, an unlimited amount of research has been focused on clarifying the underlying processes that cause UPEC disease. It is also known that these rod-shaped bacteria cause a wide variety of common human diseases, such as urinary tract infections and hemorrhagic colitis [4]. Physiological processes that are regulated include: drug resistance, development of resilient cells, stress responses, and a signaling molecule that can be produced by E. coli [5, 6]. This study aims to determine whether four plant extracts have antibacterial effects against Escherichia coli and to examine the bioactive chemical compounds produced by these bacteria.

Materials and Methods:

Optimal conditions for metabolite growth and identification

In the laboratory, some subcultures of a strain of pathogenic Escherichia coli bacteria were grown on nutritional agar for only 48 hours, specifically at a temperature of 22 degrees Celsius, after they were isolated in the laboratory. 11 minutes of incubation at 4°C after which the solution was stirred at 130 rpm for exactly another ten minutes. The important compounds were removed from the liquid medium and at the same time evaporated using a rotary evaporator that was used and set to 45 degrees Celsius [7].

Conducting a GC-MS spectrum analysis of Escherichia coli's bioactive natural chemical components.

An Agilent 789 A instrument was used to conduct the GC-MS analysis. For the gas chromatography, we used a DB-5MS column made by J&W Scientific of Folsom, California. The dimensions of this column were as follows: a sheet thickness of 0.25 um with a diameter of 30 m0.25 mm i.d. Consistent with the prior study, the oven temperature was kept constant. The carrier gas, helium, was introduced at a rate of one millilitre per minute. The gas chromatography (GC) column's effluent was immediately injected into the mass spectrometer's (MS) source via a heated transfer line set to 250 degrees Celsius [8]. The ion source was kept at 230 degrees Celsius, and ionisation occurred at a voltage of 70 electron volts (eV) .

The effectiveness of several medicinal plant extracts as antibacterial agents against Escherichia coli in a controlled laboratory environment

The agar was cut into five-millimeter-diameter wells using a sterile cork-borer. Afterwards, the wells were supplemented with 25 µl of each medicinal plant's sample solution. For 48 hours, the plates were left at room temperature to incubate. After 48 hours of incubation, the antibacterial activity was evaluated by measuring the diameter of the inhibitory zone. The solvent that was used as a control was methanol. We used Aztreonam and Ceftazidime as our reference antibacterial drugs [9, 10]. The trials were repeated twice.

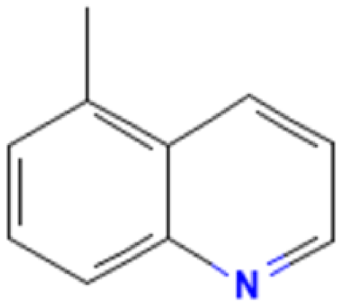
Data analysis with statistics

Data was retrieved from an SPSS (Version 11.6) database and analysed using a variety of statistical processes, including calculating the mean value and doing an analysis of variance (ANOVA).

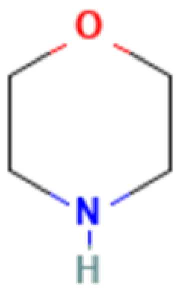
Results and Discussion:

Fifteen peaks, corresponding to the compounds in question, were seen in the GC-MS chromatogram. These chemicals are 1,2-Epithio-3-hexanol, Oxa-4-azacyclohexane,1-DL-Lysine monohydrochloride,2-Methoxy-5-methylpyridine,5-Methylquinoline, Methyl oleate hydroperoxide, methyloctadeca-9,12,15-trienoate,Nbenzylhexadecanamide,Dimethylacetamide, 2-amino-N-ethyl-N-methylacetamide, methyl(Z)-18-hydroperoxyoctadec-9-enoate, Methyl hexadecanoate, n-Butyl oleate, Methyl 16-methylheptadecanoate,2-Amino-N-methylacetamide. For the Anacardium occidentale (Crude) extract, the in vitro antimicrobial activity of conventional antibiotics was recorded as 15.91±0.12, while that of methanol, ethyl acetate, and ethanol extracts of the medicinal plant Anacardium occidentale (Crude) was 21.08±0.19, 18.57±0.15, and 15.91±0.12, respectively. Nutritional and biological benefits of anacardium plants have led to their rising profile. The plant's various sections, including its leaves and fruits, contain a number of secondary metabolites [11, 12]. Antioxidant, antibacterial, and anticancer activity are among the most studied bioactive effects of the many Anacardium species .

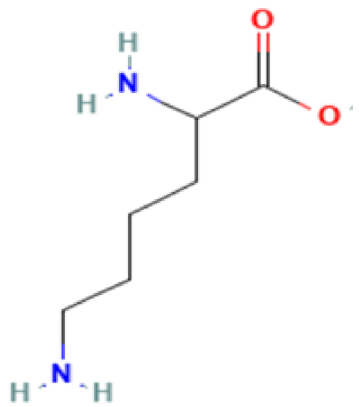
The respective values for Coriandrum sativum (Crude) extract were recorded as (23.76±0.20), 19.98±0.16, and 20.89±0.18. To develop coriander essential oil as a therapeutically established antibacterial agent, additional thorough research is needed to identify the active component responsible for the oil's antibacterial action. However, preliminary results show that coriander oil is effective against frequently microorganisms. The Origanum vulgare (Crude) antimicrobial activity was measured at 18.29±0.15, 20.64±0.19, and 24.14±0.20. The antimicrobial activity of Urtica dioica (Crude) (Crude) was compared to two conventional antibiotics, Aztreonam and Ceftazidime, with reported values of 26.09±0.21, 22.26±0.19, and 19.39±0.16, respectively. The anti-Escherichia coli activity of Urtica dioica (Crude) (26.09±0.21) was exceptionally strong, as shown in Figures 2, 3, 4, and 5. Origanum vulgare L. essential oil in particular has antibacterial characteristics, according to a number of research. The antibacterial activity of essential oils is mostly caused by phenolic compounds. When it comes to microbes, Origanum vulgare L. has you covered with its ability to combat both positive and negative bacteria, viruses, and fungi [14–17]. Origanum vulgare L. primarily affects microbial cells through interfering with cellular permeability, quorum sensing, cytoplasmic pH, and protein synthesis, as well as by interacting with the cell membrane. The majority of the antibacterial action in plants comes from their secondary metabolites. There are several classes of phytochemicals that have antimicrobial activities. Some examples include phenolics and polyphenols, terpenoids, alkaloids, lectins, polypeptides, and flavonoids, quinones, tannins, and coumarins [18-22].



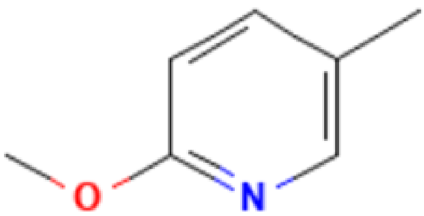
5-Methylquinoline
Molecular Formula: $C_{10}H_9N$
Molecular Weight: 143.18 g/mol



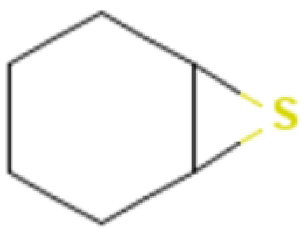
Oxa-4-azacyclohexane Diethylene oximide
Molecular Formula: C_4H_9NO
Molecular Weight: 87.12 g/mol



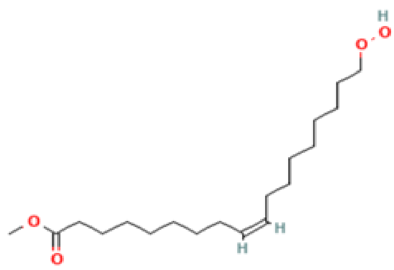
1-DL-Lysine monohydrochloride
Molecular Formula: $C_6H_{15}ClN_2O_2$
Molecular Weight: 182.65 g/mol



2-Methoxy-5-methylpyridine
Molecular Formula: C_7H_9NO
Molecular Weight: 123.15 g/mol

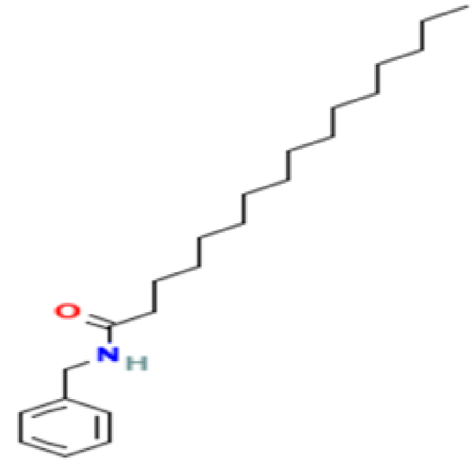


1,2-Epithio-3-hexanol
Molecular Formula: $C_6H_{10}S$
Molecular Weight: 114.21 g/mol

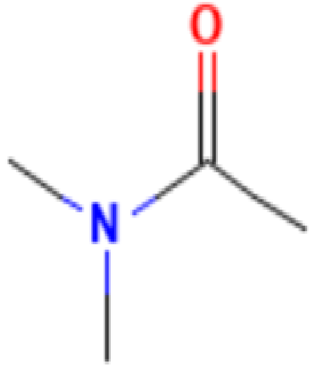


Methyl oleate hydroperoxide
Molecular Formula: $C_{19}H_{36}O_4$
Molecular Weight: 328.5 g/mol

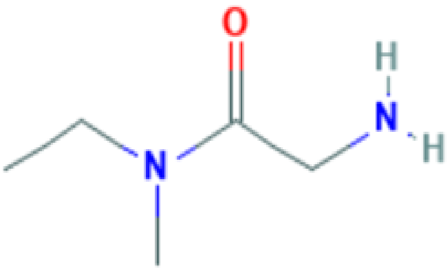
methyl octadeca-9,12,15-trienoate
Molecular Formula: $C_{19}H_{32}O_2$
Molecular Weight: 292.5 g/mol



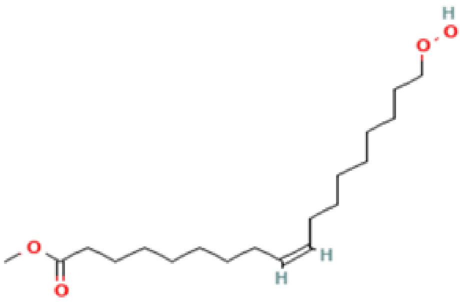
N-benzylhexadecanamide
Molecular Formula: $C_{23}H_{39}NO$
Molecular Weight: 345.6 g/mol



Dimethylacetamide
Molecular Formula: C_4H_9NO
Molecular Weight: 87.12 g/mol

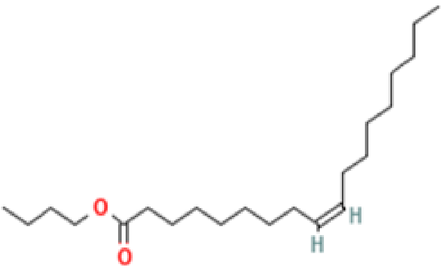


2-amino-N-ethyl-N-methylacetamide
Molecular Formula: $C_5H_{12}N_2O$
Molecular Weight: 116.16 g/mol



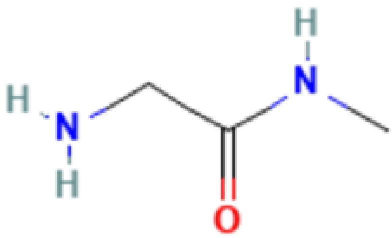
methyl (Z)-18-hydroperoxyoctadec-9-enoate
Molecular Formula: $C_{19}H_{36}O_4$
Molecular Weight: 328.5 g/mol

Methyl hexadecanoate
Molecular Formula: $C_{17}H_{34}O_2$
Molecular Weight: 270.5 g/mol



n-Butyl oleate
Molecular Formula: $C_{22}H_{42}O_2$
Molecular Weight: 338.6 g/mol

Methyl 16-methylheptadecanoate
Molecular Formula: $C_{19}H_{38}O_2$
Molecular Weight: 298.5 g/mol



2-Amino-N-methylacetamide
Molecular Formula: $C_3H_8N_2O$
Molecular Weight: 88.11 g/mol

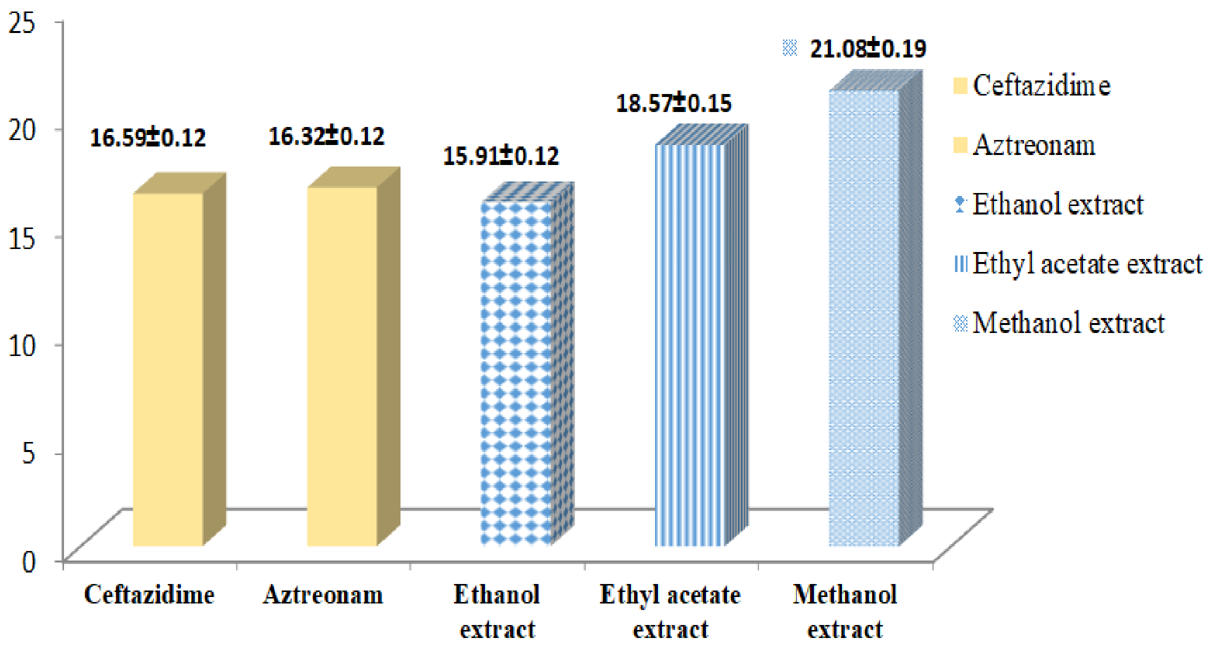


Figure 2. Zone of inhibition (mm) of various bioactive compounds derived from *Anacardium occidentale* extract (methanol , ethyl acetate and ethanol extract) and conventional antibiotics against *Escherichia coli*

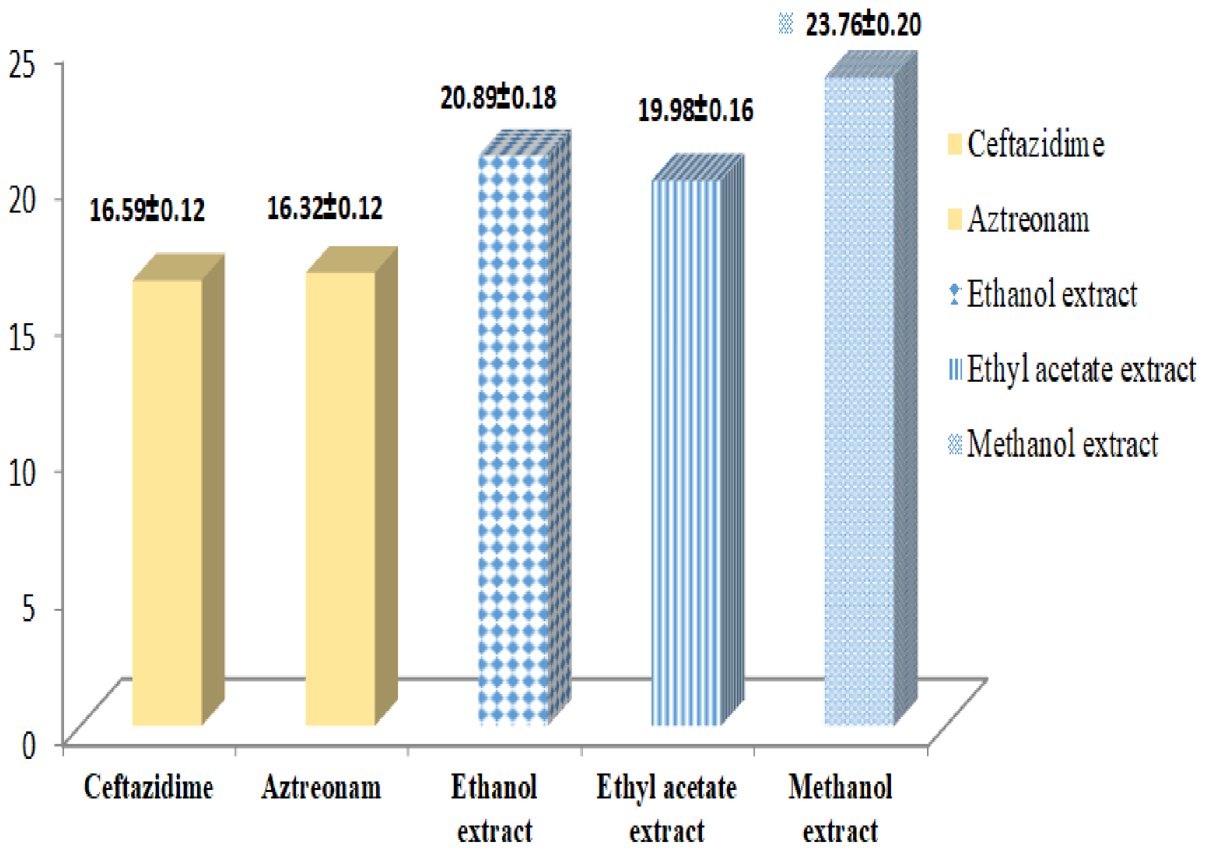


Figure 3. Zone of inhibition (mm) of various bioactive compounds derived from *Coriandrum sativum* extract (methanol , ethyl acetate and ethanol extract) and conventional antibiotics against *Escherichia coli*

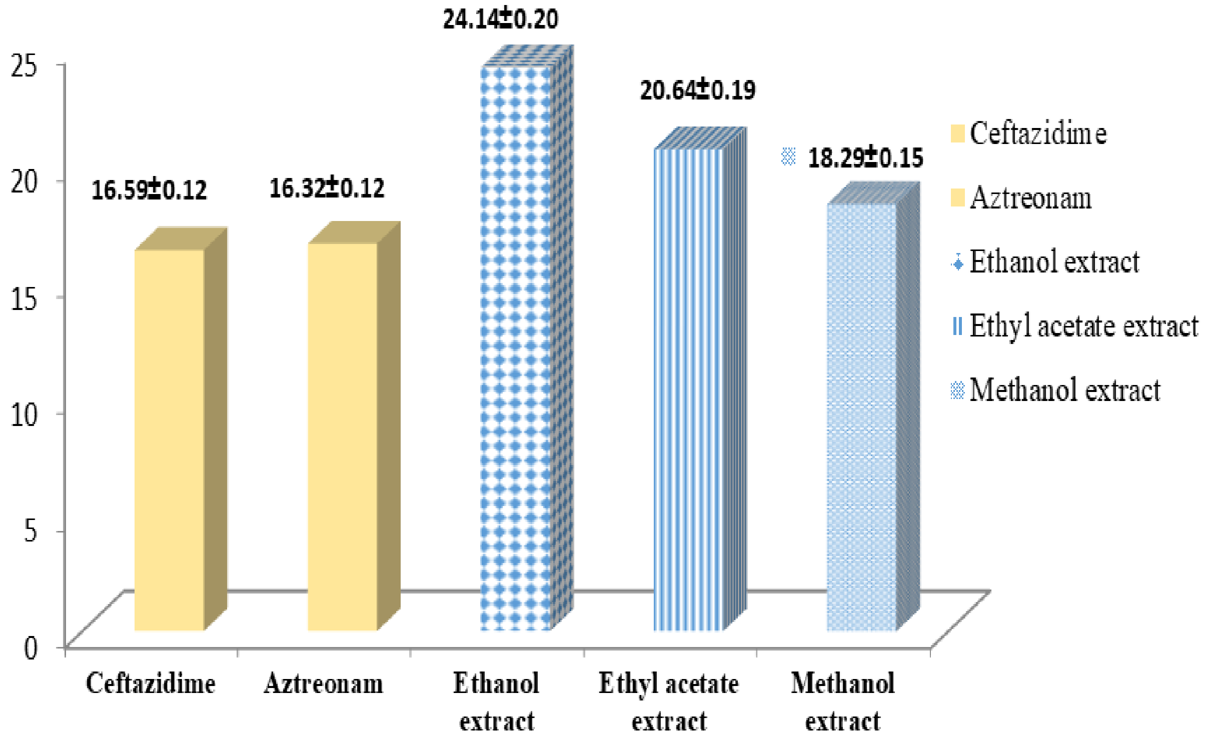


Figure 4. Zone of inhibition (mm) of various bioactive compounds derived from *Origanum vulgare* extract (methanol, ethyl acetate and ethanol extract) and conventional antibiotics against *Escherichia coli*

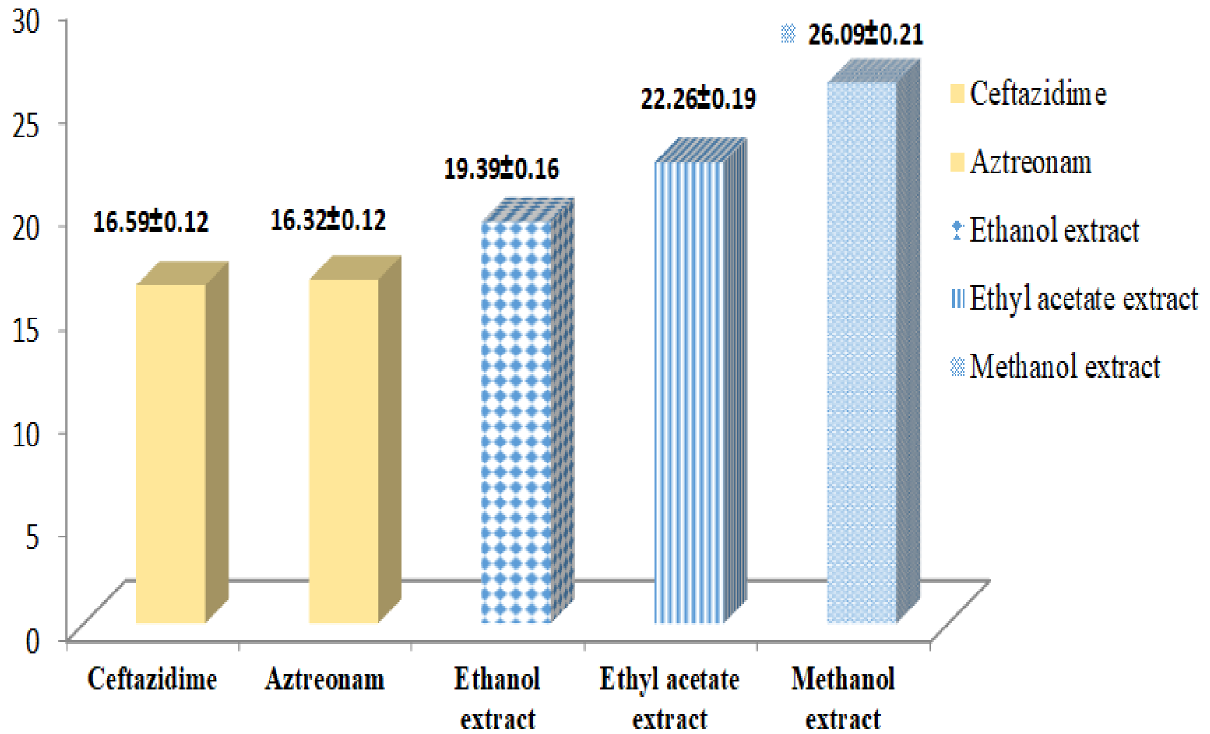


Figure 5. Zone of inhibition (mm) of various bioactive compounds derived from *Urtica dioica* extract (methanol, ethyl acetate and ethanol extract) and conventional antibiotics against *Escherichia coli*

Conclusion:

The results of all of these research point to the potential importance of secondary metabolites in the development of new medications, either as an alternative to traditional antimicrobial agents or in conjunction with them. According to the results of this study, Escherichia coli is among the most promising bacteria in terms of the bioactive compounds they produce. These compounds could be used in the pharmaceutical and medical fields either as full medications or as building blocks for even more effective drugs. A natural substitute for conventional food preservatives can be Coriandrum sativum or Origanum vulgare.

References:

1. Q. Zhang and E. Kelley, “The WHO traditional medicine strategy 2014–2023: a perspective,” Science, vol. 346, no. 6216, supplement, pp. S5–S6, 2014.
2. S. M. H. Chan and J.-M. Ye, “Strategies for the discovery and development of anti-diabetic drugs from the natural products of traditional medicines,” Journal of Pharmacy and Pharmaceutical Sciences, vol. 16, no. 2, pp. 207–216, 2013.
3. Y. Wang, X. Fan, H. Qu, X. Gao, and Y. Cheng, “Strategies and techniques for multi-component drug design from medicinal herbs and traditional Chinese medicine,” Current Topics in Medicinal Chemistry, vol. 12, no. 12, pp. 1356–1362, 2012.
4. C. Huang, C. Zheng, Y. Li, Y. Wang, A. Lu, and L. Yang, “Systems pharmacology in drug discovery and therapeutic insight for herbal medicines,” Briefings in Bioinformatics, vol. 15, no. 5, pp. 710–733, 2014.
5. J. Zou, Z. Pan, and H. Lu, “A summary of the clinical study of Sanjin tablet,” Journal of Traditional Chinese Medicine, vol. 44, no. 4, pp. 311–312, 2003.
6. L. Han, E. Liu, A. Kojo et al., “Qualitative and quantitative analysis of Eclipta prostrata L. by LC/MS,” The Scientific World Journal, vol. 2015, Article ID 980890, 15 pages, 2015.
7. J. M. Andrews, “Determination of minimum inhibitory concentrations,” Journal of Antimicrobial Chemotherapy, vol. 48, supplement 1, pp. 5–16, 2001.
8. X. Liu, S. Ouyang, B. Yu et al., “PharmMapper server: a web server for potential drug target identification using pharmacophore mapping approach,” Nucleic Acids Research, vol. 38, supplement 2, pp. W609–W614, 2010.
9. G. Li, K.D. Young. “Indole Production by the Tryptophanase TnaA in Escherichia coli is Determined by the Amount of Exogenous Tryptophan”. Microbiology. 2013. 159(Pt. 2): 402-410.
10. D. Wang, X. Ding, P.N. Rather. “Indole Can Act as an Extracellular Signal in Escherichia coli”. J. Bacteriol. Am. Soc. Microbiol. 2001. 183(14): 4210-4216.
11. H. Gaimster, D. Summers. “Regulation of Indole Signalling During the Transition of E. coli from Exponential to Stationary Phase”. PLoS One. 2015. 10(9): 4-5.
12. Burdock GA, Carabin IG. Safety assessment of coriander (Coriandrum sativum L.) essential oil as a food ingredient. Food Chem. Toxicol. 2009;47:22-34.
13. Kanimozhi D, Ratha Bai V, Baskaran C. Evaluation of Anti Microbial Activity of Acalypha indica. International Journal of Research in Pharmacy and Science. 2012;2(1):129-37.
14. Gumbo T. General Principles of Antimicrobial Therapy. In: Brunton LL, Chabner BA, Knollman BC, editors. Goodman and Gilman’s The Pharmacological Basis of Therapeutics. 12th ed. New York: Mc Graw Hill; 2011. pp. 1365-78.

15. Farrington M. Chemotherapy of infections. In: Bennet PN, Brown MJ, Sharma P, editors. Clinical Pharmacology. 11th ed. China: Churchill Livingstone Elsevier. 2012:162-72.
16. Serban ES, Ionescu M, Matinca D, Maier C, Bojita MT. Screening of the antibacterial and antifungal activity of eight volatile essential oils. Farmacia. 2011;59(3):440-6.
17. Suganya S, Bharathidasan R, Senthilkumar G, Madhanraj P, Panneerselvam A. Antibacterial activity of essential oil extracted from coriandrum sativum and GC-MS analysis. Journal of chemical and Pharmaceutical Research. 2012;4(3):1846-50.
18. Han, J., Britten, M., St-Gelais, D., Champagne, C. P., Fustier, P., Salmieri, S., et al. (2011). Effect of polyphenolic ingredients on physical characteristics of cheese. Food Research International, 44(1), 494–497.
19. Ruiz-Navajas, Y., Viuda-Martos, M., Sendra, E., Perez-Alvarez, J. A., & Fern´ andezLopez, ´ J. (2013). In vitro antibacterial and antioxidant properties of chitosan edible films incorporated with Thymus moroderi or Thymus piperella essential oils. Food Control, 30(2), 386–392
20. Worley B, Powers R (2013) Multivariate analysis in metabolomics Curr Metabol 1(1):92–107.
21. Xu YJ, Wang C, Ho WE, Ong CN (2014) Recent developments and applications of metabolomics in microbiological investigations. Trends Anal Chem 56:37–48.
22. Peix A, Ramírez-Bahena MH, Velázquez E (2018) The current status on the taxonomy of Pseudomonas revisited: an update. Infect genet envol 57:106–116.