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Original Research

In *vitro* Antimicrobial Activity of Plant Extracts on *Pseudomonas aeruginosa* and Screening of Bioactive Chemical Compounds

Lina Abdulkadhim Oudah [1], Sahba Majeed Kadhem [2]

[1]Assist. Lecturer, Department of Medical and Basic Sciences, College of Nursing, University of Al-Qadisiyah, Iraq.; e-mail: lina.abdulkadhim@qu.edu.iq

[2]Assist. Lecturer, Department of Medical and Basic Sciences, College of Nursing, University of Al-Qadisiyah, Iraq.; e-mail: sahbaaabass@gmail.com

Abstract:

Aims and Objectives: This research aimed to analyze the bioactive chemical products of *Pseudomonas aeruginosa* and evaluate the antibacterial, antifungal and in vitro antimicrobial activities of plant extracts against *Pseudomonas aeruginosa*.

Method: The chemical components known as bioactives, which are sometimes referred to as secondary metabolites, were examined using gas chromatography-mass spectrometry (GC-MS) techniques. Subsequently, the antibacterial , antifungal activity of the methanolic extract of *Pseudomonas aeruginosa* was assessed in vitro.

Results: The GC-MS analysis of *Pseudomonas aeruginosa* detected the presence of the following: The compounds listed include Nonadecanamide, N-[3-[5,8-bis[3-[acetyl(hydroxy)amino]propyl]-3,6,9,12,15,18-hexaoxo-1,4,7,10,13,16-hexazacyclooctadec-2-yl]propyl]-N-hydroxyacetamide, 16-hexazacyclooctadec-2-yl]propyl]-N-hydroxyacetamide, Methyl 2-(2-acetyl-3,5-dihydroxyphenyl)acetate, 1-Cyclohexyloctadecane,, bis(2-propylheptyl) benzene-1,2-dicarboxylate, 2-Ethyl-3,5-dimethylpyridine, decan-4-ylcyclohexane, methyl 1-methylpiperidine-3-carboxylate, beta-Methylacetylcholine, 8-hydroxy-9,11-octadecadiynoic acid, O-Acetyl-N-propionylhydroxylamine, Glycyl-D-asparagine, beta-Hydroxyasparagine, and D-Leucine, methyl ester. The metabolites of *Pseudomonas aeruginosa* exhibited significant activity against *Escherichia coli* (15.28±0.05). *Pseudomonas aeruginosa* metabolites was very highly active against *Aspergillus flavus* (12.80 ± 0.03). *Origanum vulgare* (Crude) (20.06±0.18) was very highly active against *Pseudomonas aeruginosa*.

Keywords: Pseudomonas aeruginosa, Secondary metabolites, Antibacterial, GC/MS.

Introduction:

One prevalent and well-known harmful environmental organism is pseudomonas aeruginosa. It has numerous identified metabolic pathways and is one of the primary causes of common ailments as well as resistant bacterium that is intolerant of medications and therapies. These bacteria can cause major systemic infection of known human tissues, such as pneumonia, septicemia, and respiratory infections, as well as clear and distinct localized purulent inflammation when the human body's known tissue barrier is compromised and medical processes or immune function may be significantly compromised. That is [1]. Under some conditions, P. aeruginosa might be a useful pathogen for diagnosing our often severe facultative opportunistic bacterial infections. Additionally, it is widely recognized that this bacteria causes blood infections, burn infections, dermatitis from hot tub infections, infections in the external ears, and infections of the respiratory system and urinary tract. P. aeruginosa is the most frequent and pervasive bacteria that colonizes medical equipment (humidifiers, catheters), and it is one of the pathogens that cause severe ventilator-associated pneumonia and meningoencephalitis in hospital settings [2]. Due to P. aeruginosa's innate and acquired resistance to a variety of antibiotics, treating an infection with this bacteria can be exceedingly challenging. Biofilm bacteria have the ability to proliferate and form new structures and growth patterns inside the human body [3]. There is a discernible and noteworthy rise in the resistance of these bacterial species to antimicrobial agents as a result of the increased rate of bacterial gene conversion and modification. Research has demonstrated that the development of essential cellular membranes is associated with up to 65% of bacterial resistance to antibiotics. This association may be primarily explained by the barrier that prevents drug entry, cutting off the studied bacteria's food supply, slowing down their growth, and actually decreasing Metabolites are the most active and significant component of life's activities, as opposed to gene molecules and structural and functional proteins. It must be remembered that the majority of small molecule metabolites that are now known are the outcome of complex enzyme reactions that need numerous stages and computations [4, 5]. Consequently, every environmental disruption that is identified as well as temporal and spatial alterations, in addition to the known genetic components, can actually cause urgent and quick changes in metabolites. However, it is important to note that these modifications may have a substantial impact on the metabolites' effects on gene expression, biological processes, growth, and behavior [6]. The purpose of this research is to analyze the biologically active biochemical products of *Pseudomonas aeruginosa* bacteria and to know and evaluate the in vitro antibacterial and antifungal activity of plant extracts against *Pseudomonas aeruginosa*.

Materials and Methods:

Optimal environmental conditions for growth and identification of metabolites

A strain of *Pseudomonas aeruginosa* was isolated and subcultures were obtained on nutrient agar for 48 hours at a temperature of 22°C. The solution was subjected to incubation at a temperature of 4ºC for a duration of 10 minutes, followed by agitation at a speed of 130 revolutions per minute for 10 minutes. The metabolites were isolated from the liquid culture and subjected to evaporation using a rotary evaporator at a temperature of 45ºC [7, 8].

Performing a spectral study of the bioactive natural chemical components of *Pseudomonas aeruginosa* utilizing (GC-MS).

The examination was carried out by employing a GC–MS technique with an Agilent 789 A device. The DB-5MS column from J&W Scientific in Folsom, California was utilized as the GC column. This column had the following dimensions: 30 m0.25 mm i.d. with a film thickness of 0.25 um. The temperature in the oven was maintained at the same level as in the previous investigation. Helium was used as the carrier gas, and the flow rate was set at one

milliliter per minute [9, 10]. Through a transfer line that had been heated to 250 degrees Celsius, the effluent from the gas chromatography (GC) column was directly injected into the source of the mass spectrometer (MS). Ionization took place at a voltage of 70 electron volts (eV), and the temperature of the ion source was maintained at 230 degrees Celsius (°C). The measuring range encompassed atomic mass units (amu) all the way up to 450.

Assessment of the antibacterial efficacy of secondary metabolite chemicals against three pathogenic bacteria.

Using a sterile cork-borer, wells with a diameter of five millimetres were created in the agar. Then, 25 µl of the sample solutions containing metabolites produced by *Pseudomonas aeruginosa* were added to the wells. The test pathogens, namely *E. coli*, *Proteus mirabilis*, and *Staph. Epidermidis*, were collected using swabs and applied onto Muller Hinton agar plates [11, 12]. Methanol served as the control solvent.

Determination of antifungal activity of secondary metabolite compounds of *Pseudomonas aeruginosa*

Mueller-Hinton agar plates were blotted with the tested microorganisms. In the boring wells, 70 µl of Pseudomonas aeruginosa extract was already loaded. By evaluating the studied and calculated zone of inhibition against the tested microorganisms, the antifungal bioactivity was determined and evaluated. As the solvent methanol was used as the standard antifungal agent, fluconazole and amphotericin B were used. Experiments were performed in triplicate [13]. By evaluating and measuring the diameter of the studied inhibition zone that was actually seen after 48 hours of incubation, the antifungal activity was evaluated.

Antimicrobial efficacy of selected medicinal plant extracts against *Pseudomonas aeruginosa* in a laboratory setting

Using a sterile cork-borer, wells with a diameter of five millimetres were cut from the agar. Then, 25 µl of the sample solutions of twelve medicinal plants were added to the wells. The plates were incubated for 48 h at room temperature. The antibacterial activity was assessed by measuring the diameter of the inhibitory zone seen after 48 hours of incubation. Methanol was employed as the control for the solvent. The reference antibacterial agents utilised were Rifambin and Cefotoxime [14]. The experiments were conducted in duplicate.

Statistical analysis

A number of statistical procedures, such as computing the mean value and carrying out an analysis of variance (ANOVA), were used to the examination of the data that had been collected from an SPSS (Version 11.6) database.

Results and Discussion:

The GC-MS chromatogram displayed twenty one peaks corresponding to the identified chemicals. The compounds mentioned are Nonadecanamide, N-[3-[5,8-bis[3-[acetyl (hydroxy) amino]propyl]-3,6,9,12,15, 18-hexaoxo-1,4,7,10,13,16-hexazacyclooctadec-2-yl]propyl]- N-hydroxyacetamide, 16-hexazacyclooctadec-2-yl]propyl]-N-hydroxyacetamide, Methyl 2-(2-acetyl-3,5-dihydroxyphenyl)acetate, 1-Cyclohexyloctadecane,, bis(2-propylheptyl) benzene-1,2-dicarboxylate, 2-Ethyl-3,5-dimethylpyridine, decan-4-ylcyclohexane, methyl 1-methylpiperidine-3-carboxylate, beta-Methylacetylcholine, 8-hydroxy-9,11-octadecadiynoic acid, O-Acetyl-N-propionylhydroxylamine, Glycyl-D-asparagine, beta-Hydroxyasparagine, and D-Leucine, methyl ester.

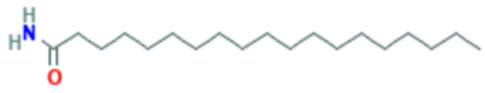
The antibacterial efficacy of secondary metabolites produced by *Pseudomonas aeruginosa* against three harmful microorganisms was investigated

The current study examined the bioactivity of the methanolic extract of Staphylococcus aureus and the standard antibiotics Rifambin and Cefotoxime against five tested pathogens: *scherichia coli* (15.28±0.05, 09.77±0.02, and 11.25±0.02), *Proteus mirabilis* (14.07±0.04, 12.07±0.03, and 08.70±0.01), and *Staph. aureus* (13.70±0.04,

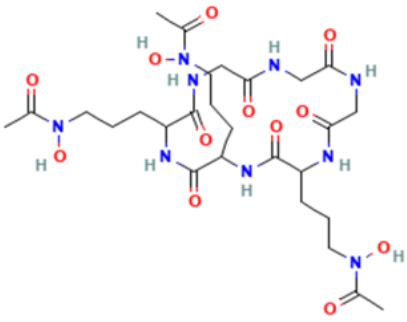
08.90±0.01, and 09.62±0.02). The metabolites of *Pseudomonas aeruginosa* exhibited significant activity against *Escherichia coli* (15.28±0.05).

Antifungal activity of secondary metabolites of *Pseudomonas aeruginosa*

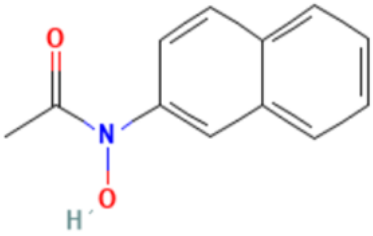
Bioactivity of the methanolic extract of *Pseudomonas aeruginosa* and standard antibiotics against three fungi. Inhibitory disc recorded (15.84 ± 0.04, 15.01 ± 0.04 and 12.10 ± 0.03) *Aspergillus flavus*, Amphotericin B and Fluconazol respectively and recorded (10.70 ± 0.02, 9.73 ± 0.01 and 07.82 ± 0.01) for *Fusarium oxyporum*, Amphotericin B and Fluconazol respectively, while recorded (14.20 ± 0.03, 12.80 ± 0.03 and 09.89 ± 0.02) *Cladosporium herbarum* Amphotericin B and Fluconazol respectively. *Pseudomonas aeruginosa* metabolites was very highly active against *Aspergillus flavus* (12.80 ± 0.03).



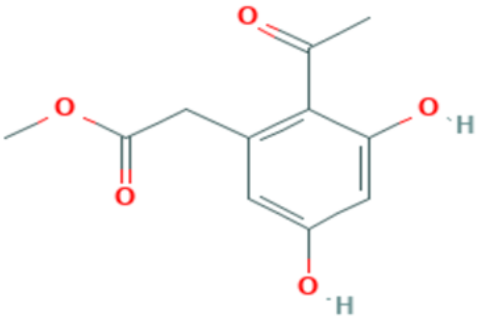
Nonadecanamide
Molecular Weight: 297.5 g/mol



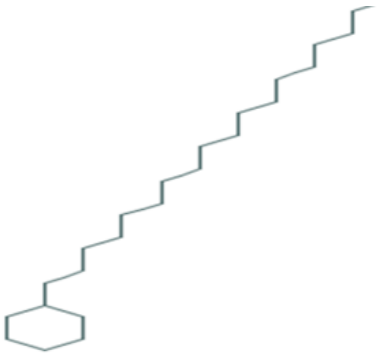
16-hexazacyclooctadec-2-yl]propyl]-N-hydroxyacetamide
Molecular Formula: $C_{27}H_{45}N_9O_{12}$
Molecular Weight: 687.7 g/mol



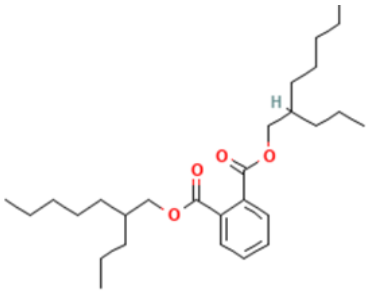
N-Hydroxy-N-2-naphthalenylacetamide
Molecular Formula: $C_{12}H_{11}NO_2$
Molecular Weight: 201.22 g/mol



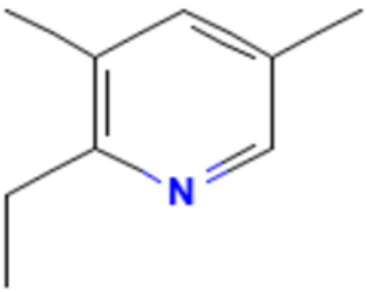
Methyl 2-(2-acetyl-3,5-dihydroxyphenyl)acetate
Molecular Formula: $C_{11}H_{12}O_5$
Molecular Weight: 224.21 g/mol



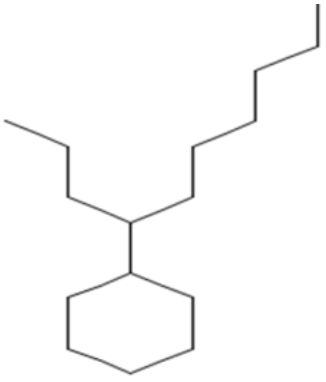
1-Cyclohexyloctadecane
Molecular Formula: $C_{24}H_{48}$
Molecular Weight: 336.6 g/mol



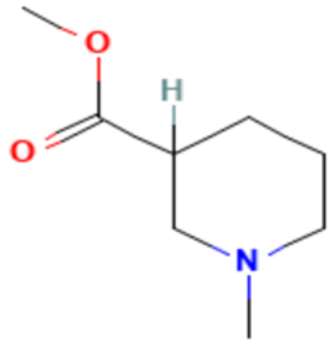
bis(2-propylheptyl) benzene-1,2-dicarboxylate
Molecular Formula: $C_{28}H_{46}O_4$
Molecular Weight: 446.7 g/mol



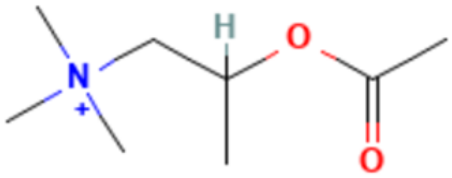
2-Ethyl-3,5-dimethylpyridine
Molecular Formula: $C_9H_{13}N$
Molecular Weight: 135.21 g/mol



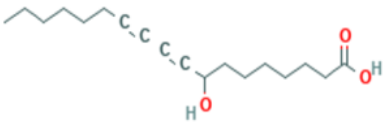
decan-4-ylcyclohexane
Molecular Formula: $C_{16}H_{32}$
Molecular Weight: 224.42 g/mol



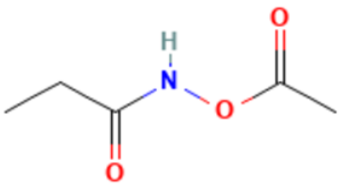
methyl 1-methylpiperidine-3-carboxylate
Molecular Formula: $C_8H_{15}NO_2$
Molecular Weight: 157.21 g/mol



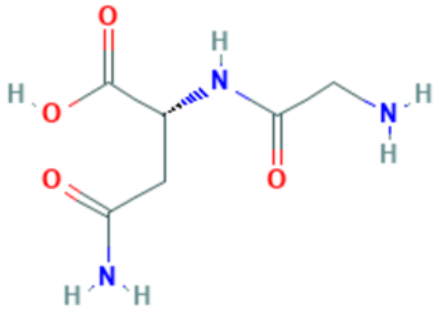
beta-Methylacetylcholine
Molecular Formula: $C_8H_{18}NO_2$
Molecular Weight: 160.23 g/mol



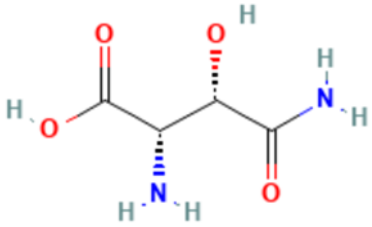
8-hydroxy-9,11-octadecadiynoic acid
Molecular Formula: C18H28O3
Molecular Weight: 292.4 g/mol



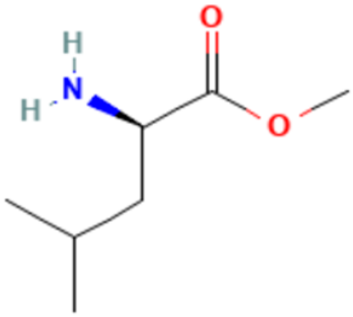
O-Acetyl-N-propionylhydroxylamine
Molecular Formula: C5H9NO3
Molecular Weight: 131.13 g/mol



Glycyl-D-asparagine
Molecular Formula: $C_6H_{11}N_3O_4$
Molecular Weight: 189.17 g/mol



beta-Hydroxyasparagine
Molecular Formula: $C_4H_8N_2O_4$
Molecular Weight: 148.12 g/mol



D-Leucine, methyl ester
Molecular Formula: $C_7H_{15}NO_2$
Molecular Weight: 145.20 g/mol

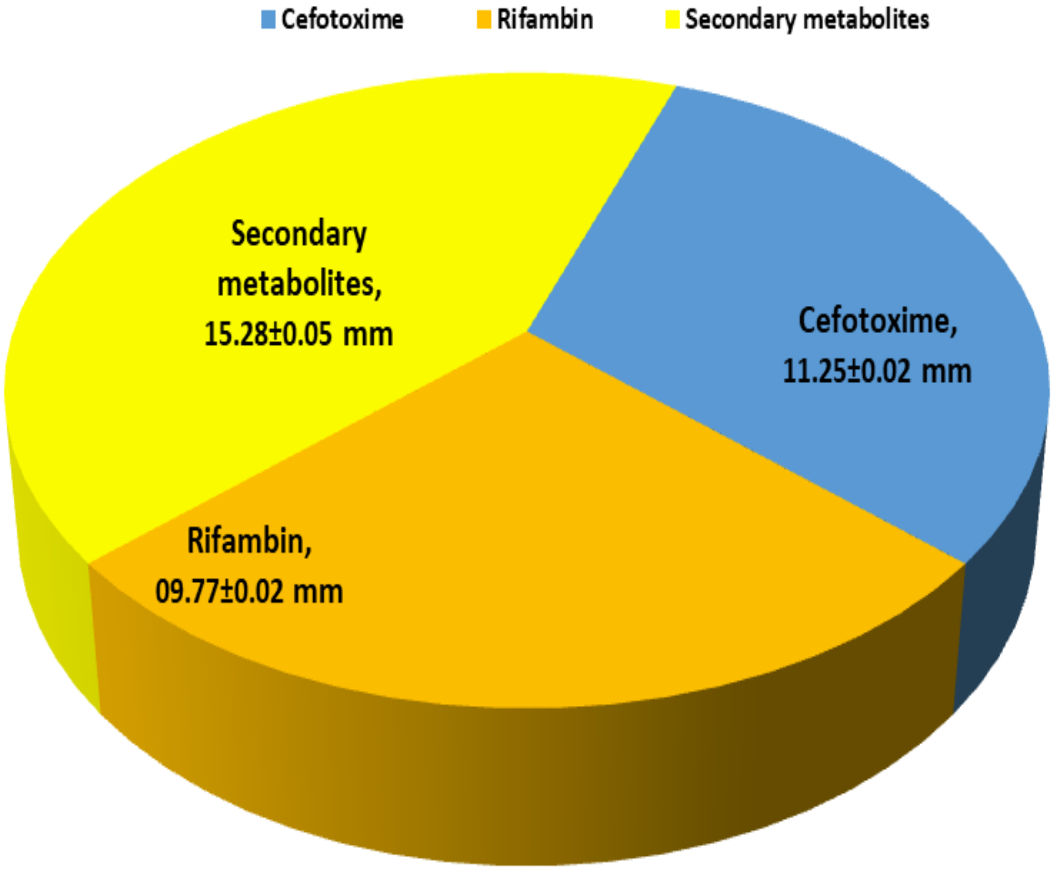


Figure 1. Metabolite products, Rifambin and Cefotoxime as anti- Bacterial activity against *Escherichia coli*

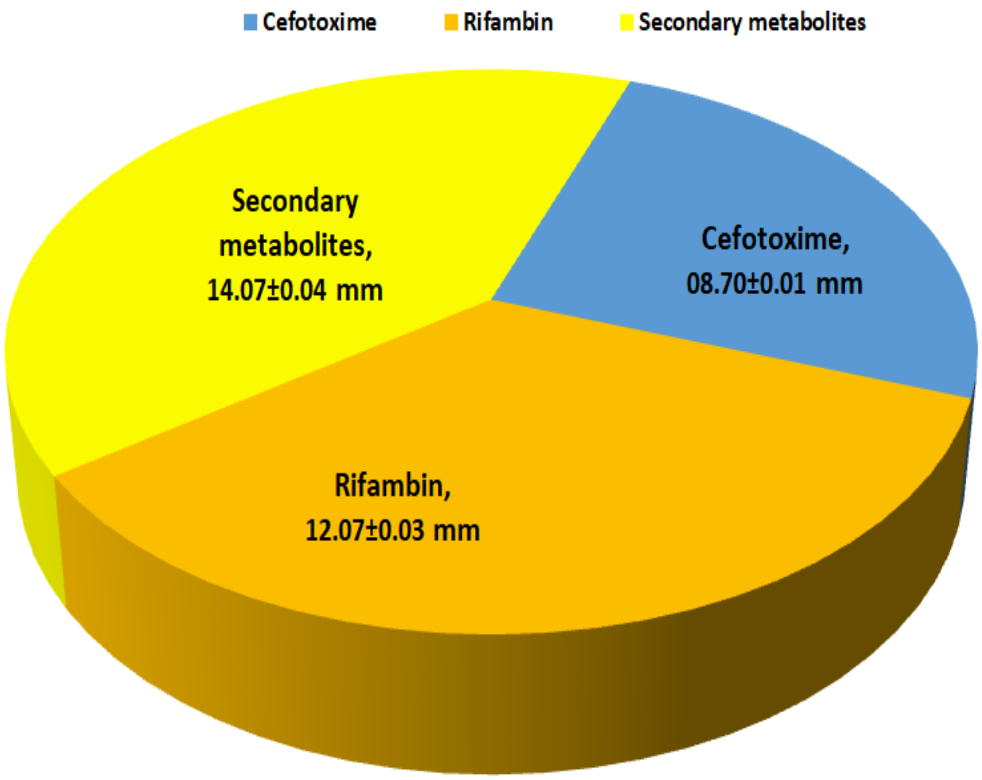


Figure 2. Metabolite products, Rifambin and Cefotoxime as anti- Bacterial activity against *Proteus mirabilis*

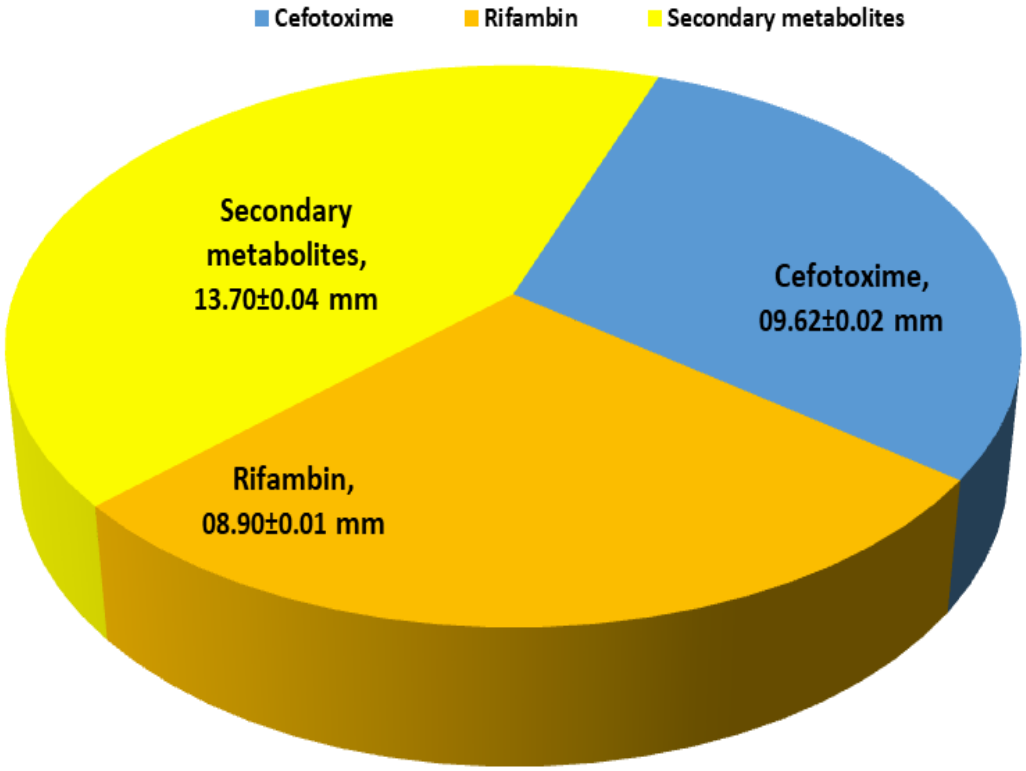


Figure 3. Metabolite products, Rifambin and Cefotoxime as anti- Bacterial activity against *Staphylococcus aureus*

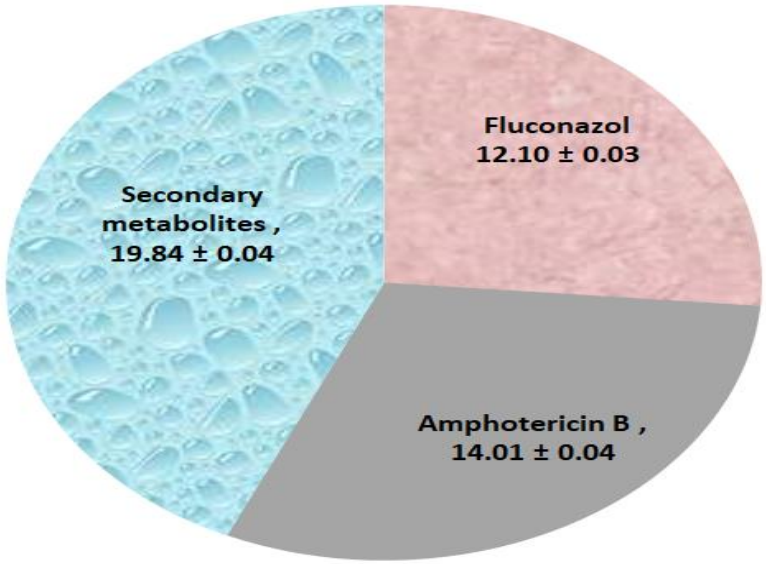


Figure 4. Metabolite products , Amphotericin B, and Fluconazol as anti- Fungal activity against *Aspergillus flavus*

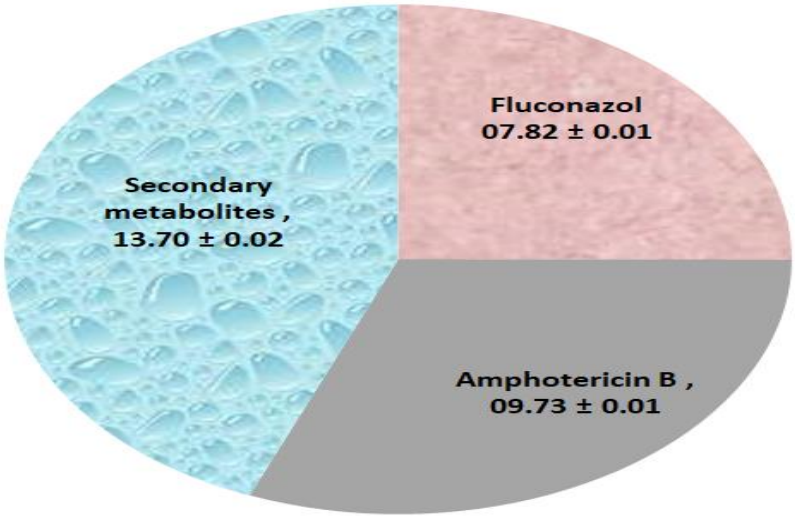


Figure 5. Metabolite products , Amphotericin B, and Fluconazol as anti- Fungal activity against *Fusarium oxyporum*

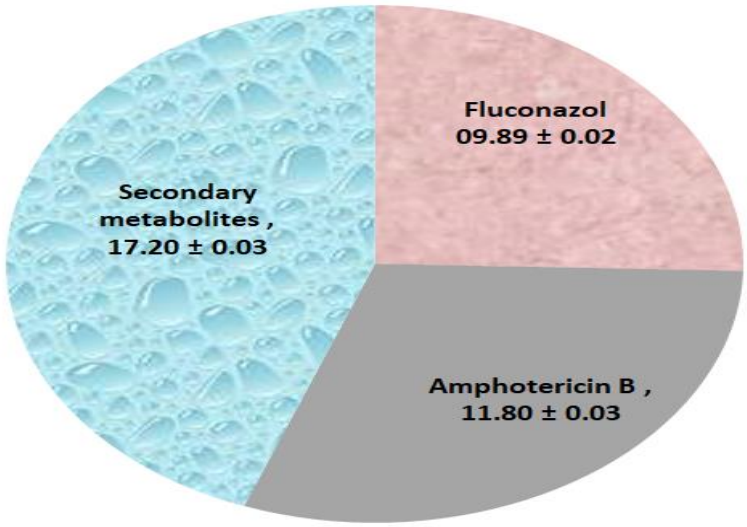


Figure 6. Metabolite products , Amphotericin B, and Fluconazol as anti- Fungal activity against *Cladosporium herbarum*

In *vitro* antimicrobial activity of various bioactive compounds derived from plant extract (methanol extract, ethyl acetate extract and ethanol extract) and conventional antibiotics against *Pseudomonas aeruginosa* recorded (17.20±0.19, 13.85±0.12 and 13.28±0.12) respectively for *Artemisia annua* (Crude) extract. While recorded (19.85±0.20, 16.52±0.19 and 17.87±0.17) respectively for *Althaea rosea* (Crude) extract. Antimicrobial activity of *Origanum vulgare* (Crude) recorded (14.44±0.13, 16.99±0.14 and 20.06±0.18). Antimicrobial activity of *Foeniculum vulgare* (Crude) recorded (19.73±0.18, 17.23±0.16 and 14.91±0.13) compare with two conventional antibiotics Rifambin and Cefotoxime. *Origanum vulgare* (Crude) (20.06±0.18) was very highly active against *Pseudomonas aeruginosa* (Figure 7, 8, 9, 10).

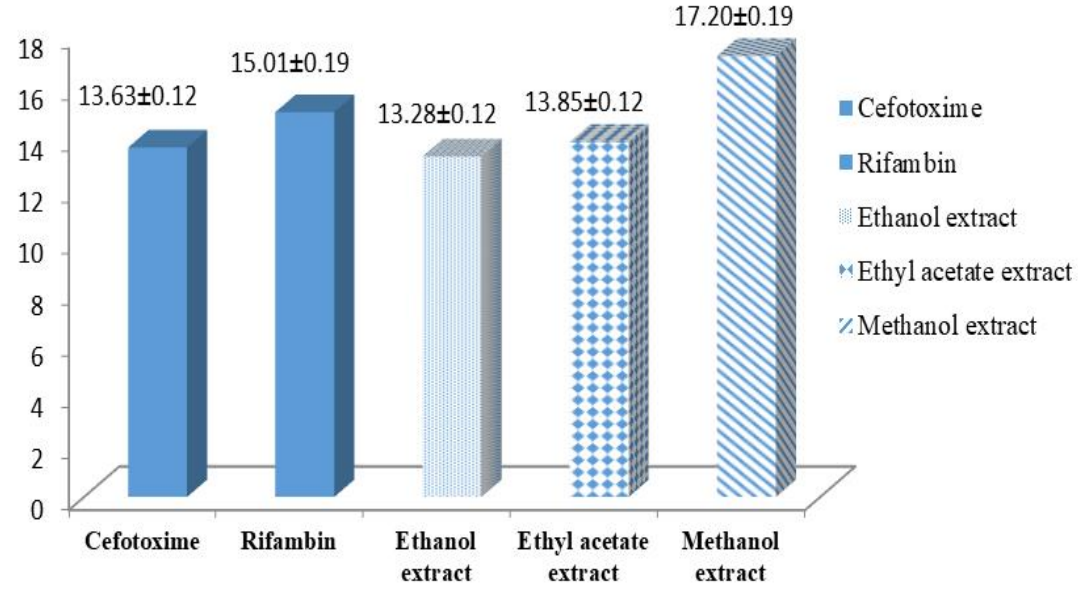


Figure 7. Zone of inhibition (mm) of various bioactive compounds derived from plant *Artemisia annua* and conventional antibiotics against *Pseudomonas aeruginosa*

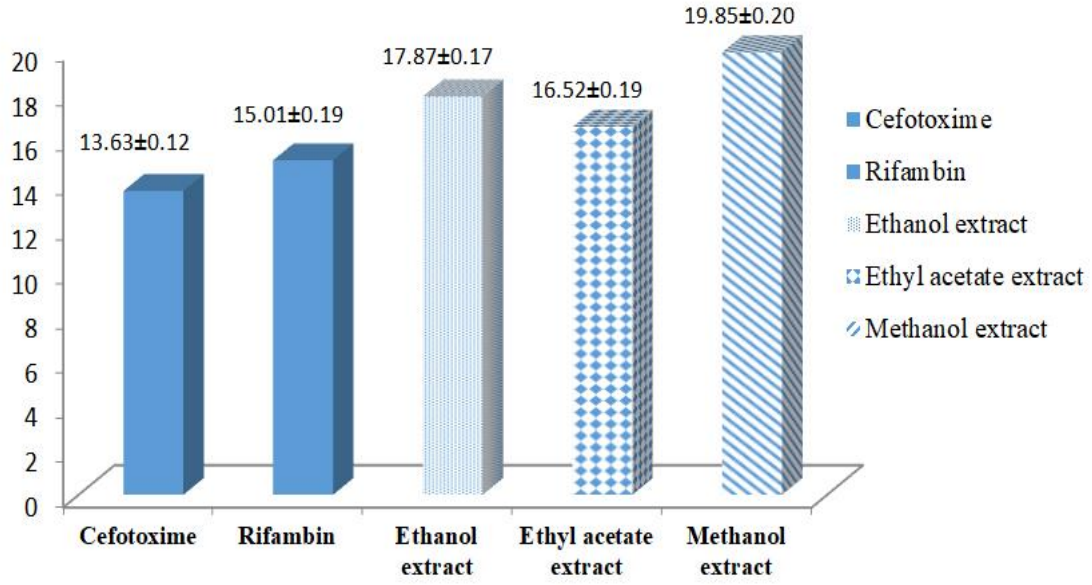


Figure 8. Zone of inhibition (mm) of various bioactive compounds derived from plant *Althaea rosea* and conventional antibiotics against *Pseudomonas aeruginosa*

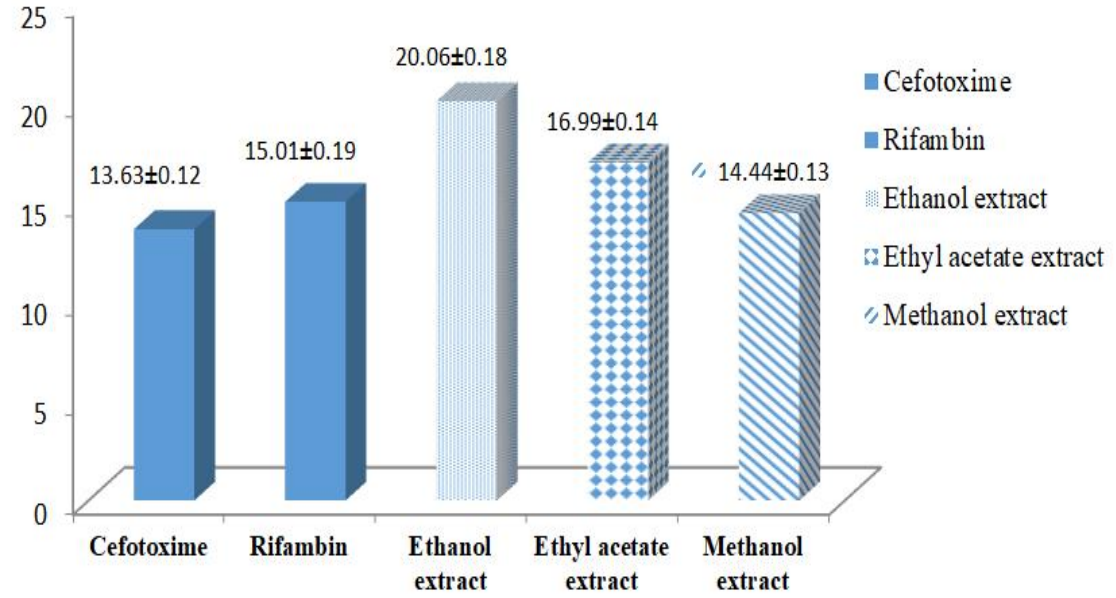


Figure 9. Zone of inhibition (mm) of various bioactive compounds derived from plant *Origanum vulgare* and conventional antibiotics against *Pseudomonas aeruginosa*

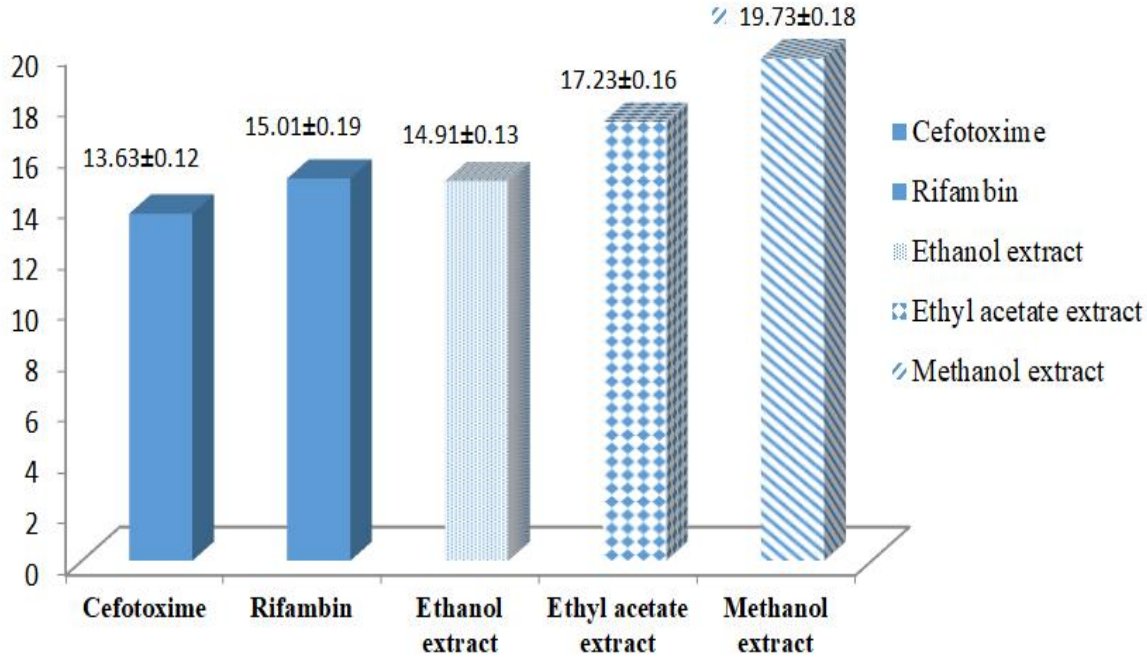


Figure 10. Zone of inhibition (mm) of various bioactive compounds derived from plant *Foeniculum vulgare* and conventional antibiotics against *Pseudomonas aeruginosa*

Bacterial metabolites are in fact more diverse, distributed and complex than those actually found in common animals and plants. The reason for this is that bacteria have a great ability to adapt to different and diverse environments with the possibility of participating in exchange between

different species by realistically changing their metabolic process. . When a person is infected with this bacteria, it is very difficult to treat and the infection recurs continuously, which is usually associated with the formation of biofilms of these bacteria. Clinical data have shown that these bacteria form an antibiotic-resistant biofilm in pulmonary cystic fibrosis, which at the same time leads to activation of the effectiveness of antibiotics and prevents the elimination of bacteria. Infection and sinusitis are also associated with PA biofilm formation [15].

These natural dyes, in particular, can be an important and distinctive alternative in various sectors of the food, pharmaceutical, medical and cosmetic industries. There is a new and important trend of global interest towards the biosynthesis of natural colorants from living organisms, the purpose of which is to reduce the negative and harmful effects of artistic colours. The toxic effects of many synthetic dyes used in pharmaceuticals and cosmetics have already been reported. Natural dyes produced from living organisms are preferred over synthetic colorants because of their good biocompatibility, bioavailability, known safety, and distinct medical benefits. Natural dyes produced from natural bio-based ingredients also have some important advantages, such as minimal health risks, simple purification process, and high sustainability [16]. Microscopic dyes are being seriously researched on a large scale and are popular due to the great demand for natural dyes in many industries, especially as is evident in foodstuffs as colorants, as well as in the textile industry as natural dyes. Studies on P. aeruginosa's metabolism have also been carried out. A collection of incredibly tiny substances found both inside and outside of cells is known as a metabolite. These substances have a molecular weight of less than 1500 Da and serve various functions as metabolic products and intermediates. We are able to observe the activity of metabolic pathways within living cells by utilizing information pertaining to metabolite concentration. These well-known metabolic studies use nuclear magnetic resonance (NMR) spectroscopy or chromatography-coupled mass spectrometry (GC/MS) as laboratory methods to detect and measure metabolites. Additionally, metabolic research studies may be able to assist in the resolution of certain scientific issues that prove challenging to resolve with the aid of biological sciences techniques, such as the identification of metabolic pathways. One popular and widely used method for identifying these compounds produced and supplied by microorganisms is metabolic analysis. Identifying and characterizing these novel compounds made by microbes is one of microbiology's major objectives. These products of microbiological production are vital to the pharmaceutical industry (new medications) and environmental conservation (biodegradable detergents). Pseudomonas strains' lipopeptides were identified using an LC/MS-based metabolic analysis technique. Additionally, 260 Pseudomonas strains that were isolated from various natural settings were studied by the researchers. Using C/MS technology, extracellular metabolic analysis of cells is performed to identify compounds that are specific to the strain of bacteria. The Global Natural Products Social Molecular Network was used to process and record all LC-MS/MS data in order to identify potential compounds.

Conclusion:

In conclusion, such a study and this work confirm that Pseudomonas species are among the most identified bacteria with high and important potential for biologically active compounds useful in pharmacy and medicine or precursors for the development of important and powerful pharmaceutical compounds. According to the findings of the antibacterial and antifungal activity test, the metabolites produced by *Pseudomonas aeruginosa* exhibited an exceptionally high degree of activity against *Escherichia coli* (15.28±0.05) and *Aspergillus flavus* (12.80 ± 0.03) respectively. Ethanolic extract of *Origanum vulgare* (Crude) (20.06±0.18) was very highly active against *Pseudomonas aeruginosa.*

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