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Proteus mirabilis Profile Metabolites Isolated in Patients with Urinary Tract Infection and Its Antibacterial Activity

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Abstract:
Examining the bioactive chemical compounds and their antibacterial activity were the goals of this research. The bioactive chemicals or secondary metabolites of the methanolic extract were analyzed using (GC-MS) techniques after which its biomedical properties of antibacterial activity in vitro were tested. *Proteus mirabilis* was analyzed by GC-MS, showing the presence of the: 1-(4-cycloprolphenyl)-ethenone, 1,3-benz-dioxole, 2-Methoxy-4-(propyl)phenol, Apiol, Pyrazolo-5-a]pyridine, 2-Methyl-phenyl-2-propanol, 2,3-Dimethoxy-5-(1-propenyl)phenol, 2-Propenamide, 2-methyl, 1-benzylindoline-2,3-dione, 4-Oxocyclohexanecarboxylic acid, 4-Amino-3,5-dinitrobenzotrifluoride, 2,5-Dimethyltetrahydrofuran-3-thiol, Hexadecanoic acid, 2-methoxyethyl ester, Isophthalaldhyde, 5-Ethoxy-4-Hydroxy, d-Methylamphetamine and 1,1,3,3-Tetramethoxypropane. The antimicrobial activity of secondary metabolites of *Proteus mirabilis* which is an isolate of a urinary tract infection was tested against six different pathogenic microorganisms. The results were compared to those of two conventional antibiotics, AP-Ampicillin and RF-Rifampicin. Antibacterial activity of bioactive secondary metabolites was (14.09±0.25, 23.07±0.34, and 16.45 ±0.29), (10.30±0.21, 17.00±0.31, and 18.96±0.34), (11.00±0.20, 23.89±0.35, and 17.45±0.30), (08.05±0.17, 19.00±0.36, and 12.01±0.24), (13.00±0.20, 23.05±0.33, and 27.81±0.40), and (15.04±0.28, 24.90±0.37, and 21.85±0.32) against *Streptococcus pyogenes*, *Enterococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus* respectively. The demonstrated results of the anti-bacterial activity indicated that the volatile chemicals produced by the *Proteus mirabilis* had a positive effect on the growth of *Staphylococcus aureus* (15.04±0.28).

Keywords: *Proteus mirabilis*, patients, UTI, Antibacterial activity.

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INTRODUCTION

The aerobic Gram-negative *Proteus spp*. bacteria are Gram-negative. Their length ranges from 1.0 to 3.0 μm and their diameter is between 0.4 and 0.8 μm. Their names are based on their ability to have morphological changes of colonies. Marmotid proteus species have peritrichouse flagella. Two characteristic features of a *Proteus* culture are swarming and an ammonia smell [1-3]. Proteus habitats may be found everywhere. *Proteus* is a human opportunistic pathogen that may occur in a wide variety of locations, such as intestines, skin, and mouth of humans and animals. It is also present in various soil, water and plant. Proteus spoils the raw meat, shellfish, veggies and canned goods. *Proteus spp.* is found in food implying that it was not cooked under clean conditions. *Proteus* is most likely to be detected during the fall. *Proteus spp.* can grow on most culture media, and do not form spores, in liquid gelatin. When Proteus is growing in it, the milk curdles and then becomes liquefied [4, 5]. A wide temperature range of 10 to 43 °C is suitable for Proteus growth. Proteus thrives at a temperature of 25 °C. In temperatures ranging from 20 to 37 degrees Celsius, swarming takes place. Various species of Proteus break down organic materials. They also exhibit proteolytic activity, in addition to hydrolyzing urea, synthesizing hemagglutinins and hemolysins, and oxidatively deaminate amino acids [6]. It described four new genomospecies, such as *Proteus hauseri* and three unidentified species belonging to a biogroup that used to contain *P. vulgaris*. Due to the lack of the universally adopted criteria of phenotypic differentiation, three genomospecies are nameless. *Proteus mirabilis* is the most prevalent species of *Proteus* which infects humans [7]. Intestinal *P. mirabilis* is carried by around a quarter of the population. Nosocomial infections are the most common way that *P. vulgaris* or *P. penneri* infect humans. The *Proteus myxofaciens* bacteria, which was found in gypsy moth larvae, is not thought to be a major pathogen in humans [8, 9].

The Possible Hazards of Proteus spp. Under the right environment, opportunistic pathogenic bacteria referred to as Proteus rods may lead to UTIs which are often associated with complex UTI. The acute pyelonephritis, cystitis, and urolithiasis of the upper urinary tract are infections caused by them and which is a common site of infection. There is also a limited number of reports of bacteraemia associated with UTIs by Proteus spp. Other infections include scepticemia, wound infections, rheumatoid arthritis and meningitis in the newborn and young children. Pseudomonas vulgaris infection of the brain with plague [10]. Regardless, the popularity of UTIs due to the Proteus bacteria deserves particular focus. Infections of this kind might be either simple or complex. Simple infections can occur in patients who are in generally good health, but complicated infections are more frequent in patients who already have a urinary catheter, structural or functional abnormalities of the urinary tract, are immunocompromised, have another disease or undergone surgery to the urogenital system. The findings indicate that *Escherichia coli* is a common cause of simple illnesses. Gram-negative Proteus spp., Gram-positive *Klebsiella pneumonia*, E. coli, *Pseudomonas aeruginosa*, *Morganella morganii*, and Providencia stuartii are some of the bacteria that can cause polymicrobial urinary tract infections (UTIs). Despite the possibility of hematogenous infections with the Proteus species [11–13], most infections are ascending. An outline of important virulence factors of *Proteus mirabilis* that lead to catheter blockage and colonization, kidney and bladder infections, and the development of stones in the urinary tract (urolithiasis).

Bioactive chemicals obtained through natural sources have a variety of food applications such as antioxidant and antibacterial features. In addition, the virulence factors produced by P. aeruginosa that are mediated by QS were inhibited by the natural extracts. One study found the environmental isolate *Proteus mirabilis* as a possible source of QS inhibitory compounds [14, 15]. But the study didn't look at the bioactive parts of that extract. Since the 1980s, the technique of (GC-MS) used as an analysis method. It is one of the most accurate, fastest and best ways of detecting a great number of different compounds including amino acids, steroids, alcohols, alkaloids, long-chain hydrocarbons, among others, and in a small amount of plant extract. Besides extract analysis, it is an appropriate tool in determining the concentration of active chemicals in the environment and other areas [16]. It is a method to analyze mixtures of compounds by combining two distinct methods of analysis. Gas chromatography can be used to separate the components of a mixture and then analyze them using mass spectroscopy [17]. Thus, the aim of the study was to

analyze the crude extract of *P. mirabilis* using the (GC-MS) technique to identify the active components in the extract that had antimicrobial effects.

Materials and Methods

Bacterial Isolates

An isolated *Proteus mirabilis* was obtained in the research. The isolation was confirmed by the Vitek-2 system.

Proteus mirabilis can be extracted using the following methods

A *Proteus mirabilis* culture was used to make the organic extract. In summary, a single colony of the *P. mirabilis* was one that was isolated in an overnight culture and then placed in Luria Bertani (LB) broth. The mixture was then left to incubate in a shaker incubator at 37 o C and leave it to incubate over a period of two days. The culture was rotated at 12,000 rpm at a temperature of 4 o C in 15 minutes. Then the liquid was filtered through a 0.22 µm membrane after the supernatant was collected. The filtrate was extracted twice with the same volume of methanol. Finally, the methanolic crude extract was reduced with a rotary evaporator and the organic crude extract was left to incubate at 40 C over a period of two days.

Identification of bacterial metabolites

Bacterial Extract purification

A bacterial isolate was fermented and incubated in a sequential of procedures in a 500 mL Erlenmeyer-flask. To prepare the broth, a mixture of sodium chloride, tryptone, and yeast extract was prepared, and transferred to the flask. Then, for three or four days, the flask was shaken in an incubator. The subsequent ingredients were added to the same flask: tryptone (5 g), sodium chloride (5 g), phenol red (0.09 g), oatmeal (0.5 g), and distilled water (500 mL) after this first incubation. A further period of incubation of the mixture with a 110-rpm shaking was then undertaken.

Separating chemicals in bacterial byproducts

Immediately the fermentation process is completed. Due to this fact, we were able to test their ability to remove some chemicals in the culture broth. The observation of the existence of different layers in the funnels used to separate overnight means that different chemicals were extracted out of the broth successfully into the different solvents. To ensure that the extracted elements were well isolated, these layers of solvents were carefully transferred into different beakers. The beakers were dried in a water bath at 60°C to remove any residue of solvent and concentrate the extracted chemicals. The goal of this step was to remove the solvent from the culture broth via evaporation. The extracts were dried and made solvent-free and transferred to test vials where further experimentation and analysis could be done. Test vials offered a convenient and logical method of storing the extracts, which ensures their availability in the future studies and characterisation.

Gas chromatography–Mass spectrophotometry

In the case of GCMS analysis using size and polarity, the raw material was exported. GCMS was used to analyze the metabolites. The GC-MS analysis was performed with a GC connected to a MS and a PerkinElmer GC Clarus 500 system. GC-MS detection was done using an electron ionization device in the electron impact mode and this device had an ionization energy of 70 eV. The carrier gas was helium gas (99.999%), and the flow rate was kept constant at 1 o mL/min, with an injection volume of 2 o L. The injector and ion-source were kept at 250o C and 200o C, respectively. Fragments in the range of 45-450 kDa were used to obtain the mass spectra. Total run time of the GC-MS was 36 minutes having a solvent delay of 0-2 minutes. The relative percent quantities of each component could be determined by comparing the average areas of each component to the total areas.

Impact of a methanolic extract from *P. mirabilis* on six different bacteria's susceptibility to antibiotics

Mueller-Hinton agar medium was used to culture each bacterial isolate, with or without the methanol extract of *P. mirabilis*. A suspension of bacteria of an equivalent concentration of McFarland tube no. 0.5 (1.5 x 10 o CFU/ml) was prepared by mixing a few bacterial colonies in a fresh-culture with sterile normal saline. *P. mirabilis* crude extract (32

µg/ml) in methanol (2 milliliters) was then mixed with an equal amount of bacterial solution in a sterile tube. A bacterial suspension was spread on the Mueller-Hinton agar in different directions with the help of a sterile cotton swab; it could also be supplemented with *P. mirabilis* extract. This was then allowed to rest after 10 minutes. The antimicrobial discs, which included the common antibiotics AP-Ampicillin and RF-Rifampicin, were then picked with sterile forceps and pressed against the agar surface at the right distances to make sure that they were touching the medium. The plates were inverted and allowed to incubate at 37 ^{o}C throughout the day. On the next day, we measured the size of the inhibition zones in millimeters with a ruler and compared them with the CLSI values.

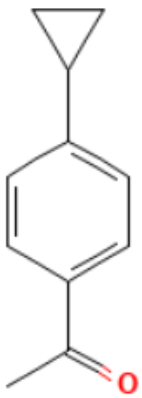
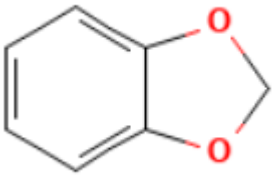
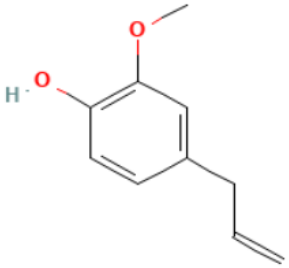
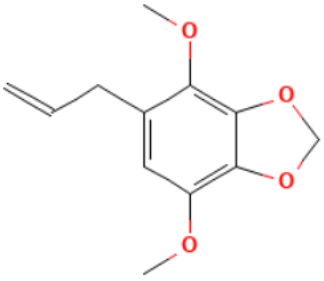
Statistical Analysis of Data

Firstly, we did one-way ANOVA to determine whether there were any significant differences between the means of the various groups. Then the HSD test of Tukey was conducted to compare all possible pairs of means where there is a significant difference at a p = 0.05 level of significance.

Results and Discussion

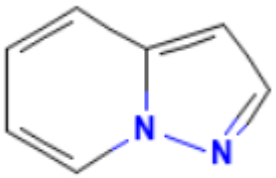
The GC-MS of the *P. mirabilis* extract in methanol had 19 different peaks and each peak corresponded to a biochemical. Table 1 shows the active ingredients and their molecular weights (MWs), MFs, and nature. The main compounds present in the extract were 1-(4-cyclopropylphenyl)-ethenone, 1,3-benzodioxole, 2-Methoxy-4-(2-propenyl)phenol, Apiol, Pyrazolo[1,5-a]pyridine, Pyrazolo[1,5-a]pyridine, 2-Methyl-1-phenyl-2-propanol, 2,3-Dimethoxy-5-(1-propenyl)phenol, 2-Propenamide, 2-methyl, 1-benzylindoline-2,3-dione, 4-Oxocyclohexanecarboxylic acid, 4-Amino-3,5-dinitrobenzotrifluoride, 2,5-Dimethyltetrahydrofuran-3-thiol, Hexadecanoic acid, 2-methoxyethyl ester, Isophthalaldehyde, 5-Ethoxy-4-Hydroxy, d-Methylamphetamine and 1,1,3,3-Tetramethoxypropane. The antimicrobial activity of the secondary metabolites of *Proteus mirabilis*, an isolate of a urinary tract infection, was tested against six different pathogenic microorganisms. The results were compared to those of two conventional antibiotics, AP-Ampicillin and RF-Rifampicin. Antibacterial activity of bioactive secondary metabolites was (14.09±0.25, 23.07±0.34, and 16.45 ±0.29), (10.30±0.21, 17.00±0.31, and 18.96±0.34), (11.00±0.20, 23.89±0.35, and 17.45±0.30), (08.05±0.17, 19.00±0.36, and 12.01±0.24), (13.00±0.20, 23.05±0.33, and 27.81±0.40), and (15.04±0.28, 24.90±0.37, and 21.85±0.32) against *Streptococcus pyogenes*, *Enterococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus* respectively. *Proteus mirabilis* metabolites had a significant impact on Staphylococcus aureus (15.04±0.28).

Table 1. The GC-MS chromatogram of the methanolic extract of *Proteus mirabilis*.

1-(4-cyclopropylphenyl)-ethenone **MF: $C_{11}H_{12}O$** **MW: 160.21 g/mol**	**1,3-benzodioxole** **MF: $C_7H_6O_2$** **MW: 122.12 g/mol**	**2-Methoxy-4-(2-propenyl) phenol** **MF: $C_{10}H_{12}O_2$** **MW: 164.2 g/mol**	**Apiol** **MF: $C_{12}H_{14}O_4$** **MW: 222.24 g/mol**
			
Pyrazolo[1,5-a]pyridine	**2-Methyl-1-phenyl-2-**	**2,3-Dimethoxy-5-(1-**	**2-Propenamide, 2-methyl**

MF: $C_7H_6N_2$

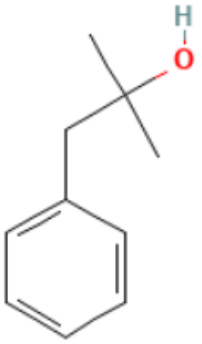
MW: 118.14 g/mol



propanol

MF: $C_{10}H_{14}O$

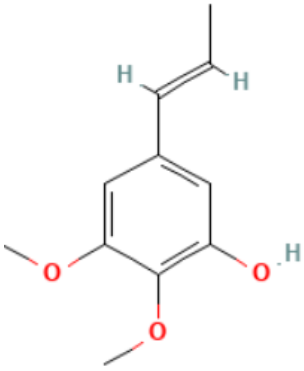
MW: 150.22 g/mol



propenyl)phenol

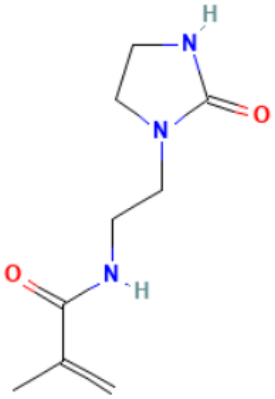
MF: $C_{11}H_{14}O3$

MW: 194.23 g/mol



MF: $C_9H_{15}N_3O_2$

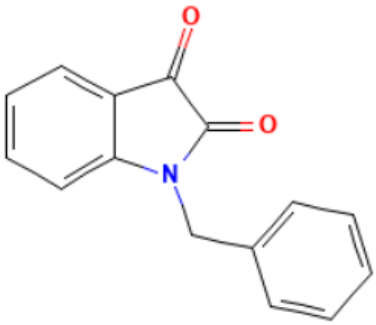
MW: 197.23 g/mol



1-benzylindoline-2,3-dione

MF: $C_{15}H_{11}NO_2$

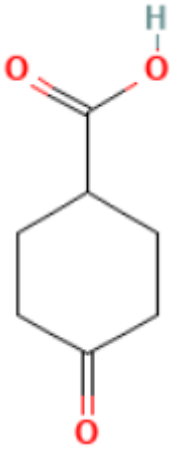
MW: 237.25 g/mol



4-Oxocyclohexanecarboxylic acid

MF: $C_7H_{10}O_3$

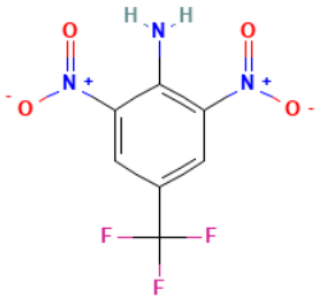
MW: 142.15 g/mol



4-Amino-3,5-dinitrobenzotrifluoride

MF: $C_7H_4F_3N_3O_4$

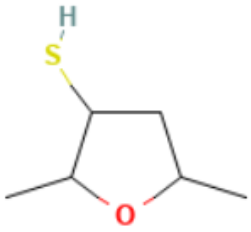
MW: 251.12 g/mol



2,5-Dimethyltetrahydrofuran-3-thiol

MF: $C_6H_{12}OS$

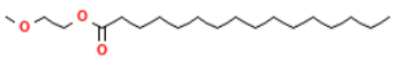
MW: 132.23 g/mol



Hexadecanoic acid, 2-methoxyethyl ester

MF: $C_{19}H_{38}O_3$

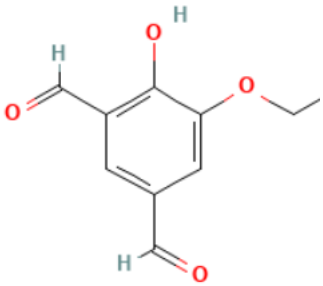
MW: 314.5 g/mol



Isophthalaldehyde, 5-Ethoxy-4-Hydroxy

MF: $C_{10}H_{10}O_4$

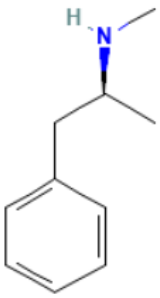
MW: 194.18 g/mol



d-Methylamphetamine

MF: $C_{10}H_{15}N$

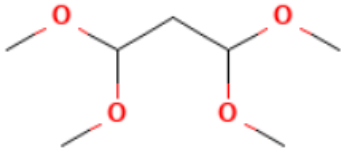
MW: 149.23 g/mol



1,1,3,3-Tetramethoxypropane

MF: $C_7H_{16}O_4$

MW: 164.2 g/mol

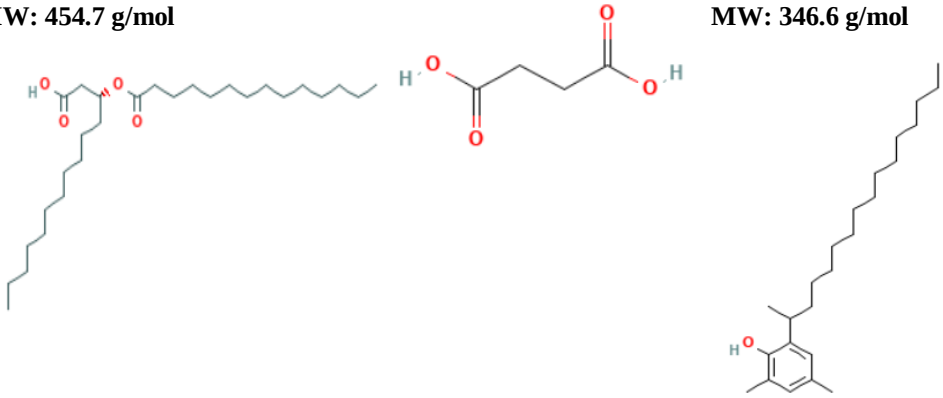


(R)-3-tetradecanoyloxytetradecanoic

succinic acid

2,4-dimethyl-6-(1-

acid	MF: $C_4H_6O_4$	methylpentadecyl)phenol
MF: $C_{28}H_{54}O_4$	MW: 118.09 g/mol	MF: $C_{24}H_{42}O$
MW: 454.7 g/mol		MW: 346.6 g/mol



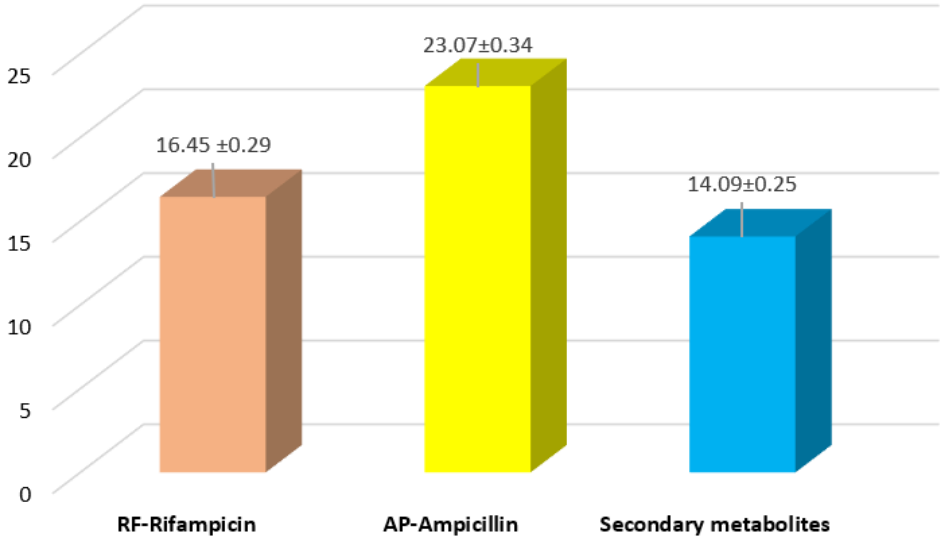


Figure 1. Antibacterial activity of bioactive secondary metabolites of *Proteus Mirabilis* isolated from urinary tract infection against *Streptococcus pyogenes*

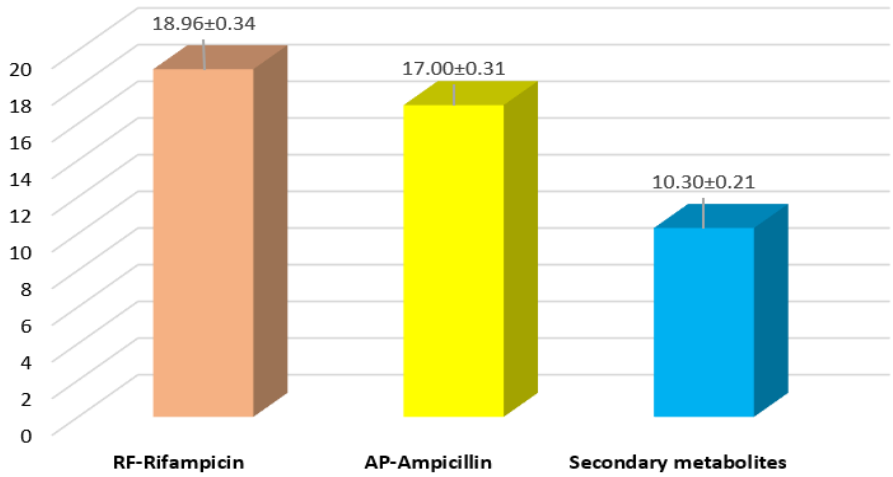


Figure 2. Antibacterial activity of bioactive secondary metabolites of *Proteus Mirabilis* isolated from urinary tract infection against *Enterococcus faecalis*

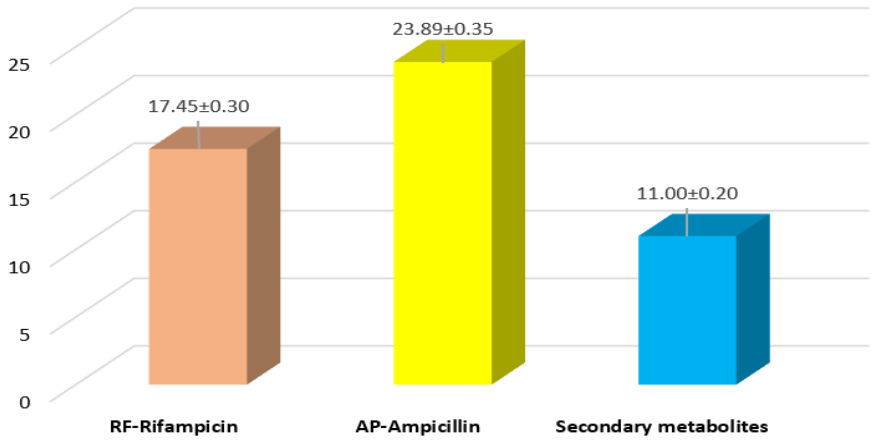


Figure 3. Antibacterial activity of bioactive secondary metabolites of *Proteus Mirabilis* isolated from urinary tract infection against *Bacillus cereus*

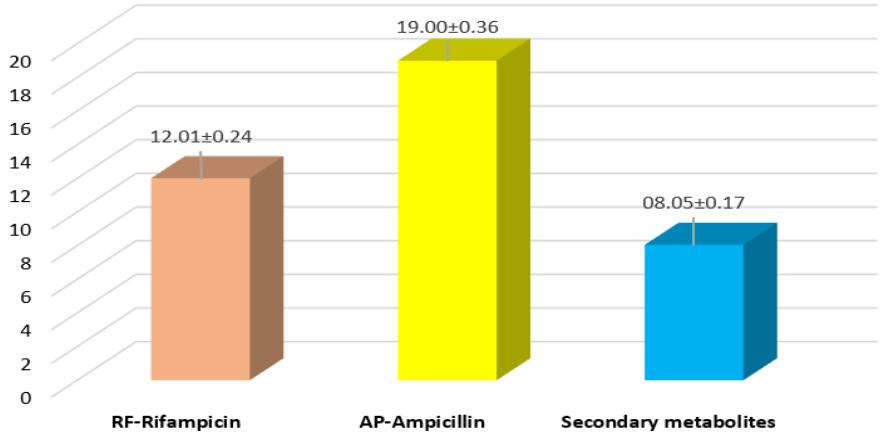


Figure 4. Antibacterial activity of bioactive secondary metabolites of *Proteus Mirabilis* isolated from urinary tract infection against *Bacillus subtilis*

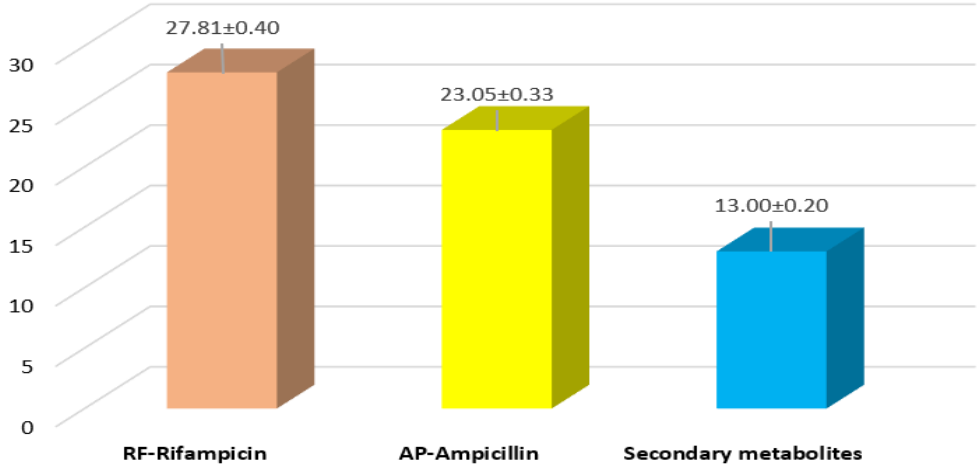


Figure 5. Antibacterial activity of bioactive secondary metabolites of Proteus Mirabilis isolated from urinary tract infection against *Staphylococcus epidermidis*

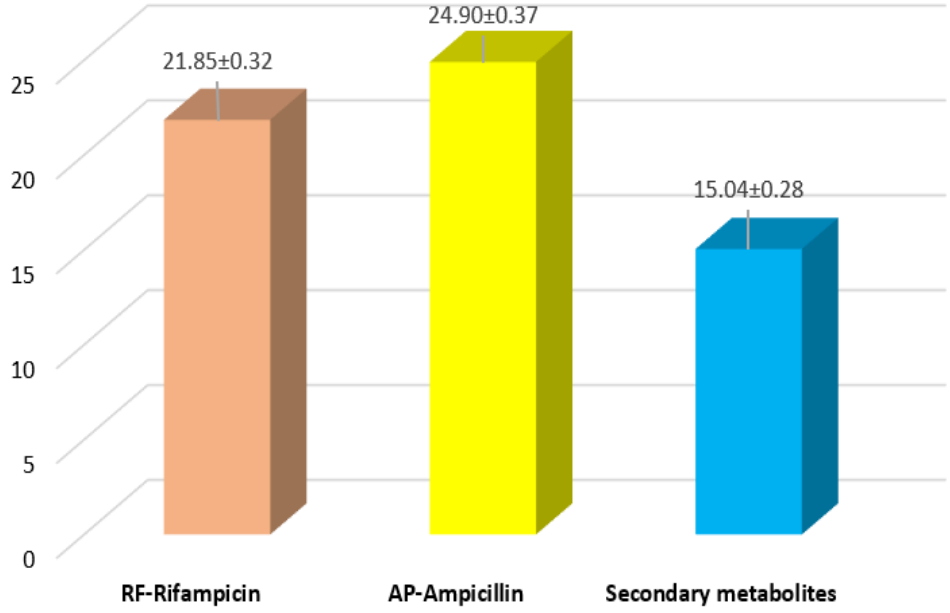


Figure 6. Antibacterial activity of bioactive secondary metabolites of Proteus Mirabilis isolated from urinary tract infection against *Staphylococcus aureus*

A specific group of chemicals known as phenylpropanoids that are involved in plant defense against living and non-living enemies. They might either physically or chemically block intruders depending on how they are composed, be involved in plant communication which triggers defensive responses, or be utilized against microbes [18]. They are likely to kill insects and bacteria by disrupting the key biological processes and machines. Importantly, some of the same chemical substances in the bacterial sample of this study, i.e. myristicin and apiol, can be found in parsley essential oil. The oil of parsley, which was essential, not only killed all the bacteria that were tested, such as *Staphylococcus aureus*, but also prevented growth throughout of all the germs. The third major component found in the water-based *P. mirabilis* extract in this study is dodecane, 1-chloro-, and an alkyl halide. Three different cancer cell lines showed anti-proliferative characteristics towards the chemical 1-chloro-dodecane. In the treatment of smallpox, cervical cancer, breast cancer, and wounds among other diseases, this medicinal plant has potential in providing new plant-based-derived pharmaceuticals [19-21]. The streptomyces spp. also produced dodecane, 1-chloro-, having both bioactive and antibacterial activity. Remarkably, this bacterium used in the experiment contained (benzo[h]quinoline, 2,4-dimethyl-, etc.) and flavonoids (2,2-diphenyl-, etc.) in its aqueous extract. In the case of quinolones, they are one of the earliest types of synthetic antibacterial agents and they are effective against a wide range of bacteria.

The structure and composition of flavonoid and their possible antibacterial properties have attracted enormous amount of quality research. Other things present in the bacterial extract in a 4.65% concentration included succinic acid, heptyl pentyl ester and succinic acid, methylbutyl pentadecyl ester. Both are succinate acid derivatives, which can be endowed with a plethora of biological functions, including antibacterial effects [22, 23]. There may be reduction in cell size and leakage of cellular components due to the effect of succinic acid on the structure of bacterial cell membranes and intracellular structures disruption. The authors' findings suggest that succinic acid can be used in food production to control the contamination of S. aureus and Pseudomonas fluorescens. Notable antibacterial activity has been found in other minor elements (varying from 1.16% to 0.04%) found in the P. mirabilis extract [24-26]. One of the few substances contained in the *P. mirabilis* extract is carotol. Several fungi and bacteria have been found to be susceptible to carotol's antimicrobial properties, which may be due to its ability to interfere with microbial metabolism or damage their membranes. Moreover, the extract of this research includes thymol that is a terpenoids. A lot of research on its antibacterial properties, particularly against bacteria and fungus, has shown that it causes destruction of cell membrane of microbes and reduction of the activity of microbial enzymes. A treatment by the crude extract of *P.*

mirabilis at a concentration lower than minimum inhibitory concentration (MIC) was identified using sensitive qPCR method. This indicates that the extract was able to molecularly disrupt the QS controlled gene's expression. In agreement with these results, Yu et al. discovered that *P. mirabilis* filtrate alone in soil significantly inhibited P. aeruginosa QS production [27-29]. This finding was phenotypically confirmed in the present study by the fact that when using the sub-MIC of *P. mirabilis* aqueous extract, the greenish dye of P. aeruginosa was completely disappeared in the pyocyanin producer isolate, i.e. isolate no. 8 of a burn patient, in comparison to the same isolate which was not treated with the extract. The extract was very effective to the MDR P. aeruginosa isolates; treatment resulted in sensitivity of Amikacin and Colistin with Amikacin and Colistin having a zone of inhibition of 25 mm and 17 mm respectively [30]. CLSI (2020) recommends Amikacin 30 μg to be used in zones with a diameter of 18-26 mm. Unlike the CLSI test, this experiment used a 10 μg of Amikacin, three times lower, and used a 25 mm zone of force. One example of a QS-regulated phenotypic is antibiotic resistance (to Kanamycin) where one study reported that an aqueous extract of *P. mirabilis* did the reverse [31, 32].

Conclusion

It is such a challenge to fight bacterial infections since bacteria can evolve so quickly and become resistant to drugs. The issue of antibiotic resistance is also increasing, and therefore, there is a need to conduct research on the possible new therapeutic targets or antibiotic adjunct drugs. The findings indicate that the AP-Ampicillin and RF-Rifampicin drugs tested were able to increase susceptibility of the six MDR clinical bacterial isolates to treatment. This happened because the crude methanolic extract of the *P. mirabilis* isolate was mixed with six bacterial isolates (MDR). The initial investigation to employ GC-MS to analyze the extract of the *P. mirabilis* isolate showed the existence of multiple elements that have antibacterial properties. The antibacterial effect was found to be even in the low concentrations of the extract. Therefore, there is a possibility that the crude bacteria extract is more effective than the processed one. The future research on the extract should focus on learning more about the properties of the extract such as interaction with other virulence factors both genetically and phenotypically. It is also important to conduct in vivo evaluations in both lab and field animals to further the research on the extract.

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