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Research Article

Screening of Metabolites Produced by Pathogenic *Escherichia coli* and Evaluation of its Antimicrobial Activity

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Abstract:

Background: First, the term 'metabolic processes' refers to all the biochemical reactions which occurs in a given organism, and this is relevant for our aims and objectives as stated below: Substrate oxidations and dissimilation reaction is the chemical variation in bacterial metabolism that energy bacteria employ in the production of energy. Bacterial metabolites play basic roles in human biochemistry; they constitute an unexploited reservoir of drugs effective against a wide spectrum of diseases. In this work, the bioactive chemical compounds produced by Escherichia coli when synthesizing BCs were important for assessing the efficacy of the synthesized BCs against different bacteria.

Methods: The primary and secondary metabolites produced and referred to as the bioactive chemical compound were analyzed using gas chromatography-mass spectrometry (GC-MS) analysis. The antibacterial activity in vitro of the ethanolic extract of Escherichia coli was also determined.In E. coli GC-MS analysis the following compounds were found: objectives. It is worth to mention that substrate oxidations and dissimilation reactions are chemically diverse aspects of bacterial metabolism as a means of producing energy. Metabolites created by bacteria are essential for human survival and are also considered to be excellent drug candidates for a large number of diseases. The researchers sought to identify the effectiveness of bioactive chemical compounds that are secreted from Escherichia coli on various bacteria types.

Methods: The bioactive chemical components also referred to as secondary metabolites were determined using gas chromatography-mass spectrometry (GC-MS) practices. The above ethanolic extract of Escherichia coli was also assessed its efficacy profiles by the in vitro antibacterial assay.

Result: Following are the list of compounds: tert-Butyl 12-aminododecanoate,1,12-Diaminododecane,Ethylidenehydroxylamine, 5,6-Diamino-2,3-dicyanopyrazine, 1 -methylpiperidine-3-Mannose,

D-Ornithine, 5-Hydroxy-L-tryptophan,Glycerophosphocholine. This research compared the efficacy of two common antibiotics, RF-Rifampicin and SF-Sulfonamide, with an ethanolic extract of Escherichia coli against five different bacteria: , Enterococcus faecalis (14.54±0.11; 23.99±0.23; 17.97±0.20), Bacillus subtilis (15.12±0.21; 25.27±0.26; 19.00±0.2), Streptococcus pyogen Comparing the average activity against all the tested microorganisms, the metabolites of Escherichia coli showed highest activity against Streptococcus pyogenes (15.12±0.21).

Keywords: Metabolites, *Escherichia coli*, Antibacterial, GC/MS.

Introduction:

Cherished by men, in clinical practice, the number one prevalence is a urinary tract infection, also known as a UTI. Even after different tries of how to minimize the rate of UTIs, these infections have remained a major public health issue today since they infect a number of people annually and complicate their management and treatment. In the US, annually, 1,00,000 people are hospitalised and 2 million visit emergency rooms while 10 million visit clinics or physician's emergency clinics for UTIs. There are a number of disorders, which can be a cause of UTIs, such as asymptomatic bacteriuria, symptomatic bacteriuria, acute UTI, chronic UTI and recurrent UTI. UTIs is also another difficult huddle due to the fact that they are hard to prevent from recurring. Infection rates are increased in the following populations: children, women, elderly people or diabetics, dogs with uroliths or that wear urinary catheters. That is why there is empirical data on simple and complex UTIs in clinical field [1, 2]. Cystitis and pyelonephritis are examples of Simple uti that occurs in normal size and normal functioning kidneys in Neurologically normal patient without any Structural urinary abnormality of urinary system. Those conditions that make it difficult for the urinary system to prevent or combat the bacteria or make it hard for the host to eliminate the bacteria or exert control over its own urinary system or suppress the bacteria include urinary obstructions, hindered urine flow, immunosuppression, renal dysfunction, pregnancy and the use of indwelling catheters are complicating UTIs.

Some microbes could only live locally like bacteria it was just a through train for the bloodstream. They wouldn't interfere with the layout of your innards [3]. Commensal E. coli and UPECs the majority of them are type 1 very closely related if not identical strains are seen in commensal E.coli and UPEC but UPEC is not pathogen. The reason for this is that due to the genetic relatedness, the identification of these pathogens is difficult hence, it suffices to search for, let us say, E.coli strains in the urines of patients exhibiting such symptoms in a particular given UPEC strain. From our clinical trials, we also established that while some of the patients who got infected with germs had sepsis, others did not. More search was needed to investigate deeper causes linked to this gap connected to the study. It seemed that there were prior philosophies that the host's reaction to the various infectious pathogenic microorganisms and their toxins together with a complex catabolic inflammatory network, genetic variance, and immunological disorder contributed to the organ predicament in the infected individuals.

In sepsis, molecules produced by bacteria may include pyrogen, toxin bureaucins. These metabolites could interfere with the normal body functions and lead to symptoms such as fever and hypotension as evidenced by authors [4; 5]. Scientists recently started waking up to the fact that sepsis was not only an issue of inflammation in a human being. Sepsis was also strongly correlated with the coupling coefficients of the high stress state of other cells and the cells' metabolism. Seeing the propitious potential of metabolomics for tracking the changes of sepsis and reviewing the sepsis patients' situations, some researchers began to try to use it. They concluded that septic patients serum glucose, glycine and 3-

hydroxybutyric acid was comparatively higher than the control group while citric acid and histidine was significantly lower than the control group. This process manifested an ability for 3-hydroxybutyric acid, citric acid and glycine to be specifically detected or potential metabolites in a sepsis individual. Using the indexes the synthetic ability of protein and degree of liver function was diagnosed and it was inferred that the concentration of amino acid such as alanine, glutamic acid, glutamine and methionine, which formed part of the tricarboxylic acid cyclic tact circulating intermediates, lactate and pyruvate were higher in the serum of patients A,B who had poor prognosis. The overall kings and queens' metabolic signature demonstrated significant differences with the dead and moreover highlighted regarding the point, that precise monitoring of alterations in the related metabolites was feasible to determine the state of the disease.

Among human infections, bacterial infections of the urinary tract IUTI are common especially to females. According to the current data, half of the female population will get a urinary tract infection at one time in their life span. Some women will develop persistent use of UTIs and around half of them may have complex UTIs. Community-acquired UTIs and hospital-acquired UTI are mainly due to Escherichia coli organism in most cases. In another study of midstream urine samples collected in 252 centres in 17 countries worldwide 80 % of all general infections, 40 % of nosocomial infections and 77,2 % of all isolates were found to be due to E. coli [6–9]. Recent molecular epidemiologic investigations have demonstrated that most UTIs derive from a limited repertoire of UPEC clones; a large proportion of these clones has been found to be multidrug resistant. Analysis of individual chemical compounds regarding their bioactivity and activities on bacteria was the main aim of this research.

Materials and Methods:

Extracting Metabolites via Separation:

Patients with positive E. coli in the results of their UTI culture were contacted through Marjan Hospital's clinical laboratory to obtain their records of hospitalization. The cell pellets were centrifuged and two washes using phosphate buffer saline (PBS) were done on the resulting samples. In this study, we used an enzymatic lysis mechanism, lysozyme, to break down the target protein in order to investigate the mipo protein's properties. The treatments were given for a 30 minute incubation time. Metabolite extraction from cellular lysate was carried out using a 9:1 volume/volume co-solvent solution of methanol and water. Thus, to 150 µL of sample, 850 µL of extraction solvent were added and the samples were vortexed for 1 minute. The samples were spun at 15,000g for 10minutes. The next procedure was the gentle evaporation of the supernatant under a vacuum centrifuge [10]. Before subjecting the dried extract to incubation at 70oC for 90 minutes, 20µL of methoxyamine hydrochloride reagent in pyridine (20 mg/mL) (Sigma) was added as the first process of the derivatization step. After that, 100 µl of Sigma-brand MSTFA was mixed and allowed to heat at 37o C for half an hour. Acidified methanol auto sampler vials were used for GC/MS analysis after derivatization.

Gas chromatography-mass spectrometry

In the following manner explained above, the metabolomics analysis proceeded with GC-MS. Concentrations of metabolites were measured with the Shimadzu GCMS-QP2010 Ultra gas chromatography extreme – mass spectrometry using the DB5MS stationary phase column = 30 m + 10 m DuraGuard × 0.25 mm i.d. and 0.25-µm film thickness. The carrier gas used was helium and the flow rate was set at 1 ml/min The samples were delivered without splitting them. The amount of injection was optimized down to 2 µl. There was the agreement to maintain the injector temperature at 250 °C [11]. Under the gradient system, the temperature in the oven was set to 70°C then increased to 325°C with a ramp up time of one minute at 70°C and the temperature there after increasing at the rate at 10°C/minute. It was followed by heating at 325 degrees Celsius for other ten minutes of the experiment The

temperature was then maintained at 325 degrees Celsius for another ten minutes. We operated the MS detector in the EI mode, In the next step, we scanned the mass detector. The data was acquired in full scan mode, mass range of 50-650 m/z.

Investigating whether an active secondary metabolite from ethanolic extract of E. coli is capable of eradicating five different pathogenic bacteria

For making the agar, a sterile cork-borer was used to puncture Petridishes whose diameters were standard five millimeters. Subsequently, equal volume of 25 µl of all the sample solution containing the metabolites synthesizing E. coli were aliquoted to the wells. Microorganisms tested for were E. coli, B. subtilis, E. pyogenes, E. aerogenes and S. aureus of which samples were collected using swabs. Third, the pathogens were proceeded by using Muller Hinton agar plates. The standard used were the antibacterial agents RF-Rifampicin and SF-Sulfonamide. We did each experiment twic

After data collection, conducted statistical analysis

Most of the data analysis were done using the Statistical Package for Social Sciences (SPSS) with version 11.6 where most statistical tests such as Analysis of variance (ANOVA) and computation of means

Results and Discussion:

As metabolites serve as biological information carriers, the information may open the lights on both the body's healthy and diseased, physiological and pathological status. This has in turn promoted a growing focus on metabonomic technologies that deals with an analysis of small molecular metabolites. While on the other hand, some problems were still experienced, for instance with KEGG metabolic pathway database [12- 15]. There was not a metabolic route map that belonged to every molecule. Eleven peaks were used in the identification of the chemicals in the GC-MS chromatogram that showed above. tert-Butyl 12-aminododecanoate, 1,12-Diaminododecane, Ethylidenehydroxylamine, 5,6-Diamino-2,3-dicyanopyrazine, 1-methylpiperidine-3-carboxylic acid, 3,3-Dimethyl-2-acetyloxirane, 8-Hexadecanol, 4-methyl-hexadecane, 5,7-Octadecadiynoic acid and 4,6-dimethylpyridin-2-amine, D-Ornithine, D-Mannose, D-Ornithine, 5-Hydroxy-L-tryptophan, Glycerophosphocholine. We identified that Natamycin, Tyramine, D-Tagatose, Tryptophol, Glycerophosphocholine, 5-Hydroxy-L-tryptophan, Hypoxanthine and Uracil to have positive correlation with SOFA score, APPACHE II score and creatine in prior studies ($p < 0.01$). Fructose, glucose, fructose monohydrate, indole, ornithine, 5-hydroxy-L-tryptophan, glycerophosphocholine, and tyrosine positively correlated with C-reactive protein ($p<0.01$). The link between the patient's temperature and 5-Hydroxy-L-tryptophan existed at once. The performed quantity of erythrocytes was proved to depend on Indole and 3-Hydroxypropanal ($p<0.01$). Significant positive correlation ($p < 0.00$) was established between the amount of platelet and the level of Glycerol, D-biotin, D-Mannose, D-Ornithine and 3-Hydroxypropanal. Thus, significant near perfect correlation ($P < 0.01$) existed between aspartate aminotransferase and L-Glutamic Acid, Glycerol and 3-Hydroxypropanal. The coagulation function of the patient experienced adverse changes in Glycerol, 4-Hydroxybenzaldehyde, Glycerophosphocholine, Natamycin (all $p<0.01$). The results of lactic acid changes were significant for sucrose $r=0.33$, $p<0.01$, D-biotin $r=0.37$, $p<0.01$, and D-ornithine $r=0.30$, $p<0.01$. Altogether these studies indicated the possible role of E. coli metabolites in the onset and incidence of UTI.

Education, health care, agriculture, and commerce are but a few fields that will reap a lot of gains from this versatile and comprehensive approach to researching bacterial extracts. To this end, searching for and analyzing chemicals and biological processes to address issues or leverage opportunities in these areas is the mission. Due to the enormous number of compounds that bacteria synthesize, it is routine to look at crude bacterial extract for novel antibiotic compound [16–19] and

Antimicrobial peptides (AMPs) that are of medicinal value. Furthermore, there are some bacterial extracts that can be utilized as source of drugs that are likely to cure cancer and infective disorders. Some of the researches have established importance of the bacterial extracts in controlling agricultural pests and diseases; other researches have used them in assay tests to determine biological activity of chemicals such as screening for inhibitors [20–23] or activators. For instance, Bacillus species have been reported to produce many different types of bioactive metabolites that have given them a great reputation in the fields of agriculture and biotechnology; these metabolites promise better plant health and farming efficiency. Furthermore, there are bacterial extracts of probable therapeutic efficacy which enhance a healthy gut; thus these probiotic strains are characterized.

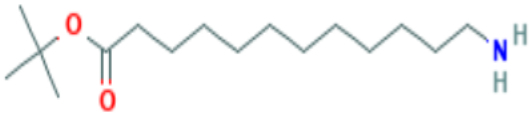
There was constant and fast turnover of the materials exchanged. This would mean that as a result of European involvement in response to changes in their environment, E. coli would change the source of energy, their enzyme systems and nutritional needs to produce different metabolites [24-27]. Metabolites in each of the E. Coli septic samples were different from that of each of non septic samples. It could once again be attributed to the fact that E. coli actively adapts to its external environment. Future study will perhaps be able to pinpoint if E. coli is in the sepsis setting or out of it by detecting its metabolites in serum. All of these may be fast possible allowing for an almost incidental identification [28-30] of whether the host is a sepsis patient when integrated with such options as a machine learning model. This research compared the efficacy of two common antibiotics, RF-Rifampicin and SF-Sulfonamide, with an ethanolic extract of Escherichia coli against five different bacteria: *Enterococcus faecalis* (14.54±0.11, 23.99±0.23, and 17.97±0.20), *Bacillus subtilis* (15.12±0.21, 25.27±0.26, and 19.00±0.2), *Streptococcus pyogenes* (17.00±0.20, 24.17±0.25, and 19.09±0.21), *Enterobacter aerogenes* (13.00±0.10, 22.07±0.22 and 24.71±0.25) and *Staphylococcus aureus* (14.98±0.12, 26.00±0.28, and 21.75±0.27). The metabolites of *E. coli* exhibited significant activity against *Streptococcus pyogenes* (15.12±0.21).

Table 1. Bioactive chemical compounds of ethanolic extract of *Escherichia coli*.

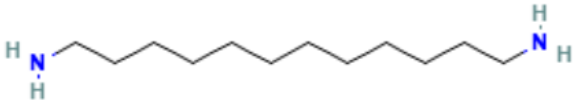
Compounds	Molecular Formula	Molecular Weight
tert-Butyl 12-aminododecanoate	**$C_{16}H_{33}NO_2$**	**271.44 g/mol**
1,12-Diaminododecane	**$C_{12}H_{28}N_2$**	**200.36 g/mol**
Ethylidenehydroxylamine	**C_2H_5NO**	**59.07 g/mol**
5,6-Diamino-2,3-dicyanopyrazine	**$C_6H_4N_6$**	**160.14 g/mol**
D-ornithine	**C5H12N2O2**	**132.16 g/mol**
1-methylpiperidine-3-carboxylic Acid	**$C_7H_{13}NO_2$**	**143.18 g/mol**
3,3-Dimethyl-2-acetyloxirane	**$C_6H_{10}O_2$**	**114.14 g/mol**
8-Hexadecanol	**$C_{16}H_{34}O$**	**242.44 g/mol**
4-methyl-hexadecane	**$C_{17}H_{36}$**	**240.5 g/mol**
5,7-Octadecadiynoic acid	**$C_{18}H_{28}O_2$**	**276.4 g/mol**

4,6-dimethylpyridin-2-amine	**$C_7H_{10}N_2$**	**122.17 g/mol**
D-Mannose 1-phosphate	**C6H13O9P**	**260.14 g/mol**
5-Hydroxy-L-tryptophan	**C11H12N2O3**	**220.22 g/mol**
Glycerophosphocholine	**C8H20NO6P**	**257.22 g/mol**

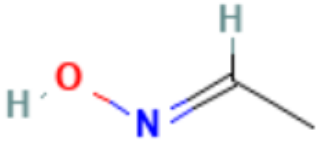
tert-Butyl 12-aminododecanoate



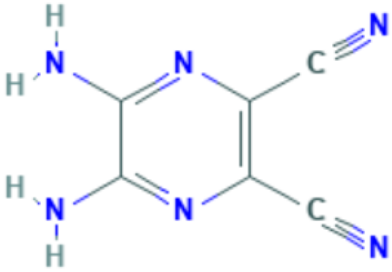
1,12-Diaminododecane



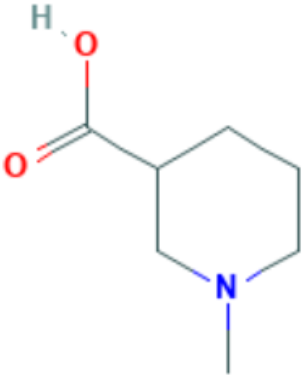
Ethylidenehydroxylamine



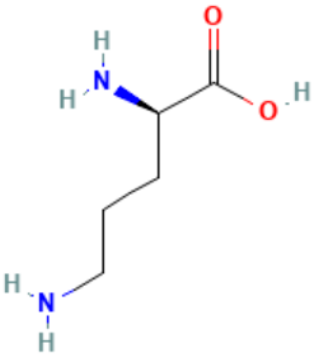
5,6-Diamino-2,3-dicyanopyrazine



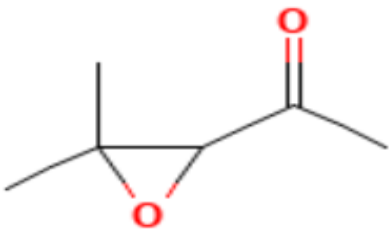
1-methylpiperidine-3-carboxylic Acid



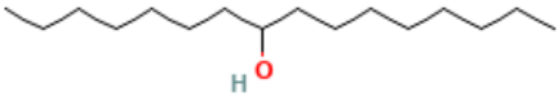
D-ornithine



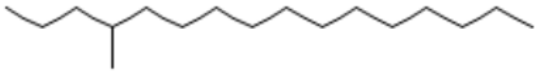
3,3-Dimethyl-2-acetyloxirane



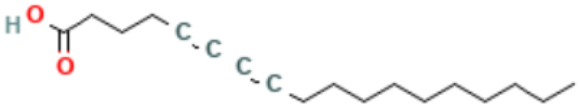
8-Hexadecanol



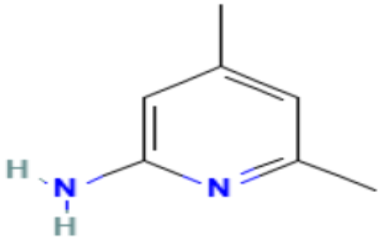
4-methyl-hexadecane



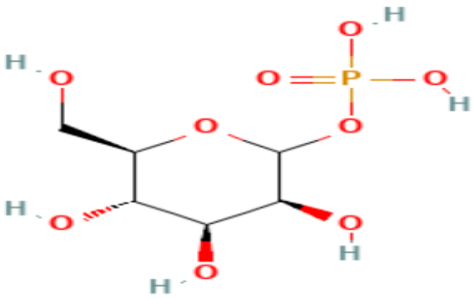
5,7-Octadecadiynoic acid



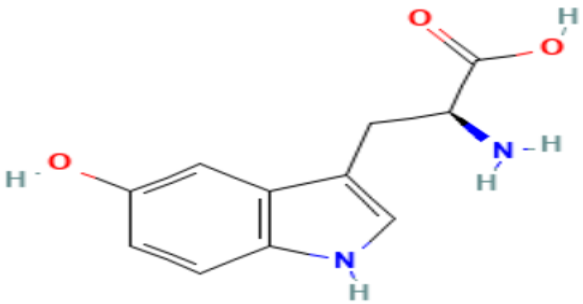
4,6-dimethylpyridin-2-amine



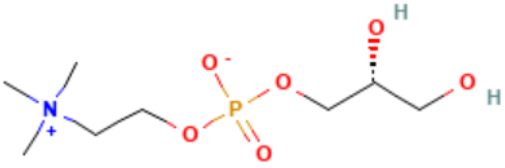
D-Mannose 1-phosphate



5-Hydroxy-L-tryptophan



Glycerophosphocholine



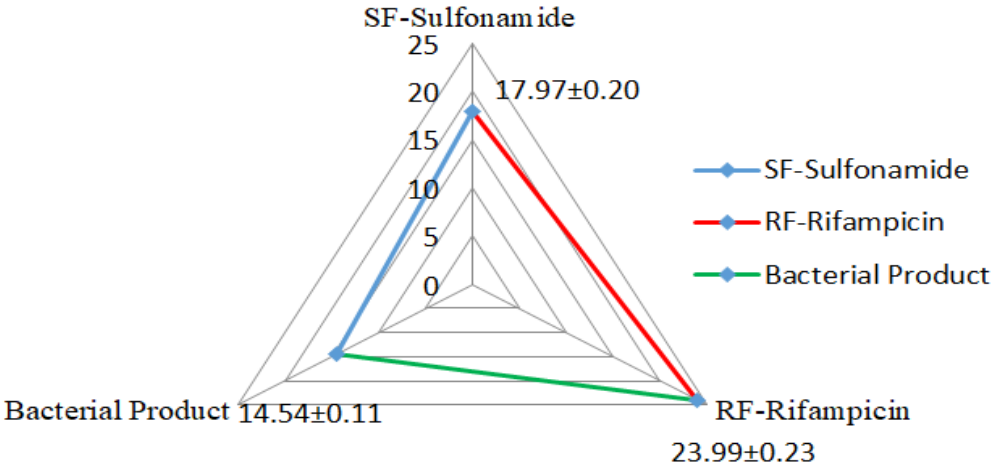


Figure 1. Bioactive secondary metabolites of *Escherichia coli* and standard antibiotics RF-Rifampicin, and SF-Sulfonamide against *Enterococcus faecalis*

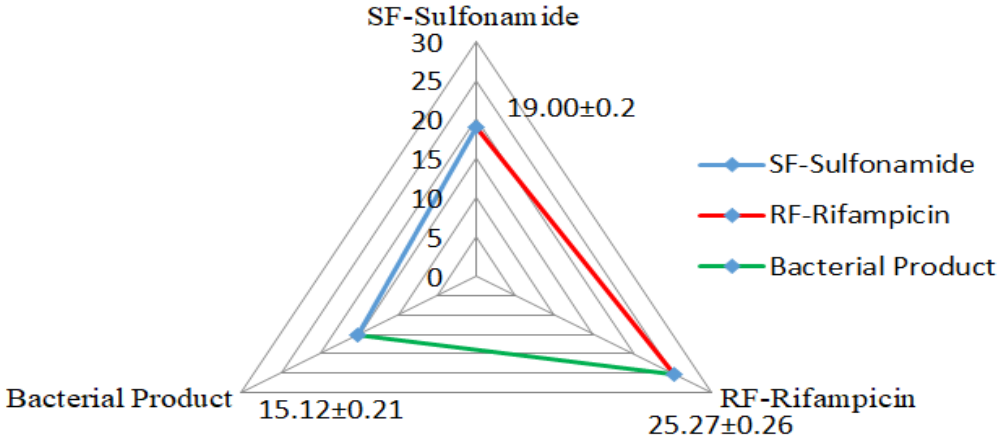


Figure 2. Bioactive secondary metabolites of *Escherichia coli* and standard antibiotics RF-Rifampicin, and SF-Sulfonamide against *Bacillus subtilis*

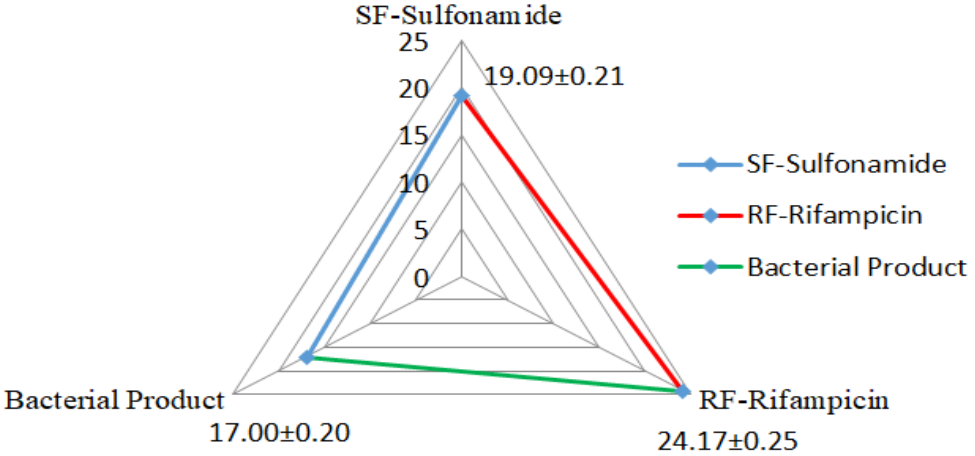


Figure 3. Bioactive secondary metabolites of *Escherichia coli* and standard antibiotics RF-Rifampicin, and SF-Sulfonamide against *Streptococcus pyogenes*

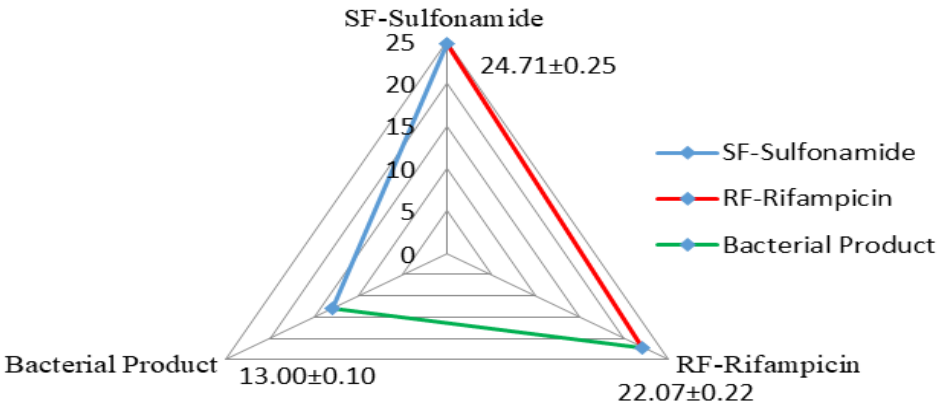


Figure 4. Bioactive secondary metabolites of *Escherichia coli* and standard antibiotics RF-Rifampicin, and SF-Sulfonamide against *Enterobacter aerogenes*

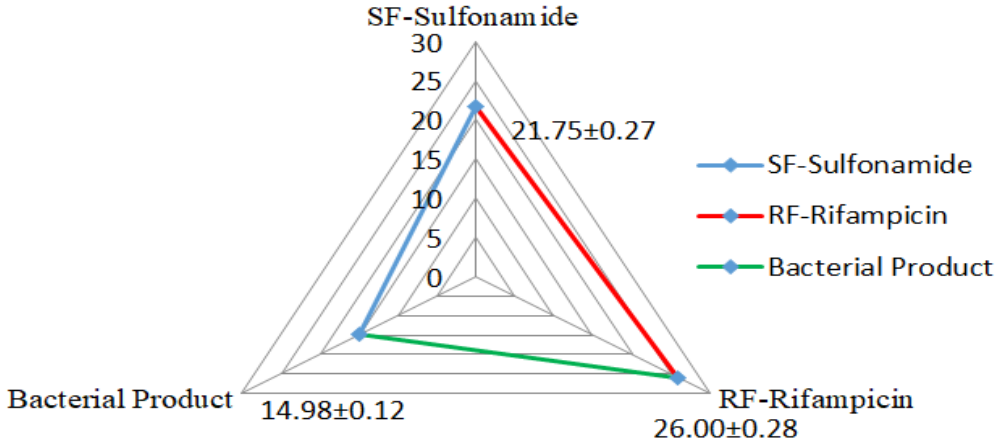


Figure 5. Bioactive secondary metabolites of *Escherichia coli* and standard antibiotics RF-Rifampicin, and SF-Sulfonamide against *Staphylococcus aureus*

Conclusion:

Antimicrobial activity may be expressed by bacterial extracts which contain production of bioactive compounds by some Bacillus strains. We can only hope that similar future comprehensive investigations to the one given here will eventually reveal new hitherto unknown compounds or metabolites with extremely strong antimicrobial activity, and further reveal new microbiological possibilities. Out of all the bacterial species, only Escherichia coli produced metabolites with an inhibitory effect on Streptococcus pyogenes (15.12±0.21).

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