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Original Article

Characterization of Bioactive Metabolites Released from Proteus mirabilis isolated from UTI patients and Evaluation of Antibacterial activity Using Trigonella foenum-graecum, Petroselinum crispum, and Hordeum vulgare Medicinal Plants

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Abstract: Background: *Proteus mirabilis* is a Gram-negative bacterium which is well-known for its ability to robustly swarm across surfaces in a striking bulls'-eye pattern. Clinically, this organism is most frequently a pathogen of the urinary tract, particularly in patients undergoing long-term catheterization. *P. mirabilis* with a focus on urinary tract infections (UTI), including disease models, vaccine development efforts, and clinical perspectives. **The purpose of this study was to :** Investigate the antibacterial effects of the medicinal herbs *Trigonella foenum-graecum*, *Petroselinum crispum*, and *Hordeum vulgare* by analyzing the bioactive volatile compounds generated by Proteus mirabilis.

Materials and Methods: The isolates were diagnosed as Proteus mirabilis species based on the findings of non-lactose fermenting colonies on MacConkey agar with swarming on blood agar plate, characteristic fishy smell, microscopic examination show gram negative pleomorphic bacilli with active motility and the results of biochemical tests were negative for Gram stain, Oxidase and Indol. While, were positive for Catalase, Methyl red, Voscproscouer, Citrate and Urease. An Agilent 789 A instrument was used to perform the examination, which was performed using a GC–MS approach.

Results: GC-MS analysis of Proteus mirabilis found: Ethylsulfonylpropanone, Pyrazolo[1.5-a]pyridine , 3-methyl-2-phenyl, 3-Methyl-2-phenylbutanal, 1- Benzylindole-3-carboxylic acid, L-proline, 1-cyclohexanecarbonylpiperidin-4-amine, 2,4-dimethyl-4-phenyloxolane, mono-Methyl isophthalate, 3-(benzyloxymethyl)cyclobutanone, 1-(4-methoxyphenoxy)propan-2-amine, and 2-cyclopropylpropan-2-amine.

In vitro antimicrobial activity of three plant extracts on *Proteus mirabilis*: The inhibition zone (mm) of bioactive components of *Trigonella foenum-graecum*, *Petroselinum crispum*, and *Hordeum vulgare* medicinal plants ethanolic, and ethyl acetate, extract comparison with Vancomycin (standard antibiotics) against *Proteus mirabilis* was recorded as follows: 19.71±0.25, 15.00±0.21 and 23.09±0.16 mm respectively for *Trigonella foenum-graecum.* While recorded 16.00±0.23, 10.81±0.11 and 23.09±0.16 mm respectively for *Petroselinum crispum.* In vitro antimicrobial activity of plant extracts on *Proteus mirabilis* recorded 15.04±0.21, 09.00±0.01 and 23.09±0.16 mm respectively for *Hordeum vulgare*. *Trigonella foenum-graecum* 19.71±0.25 mm was very highly active against *Proteus mirabilis.*

Keywords: Proteus mirabilis, Bioactive metabolites, Antibacterial, GC/MS

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Introduction:

P. mirabilis is capable of causing symptomatic infections of the urinary tract including cystitis and pyelonephritis and is present in cases of asymptomatic bacteriuria, particularly in the elderly and patients with type 2 diabetes. These infections can also cause bacteremia and progress to potentially life-threatening urosepsis. Additionally, P. mirabilis infections can cause the formation of urinary stones (urolithiasis). P. mirabilis is often isolated from the gastrointestinal tract, although whether it is a commensal, a pathogen, or a transient organism, is somewhat controversial [1, 2]. It is thought that the majority of P. mirabilis urinary tract infections (UTI) result from ascension of bacteria from the gastrointestinal tract while others are due to person-to-person transmission, particularly in healthcare settings [3]. This is supported by evidence that some patients with P. mirabilis UTI have the same strain of P. mirabilis in their stool, while others have no P. mirabilis in their stools [4, 5]. In addition to urinary tract infection, this species can also cause infection in the respiratory tract, eye, ear, nose, skin, throat, burns, and wounds and has been implicated in neonatal meningoencephalitis, empyema, and osteomyelitis. Several studies have linked P. mirabilis to rheumatoid arthritis, although others have failed to find an association [6, 7]. It is thought that antibodies against hemolysin and urease enzymes are subsequently able to recognize self antigens targeted in rheumatoid arthritis patients [8, 9].

Trigonelline is known to have some hypoglycemic effect. The effect of an alkaloid extract of fenugreek dried seeds (Trigonella foenum-graecum L.) on blood glucose, serum insulin, serum lipid profile and [10] lipid peroxidation in addition to histological and histochemical study of liver and kidney in streptozotocin induced diabetic albino rats have been studied.

Trigonella foenum-graecum L. on the histological structures and function of liver, kidney and pancreas of induced diabetes were studied. A growing interest for the rapid extraction from different matrices and for the precise analyses of annual plants compounds increased the need for optimization of the experimental design, to obtain better recoveries, low solvent consumption and reduced extraction times [11] . In the latest years the interest for the study of the organic compounds from plants and their activity has increased.

A lot of extraction methods and analytical methods as spectrophotometry, high performance liquid chromatography, capillary electrophoresis [12, 13], gas chromatography (GC) with flame ionization detection (FID), gas chromatography–mass spectrometry (GC–MS) are developed for plant active compounds study. The combination of an ideal separation technique (GC) with the best identification technique (MS) made GC/MS an ideal technique for qualitative and quantitative for volatile and semi-volatile compounds. In addition, the use of a proper extraction method is needed [14].

Parsley (Petroselinum crispum) is a member of the Umbelliferae family, and it has been employed in the food, pharmaceutical, perfume, and cosmetics industries [15]. It is popularly known as cilantro, salsa, and salsa-pea. In traditional medicine, it is considered to be a di- uretic, uterine stimulant, sedative, emollient, and anti-para- sitic agent, and it is commonly employed for the treatment of chronic bronchitis, bronchial asthma, and dyspepsia. Its leaves and stems are indicated in the cases of menstrual problems [16], cystitis, edema, kidney stones, prostatitis, cramps, indigestion, anorexia, arthritis, and rheumatism [17]. The constituents of parsley, which include ascorbic acid, carotenoids, flavonoids, coumarins, myristicin, apiole, various terpenoic compounds, phenyl propanoids, phthalides, furano coumarins and tocopherol, have been chemically investigated.

Barley is an annual herbaceous monocotyledonous grass. During germination the barley caryopsis develops seedling roots followed by emergence of a coleoptile through the soil surface , the origins of the leaf sheaths and range from 70 cm for dwarf types to >150 cm in Length. On the basis of scientific research barley is considered to be most useful grain. As it is easily digestible compared to wheat, therefore, it is the best diet for patients or those cured persons which are still weak. Many studies used this plant extract in treated inflammation, in Iran dried seeds used orally to treated bladder inflammation [18]. Thaumatin like proteins was extracted from barley have antimicrobial activity against Candida albicans, Bacillus subtilis, E.coli, Saccharomyces cerevisiae as they are pathogenic organisms. The purpose of this study was to investigate the antibacterial effects of the medicinal herbs *Trigonella foenum-graecum, Petroselinum crispum, and Hordeum vulgare* by analyzing the bioactive volatile compounds generated by *Proteus mirabilis*.

Materials and Methods

Isolation and Identification of *Proteus Mirabilis*

The isolates were diagnosed as Proteus mirabilis species based on the findings of non-lactose fermenting [19] colonies on MacConkey agar with swarming on blood agar plate, characteristic fishy smell, microscopic examination show gram negative pleomorphic bacilli with active motility and the results of biochemical tests were negative for Gram stain, Oxi- dase and Indol. While, were positive for Catalase, Methyl red, Voscproscouer, Citrate and Urease.

Preparation of extracts

The Fresh of *Trigonella foenum-graecum*, *Petroselinum crispum*, and *Hordeum vulgare*, was purchased from a local market at Hilla city , Iraq. About 100 g of each plant were crushed in a mortar. Exactly 10 g of each plant powder were soaked in 100 ml of ethanol extract was then filtered, evaporated.

Performing spectral analysis of naturally occurring bioactive chemical components of Proteus mirabilis using gas chromatography and mass spectrometry (GC-MS).

An Agilent 789 A instrument was used to perform the examination, which was performed using a GC–MS approach. The gas chromatography column used was a DB-5MS column purchased from J&W Scientific in Folsom, California. The following measurements were made for this column: The film thickness is 0.25 µm and the diameter is 30 m with an internal diameter of 0.25 mm. Compared to the previous experiment, the temperature in the furnace was kept at the same level throughout the process. The carrier gas used was helium and the flow rate was set at one milliliter per minute each time. Effluent from the gas chromatography (GC) column was directly injected into the mass spectrometer (MS) source via a transfer line that was heated to 250 degrees Celsius. 230 degrees Celsius (°C) was the temperature that was maintained at the ion source while the ionization process took place at a voltage of 70 electron volts (eV). A total of 41 atomic mass units (amu) were included in the measuring range, which reached up to 450.

Antibacterial *Proteus mirabilis* activity Using three medicinal plants *Trigonella foenum-graecum*, *Petroselinum crispum*, and *Hordeum vulgare*

The extracts were prepared and the sterile blotting paper disc (5 mm) was soaked in the diluted extract in two different final concentrations (50 µl and 100 µl/disc). The prepared disc were dried in controlled temperature (at 37^{o}C overnight) to remove excess of solvent and used for study.

Statistical analysis

A number of statistical procedures were employed during the data analysis process that was carried out on the information that had been extracted from an SPSS (Version 11.6) database. The steps involved in these processes included calculating the average and doing an ANOVA.

RESULTS and DISCUSSION

The identified chemicals were represented by thirteen peaks in the GC-MS chromatogram. Said compounds are : Ethylsulfonylpropanone, Pyrazolo[1.5-a]pyridine , 3-methyl-2-phenyl, 3-Methyl-2-phenylbutanal, 1-Benzylindole-3-carboxylic acid, L-proline, 1-cyclohexanecarbonylpiperidin-4-amine, 2,4-dimethyl-4-phenyloxolane, mono-Methyl isophthalate, 3-(benzyloxymethyl)cyclobutanone, 1-(4-methoxyphenoxy)propan-2-amine, and 2-cyclopropylpropan-2-amine.

In vitro antimicrobial activity of three plant extracts on *Proteus mirabilis*: The inhibition zone (mm) of bioactive components of *Trigonella foenum-graecum*, *Petroselinum crispum*, and *Hordeum vulgare* medicinal plants ethanolic, and ethyl acetate, extract comparison with Vancomycin (standard antibiotics) against *Proteus mirabilis* was recorded as follows: 19.71±0.25, 15.00±0.21 and 23.09±0.16 mm respectively for *Trigonella foenum-graecum.* Figure 1. While recorded 16.00±0.23, 10.81±0.11 and 23.09±0.16 mm respectively for *Petroselinum crispum* Figure 2. In vitro antimicrobial activity of plant extracts on *Proteus mirabilis* recorded 15.04±0.21, 09.00±0.01 and 23.09±0.16 mm respectively for *Hordeum vulgare* Figure 3. *Trigonella foenum-graecum* 19.71±0.25 mm was very highly active

against *Proteus mirabilis.*

Recently, the study of the therapeutic effects of plants has increased due to their inclusive medicinal and economical properties and the successful utilization of some of these plants in treating human diseases. Some investigations have led to discovery of restorative properties of fenugreek seed. Fenugreek (Trigonella Foenum-gracium), also known in Arab countries as “Helba”, is a plant from the family of Leguminosae. It grows annually and is being planted in the Mediterranean countries and Asia.

Fenugreek dried seed are known for their valuable antibacterial, anticancer and anti-inflammatory properties in India, Egypt and some European countries. These seed are also known to work as anti-oxidants having reviving properties. Fenugreek is rich with a wide variety of metabolites such as tannins, alkaloids, flavonoids, terpenoids and glycosides which are known to have antimicrobial properties [20].

Fenugreek *Trigonella foenum-graecum* is a herbal plant. Its fresh seed, twigs, roots, and leaves can be used directly and after being dried as flavoring, supplements and spices. Its medical advantages have been reported in many studies. Infections by Staphylococcus aureus and Pseudomonas aeruginosa has become a serious issue in sick and immune-compromised patients. For this reason, the antibacterial effect of fenugreek ethanol extract on these strains receives significant interest. The serious issue prompting high mortality lies in the presence of drug-resistant strains [21]. In the present investigation both Gram-positive and Gram-negative bacterial strains exhibited similar response after exposure to fenugreek *Trigonella foenum-graecum* seed extract [22]. On the other hand, ethanol extract exhibited higher activity on most of the bacterial strains except E. coli compared with the aqueous extract.

The antimicrobial properties of plant extracts and isolated compounds have been investigated by a number of researchers worldwide [23]. In Brazil, the consumption of herbal medicines is growing at a rate of 20% a year, following the re-evaluation of the global use of medicinal plants for the treatment of several diseases [24].

Parsley (*Petroselinum crispum*) is a member of the Umbelliferae family, and it has been employed in the food, pharmaceutical, perfume, and cosmetics industries [25]. It is popularly known as cilantro, salsa, and salsa-pea. In traditional medicine, it is considered to be a diuretic, uterine stimulant, sedative, emollient, and anti-parasitic agent, and it is commonly employed for the treatment of chronic bronchitis, bronchial asthma, and dyspepsia. Its leaves and stems are indicated in the cases of menstrual problems, cystitis, edema, kidney stones, prostatitis, cramps, indigestion, anorexia, arthritis, and rheumatism.

Hormonal changes and repositioning of the urinary tract during pregnancy may make it easier for bacteria to invade the kidneys.The antibacterial activity was evaluated by disc diffusion method against isolated pathogens using Hordeum vulgare extracts such as water, Methanol, Chloroform and Petroleum ether at a concentration of 100 mg/disc. The activity revealed that methanol extract was highly sensitive against E. coli, Klebsiella and Staphylococcus where as water extract was highly sensitive against Proteus mirabilis which was well compared with the standard antibacterial agents such as streptomycin at a concentration of 10 mcg/ml [26].

The solvent extract of T. foenum-graecum showed broad spectrum of antibacterial activity against pathogenic bacteria [27]. Sudharsan et al., 2011 observed a strong activity of T. foenum-graecum against B. subtilis and P. aeruginosa where as weak activity in S. aureus and E. coli [28].

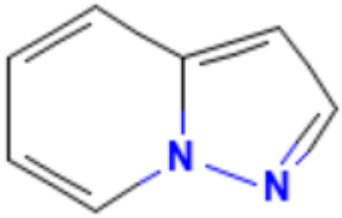
On the other hand Dash et al., 2011 reported that the methanol and acetone extracts of fenugreek showed antimicrobial activity against Pseudomonas spp. whereas acetone extract of spices exhibited highest activity against E. coli [29].

Acetone extract of Fenugreek showed no activity against Salmonella typhi. Nandagopal et al., (2012) reported that the presence of alkaloids, anthracene glycosides, flavonoids, phenolics, saponins, tannin, volatile oils and phenolics in the fenugreek were responsible for the antibacterial activity [30, 31].

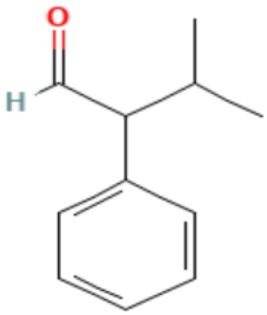
Table 1. GC-MS analysis of Proteus mirabilis

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

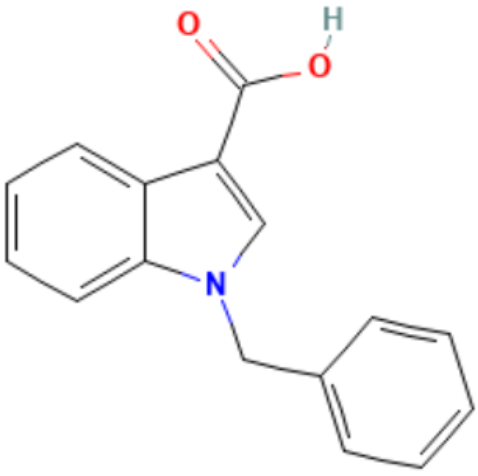
Pyrazolo[1.5-a]pyridine , 3-methyl-2-phenyl



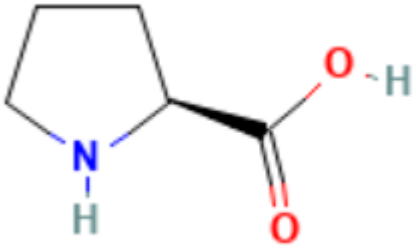
3-Methyl-2-phenylbutanal



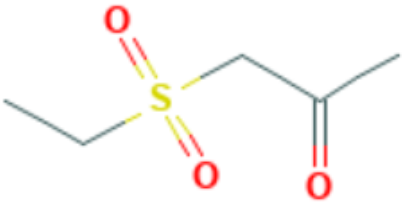
1-Benzylindole-3-carboxylic acid



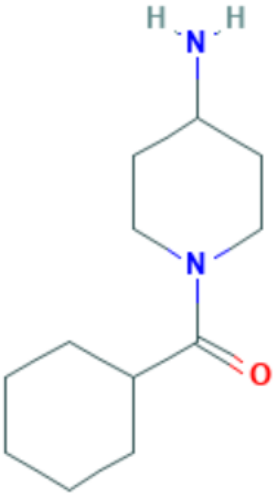
L-proline



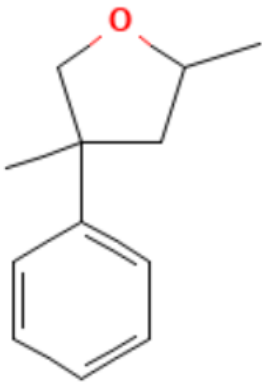
Ethylsulfonylpropanone



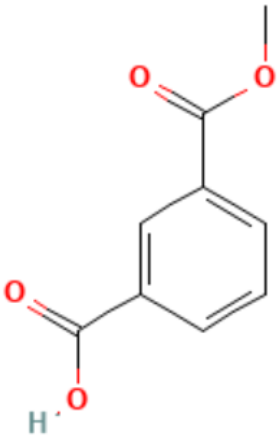
1-cyclohexanecarbonylpiperidin-4-amine



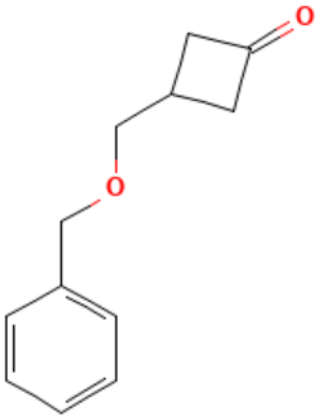
2,4-dimethyl-4-phenyloxolane



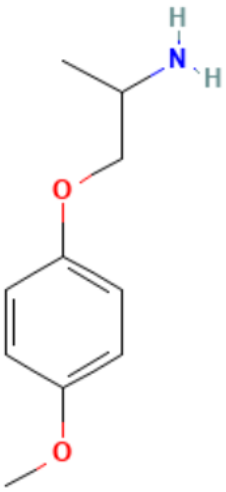
mono-Methyl isophthalate



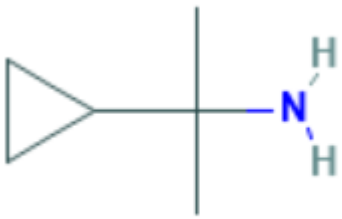
3-(benzyloxymethyl)cyclobutanone



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



2-cyclopropylpropan-2-amine



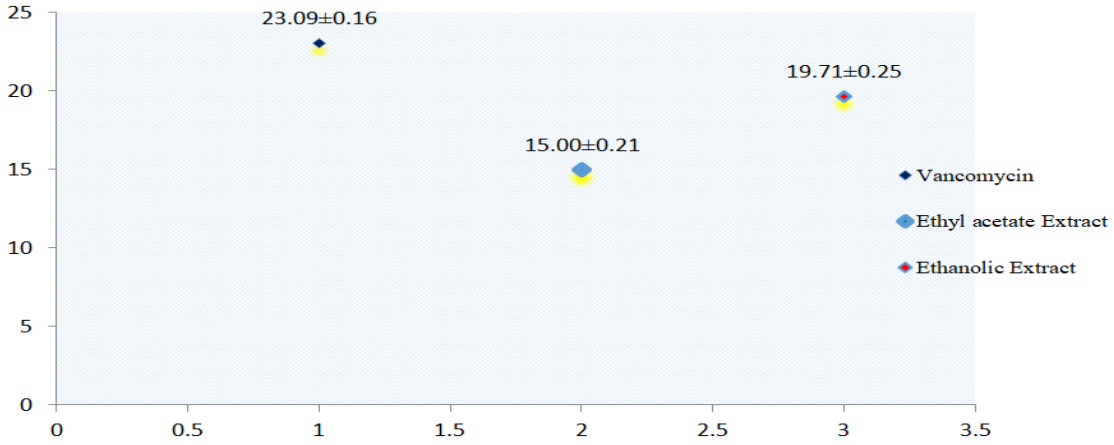


Figure 1. Inhibition Zone (mm) of Ethanolic, and Ethyl acetate extract of *Trigonella foenum-graecumand* comparison with Vancomycin (standard antibiotics) against *Proteus mirabilis*

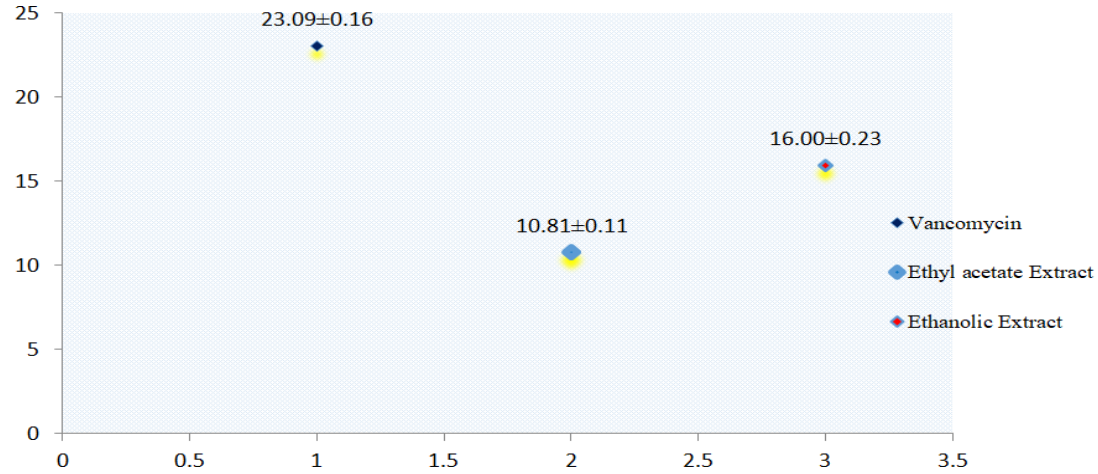


Figure 2. Inhibition Zone (mm) of Ethanolic, and Ethyl acetate extract of *Petroselinum crispum* comparison with Vancomycin (standard antibiotics) against *Proteus mirabilis*

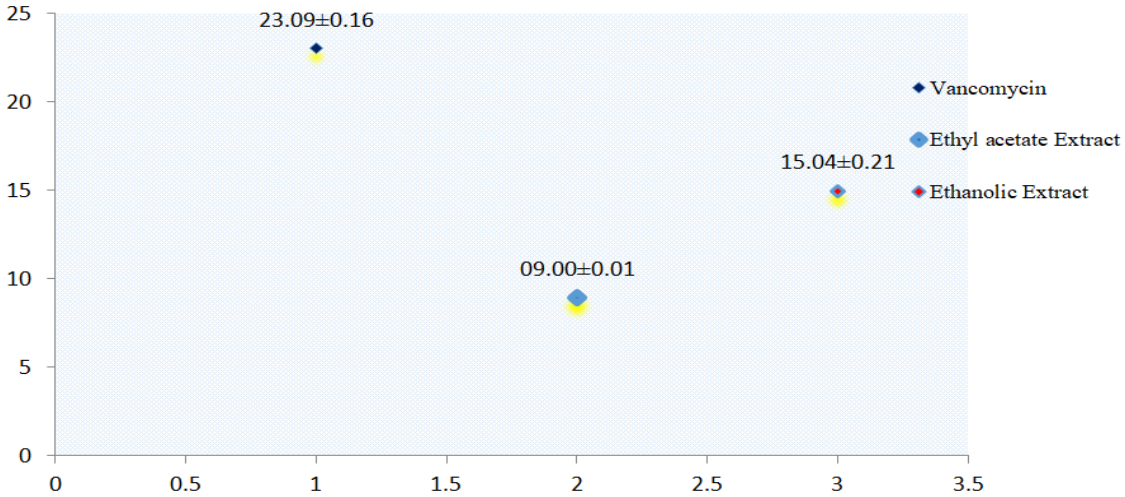


Figure 3. Inhibition Zone (mm) of Ethanolic, and Ethyl acetate extract of *Hordeum vulgare* comparison with Vancomycin (standard antibiotics) against *Proteus mirabilis*

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CONCLUSION

Using GC-MS, the chemical components contained in the bacterial species were identified and quantified. Fenugreek *Trigonella foenum-graecum* seed are potential sources to new antibacterial compounds as emphasized from the antibacterial activity of their aqueous and ethanol extract on many pathogenic bacterial strains. *Trigonella foenum-graecum* 19.71±0.25 mm was very highly active against *Proteus mirabilis*. The chemical composition of the volatile molecules varies between the tested samples, raising the possibility that these differences affect antibiotic efficacy and antibacterial activity. Some strains of *Proteus mirabilis* are able to produce a wide variety of secondary metabolites that have antibacterial effects.

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