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Original Research

Secondary Metabolite Products from *Enterococcus faecalis* Using GC/MS and In *vitro* Evaluation of Its Antimicrobial Activity of Some Medicinal Plant Extracts

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Abstract:

Aims and Objective: The objectives of this study were to analyze *Enterococcus faecalis* bioactive chemical compounds and assess the antibacterial, antifungal, and in vitro antimicrobial activity of plant extracts on this organism.

Method: Gas chromatography-mass spectrometry (GC-MS) methods were used to investigate bioactives, which are chemical substances frequently referred to as secondary metabolites, before the in vitro antibacterial and antibacterial activity of the *Enterococcus faecalis* methanolic extract was assessed.

Results: The GC-MS investigation of *Enterococcus faecalis* demonstrated the presence of the following: 2-Butanol , 8,11-Octadecadiynoic acid , 6-Acetyl-β-d-mannose , Lactose , Cyclopropanebutanoic acid , 9-Hexadecenoic acid , E-11-Hexadecenoic acid , D-Glucose , α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β- ,2,3-dihydro-3,5-dihydroxy-6-methyl-, n-Hexadecanoic acid , 9-Octadecenoic acid , Octadecanoic acid , 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-2(5H)-Furanone, D-Glucose, 6-O-α-D-galactopyranosyl, α-D-Glucopyranoside, Bicyclo[2.2.1]heptane-2-carboxylic acid isobutyl-amide, 2H-Oxecin-2-one.3.4.7.8.9.10-hexahydro-4-hydroxy-10-methyl, Maltol, D-Glucose,6-O-α-D-galactopyranosyl, 1-Gala-l-ido-octonic lactone, Acetamide , l-(+)-Ascorbic acid 2,6-dihexadecanoate, D-fructose , diethyl mercaptal , pentaacetate, Octadecanal ,2 –bromo, L-Ascorbic acid , 6-octadecanoate. *Enterococcus faecalis* metabolites was very highly active against *Staphylococcus epidermidis* (9.92±0.05). *Enterococcus faecalis* metabolites was very highly active against *Alternaria alternate* (10.00 ± 0.07). *Cassia angustifolia* (Crude) (Crude) (10.48±0.13) was very highly active against *Enterococcus faecalis*.

Keywords: Enterococcus faecalis, GC/MS, Metabolites, Antimicrobial activity.

Introduction:

Enterococcus faecalis is a type of gram-positive bacteria that is widespread and may be found in both soil and water in its natural environments [1,2]. *Enterococcus faecalis* is a significant pathogen in hospital settings because it can cause infections in a variety of body systems, including the gastrointestinal tract, the skin and skin structures, the urinary tract, the bloodstream, and the heart. Due to its resistance to a large array of antimicrobial medications (cell wall-active compounds, all commercially available aminoglycosides, penicillin, ampicillin, and vancomycin), *Enterococcus faecalis* provides a considerable obstacle to the therapeutic process [3]. The ability of *Enterococcus faecalis* to participate in various forms of conjugation, which can result in the transmission of genes as part of conjugative transposons, pheromone-responsive plasmids, or broad-host-range plasmids, may be related to its propensity to develop resistance [4]. The clinical significance of the genus Enterococcus is directly proportional to the level of antibiotic resistance it possesses. Antibiotic resistance is a factor that adds to the risk of colonization and infection [5]. Both *Enterococcus faecalis* and *Enterococcus faecium* are considered to be of the utmost significance from a therapeutic standpoint. Even if the resistance characteristics of these two species differ significantly in essential respects, they are nonetheless able to be classified as either intrinsic resistance, acquired resistance, or tolerance in a general sense [6]. When compared to streptococci, enterococci possess a natural resistance to a greater number of antimicrobial drugs that are routinely employed. The expression of low-affinity penicillin-binding proteins causes all enterococci to display decreased susceptibility to penicillin and ampicillin, as well as high-level resistance to the majority of cephalosporins and all semi-synthetic penicillins. This resistance is caused by the enterococci's ability to withstand all semi-synthetic penicillins. The fact that many bacteria have some level of resistance to ampicillin does not rule out the possibility of using this antibiotic in therapeutic settings. In point of fact, ampicillin is still the drug of choice for treating enterococcal infections in patients who do not have any other pathways for high-level resistance [7]. In addition, enterococci have an innate resistance to the antibiotic clindamycin. This resistance is caused by the product of the lsa gene, but the mechanism behind it is not completely understood. It would appear that trimethoprim-sulfamethoxazole is effective against enterococci when it is tested in vitro on folate-deficient media; however, it is not effective when tested on animal models. Because enterococci also have a natural resistance to aminoglycosides at concentrations that are feasible in clinical settings, it is impossible to utilize these drugs alone to treat infections caused by enterococci. In spite of the fact that *E. faecalis* possesses an innate resistance to quinupristin-dalfopristin, Therefore, the widespread resistance of enterococci has had a significant impact on our utilization of both empirical and definitive antibiotics for the treatment of enterococcal infections, which is a condition that is expected to continue for the foreseeable future. The objectives of this study were to analyze *Enterococcus faecalis* bioactive chemical compounds and assess the antibacterial, antifungal, and in vitro antimicrobial activity of plant extracts on this organism.

Materials and Methods:

Growth conditions and determination of metabolites

A strain of *Enterococcus faecalis* was isolated, and subcultures were grown for 48 hours at 22°C on nutrient agar. The mixture was shaken for 10 minutes at 130 rpm after being incubated at 4°C for 10 min [8]. By separating the metabolites from the liquid culture and drying them with a rotary evaporator at 45 °C.

Spectral analysis of bioactive natural chemical compounds of *Enterococcus faecalis* using (GC/MS)

A 30 m x 0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA, DB-5MS column

was used for the analysis, which was performed using a GC-MS (Agilent 789 A) connected with the column. The oven temperature was set according to the results of the prior analysis. The carrier gas, helium, was employed at a rate of 1.0 mL/min. A transfer line (250oC) was used to directly inject GC column effluent into the MS source. Ion source temperature was 230 ^{o}C, and the ionization voltage was 70 eV. The amu range of the scan was 41-450 [9]. By contrasting their retention times with those of genuine samples from the WILEY MASS SPECTRAL DATA BASE Library, the components were identified.

Determination of antibacterial activity of secondary metabolite compounds of *Enterococcus faecalis*

Using a sterile cork-borer, five-millimeter-diameter wells were made in the agar, and 25 µl of the sample solution (*Enterococcus faecalis* Metabolites) was injected into each well. In Muller Hinton agar plates, the test pathogens (*Streptococcus pneumonia, Proteus mirabilis*, and *Staphylococcus epidermidis*) were swabbed. On the drilled wells, 90 µl of fungus extracts were loaded. The wells were drilled with a 0.5 cm diameter hole. The plates were evaluated 24 hours after being incubated at 37C° [10]. Methanol was utilized to regulate the solvent.

Determination of antifungal activity of secondary metabolite compounds of *Enterococcus faecalis*

The test microorganisms were swabbed and spread out across plates of Muller-Hinton agar. On the wells that had been bored, 70µL of extract from *Enterococcus faecalis* was placed. Antifungal activity was evaluated by measuring the size of the zone of inhibition produced against the various test bacteria. Methanol was utilized so that the solvent could be controlled. Fluconazole and amphotericin B were utilized in this antifungal treatment as the gold standard. The experiments were carried out three times to ensure accuracy. The antifungal activity was evaluated by measuring the diameter of the inhibitory zone that was produced after incubation for a period of 48 hours.

In *vitro* antimicrobial activity of some medicinal plant extracts on *Enterococcus faecalis*

Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions (*Gramineae poaceae, Nerium olender, Ricinus communis, Datura stramonium, Linum usitatissimum, Anastatica hierochuntica, Cassia angustifolia, Euphorbia lathyrus, Rosmarinus oficinalis, Mentha viridis, Artemisia annua, Quercus infectoria, Citrullus colocynthis, Althaea rosea, Coriandrum sativum, Origanum vulgare, Urtica dioica, Equisetum arvense, Foeniculum vulgare, Nigella sativa*, and *Ocimum basilicum*,)

Statistical analysis

The gathered data were investigated using statistical methods such as mean value and analysis of variance (ANOVA), which were performed on a database built using SPSS Version 11.6.

Results and Discussion:

The GC-MS chromatogram of the thirty one peaks of the compounds detected were : 2-Butanol , 8,11-Octadecadiynoic acid , 6-Acetyl-β-d-mannose , Lactose , Cyclopropanebutanoic acid , 9-Hexadecenoic acid , E-11-Hexadecenoic acid , D-Glucose , α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β- ,2,3-dihydro-3,5-dihydroxy-6-methyl-, n-Hexadecanoic acid , 9-Octadecenoic acid , Octadecanoic acid , 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)- 2(5H)-Furanone, D-Glucose, 6-O-α-D-galactopyranosyl, α-D-Glucopyranoside, Bicyclo[2.2.1]heptane-2-carboxylic acid isobutyl-amide, 2H-Oxecin-2-one.3.4.7.8.9.10-hexahydro-4-hydroxy-10-methyl, Maltol, D-Glucose,6-O-α-D-galactopyranosyl, 1-Gala-l-ido-octonic lactone, Acetamide , l-(+)-Ascorbic acid 2,6-dihexadecanoate, D-fructose , diethyl mercaptal , pentaacetate, Octadecanal ,2 –bromo, L-Ascorbic acid , 6-octadecanoate.

Antibacterial activity of secondary metabolites of *Enterococcus faecalis* on three pathogenic bacteria

In the current study, Bioactivity of the methanolic extract of *Enterococcus faecalis* and standard antibiotics Rifambin and Cefotoxime against the five tested pathogens *Streptococcus pneumonia* (7.00±0.03 , 6.90±0.02 and 5.04±0.01), *Proteus mirabilis* (6.03±0.03, 4.61±0.02 and 4.00±0.01), *Staphylococcus epidermidis* (9.92±0.05, 7.33±0.03 and 5.08±0.02). *Enterococcus faecalis* metabolites was very highly active against *Staphylococcus epidermidis* (9.92±0.05).

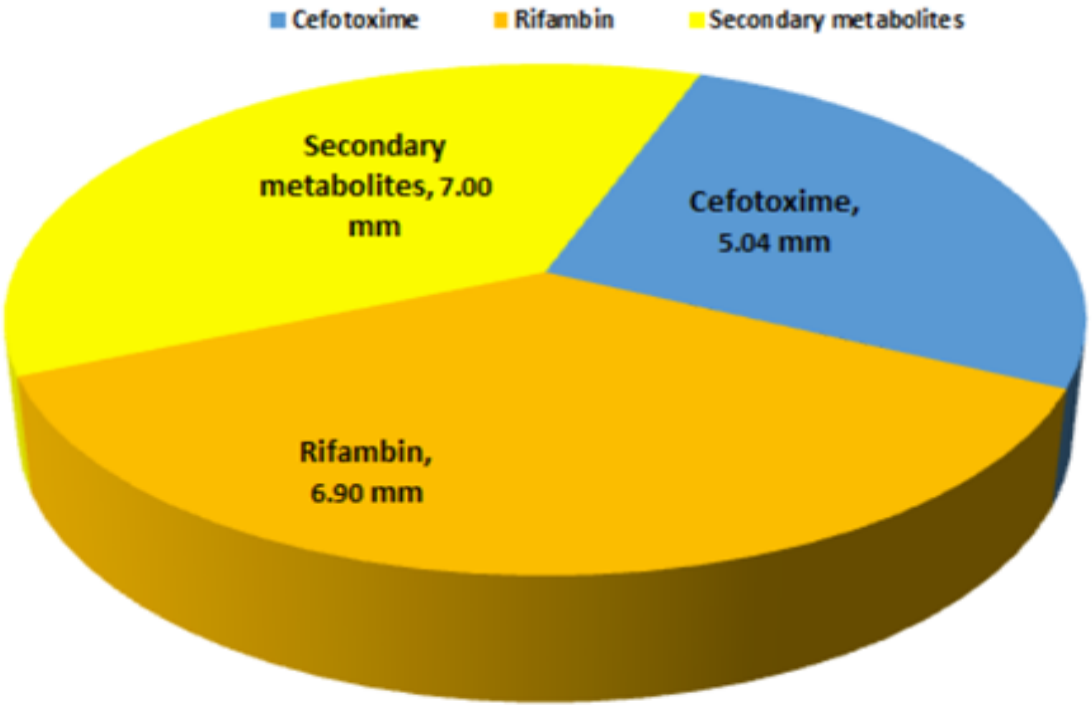

Figure 1. Metabolite products, Rifambin and Cefotoxime as anti- Bacterial activity against *Streptococcus pneumonia*

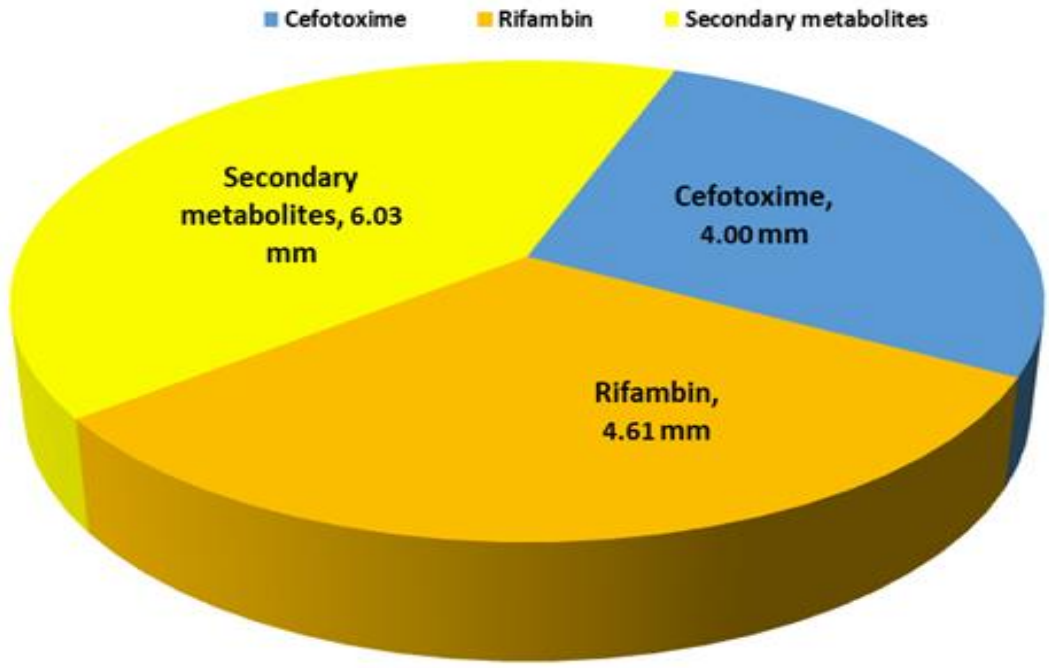

Figure 2. Metabolite products, Rifambin and Cefotoxime as anti- Bacterial activity against *Proteus mirabilis*

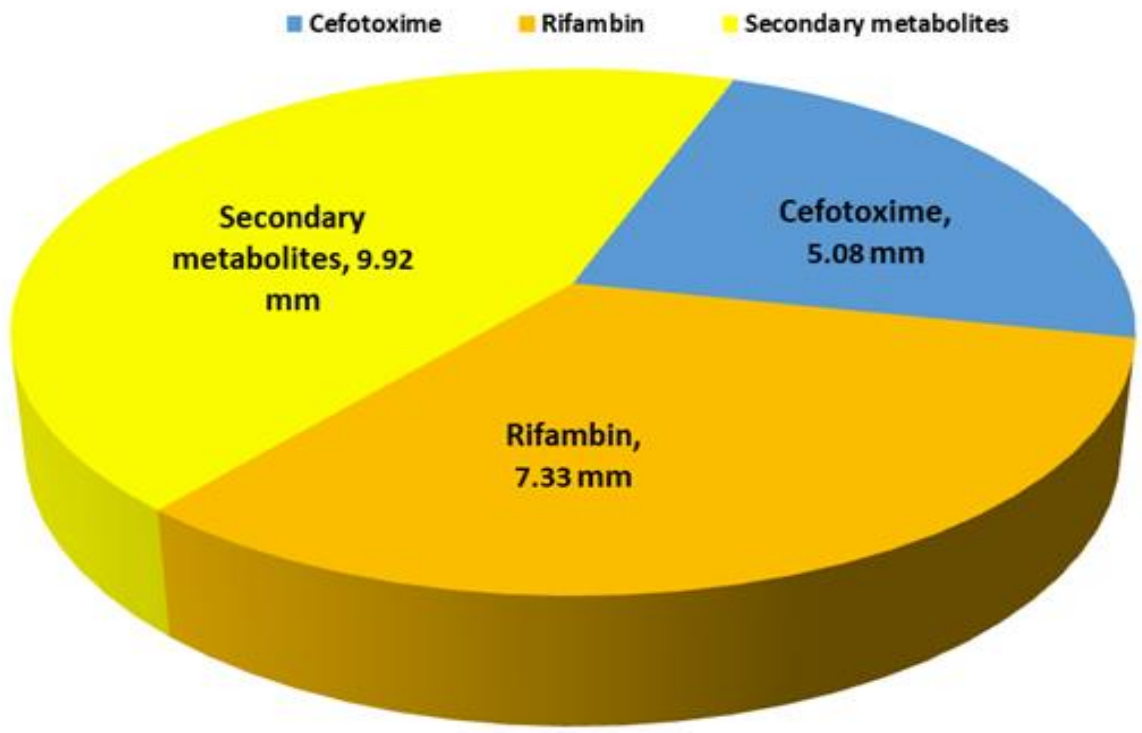

Figure 3. Metabolite products, Rifambin and Cefotoxime as anti- Bacterial activity against *Staphylococcus epidermidis*

Antifungal activity of secondary metabolites of *Enterococcus faecalis*

Bioactivity of the methanolic extract of *Enterococcus faecalis* and standard antibiotics against four fungi and yeast. *Alternaria alternate* (10.00 ± 0.07 , 8.03 ± 0.06 and 8.09 ± 0.05), *Candida albicans* (7.80 ± 0.05 , 6.00 ± 0.03 and 5.00 ± 0.02), *Cladosporium herbarum* (6.05 ± 0.04, 7.00 ± 0.04 and 4.20 ± 0.02), *Fusarium oxyporum* (7.59 ± 0.04 , 5.07 ± 0.03 and 3.00 ± 0.01). *Enterococcus faecalis* metabolites was very highly active against *Alternaria alternate* (10.00 ± 0.07).

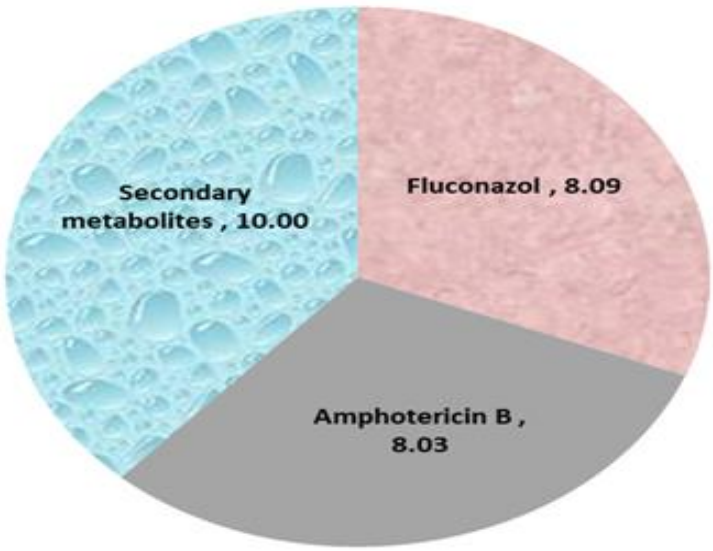

Figure 4. Metabolite products , Amphotericin B, and Fluconazol as anti- Fungal activity against *Alternaria alternata*

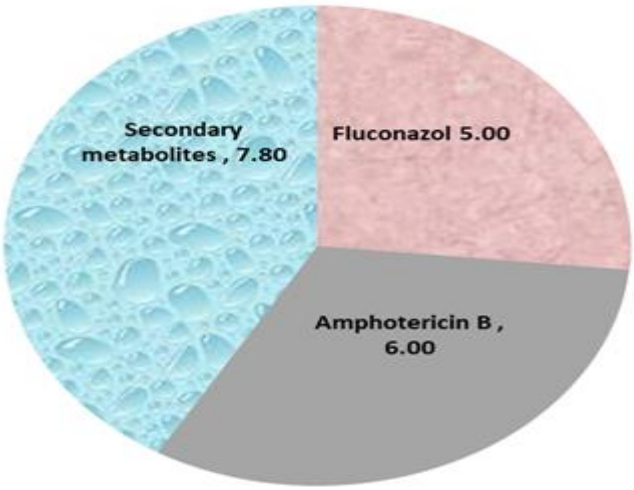

Figure 5. Metabolite products , Amphotericin B, and Fluconazol as anti- Fungal activity against *Candida albicans*

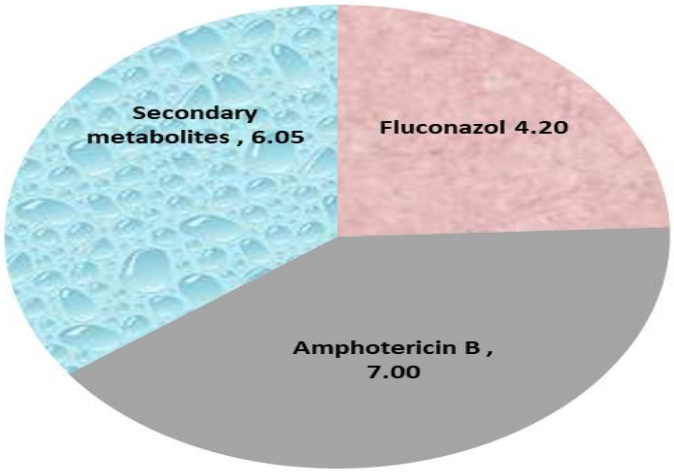

Figure 6. Metabolite products , Amphotericin B, and Fluconazol as anti- Fungal activity against *Cladosporium herbarum*

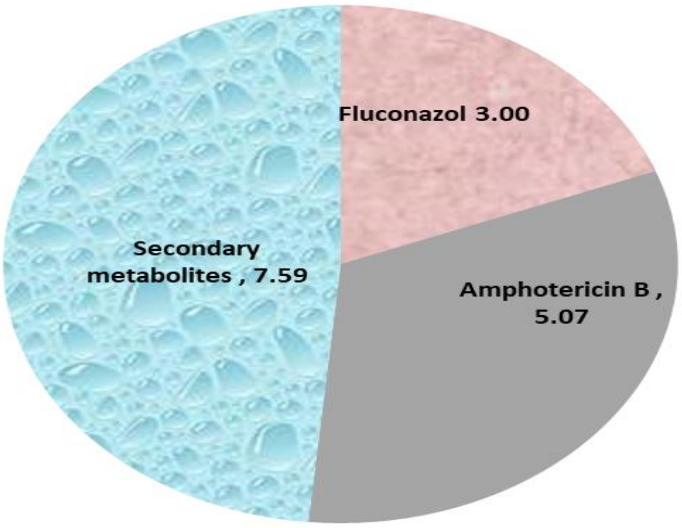

Figure 7. Metabolite products , Amphotericin B, and Fluconazol as anti- Fungal activity against *Fusarium oxyporum*

Table 1. Zone of inhibition (mm) of test different bioactive compounds and standard antibiotics of plants to *Enterococcus faecalis*.

S. No.	Plant extract	Diameter of zones of inhibition (mm) After 48 hr.		Mean Standard Deviation
		Replicate 1	Replicate 2	
1.	*Gramineae poaceae* (Crude)	8.06	9.05	8.56±0.12
2.	*Nerium olender* (Alkaloids)	9.27	9.06	9.17±0.11
3.	*Ricinus communis* (Alkaloids)	6.00	6.50	6.25±0.19
4.	*Linum usitatissimum* (Crude)	8.05	8.07	8.33±0.14
5.	*Anastatica hierochuntica* (Crude)	7.00	7.09	7.05±0.17
7.	*Cassia angustifolia* (Crude)	11.90	9.06	10.48±0.13
8.	*Mentha viridis* (Crude)	10.96	10.08	10.52±0.14
9.	*Artemisia annua* (Crude)	9.38	8.75	9.07±0.19
10.	*Quercus infectoria* (Crude)	11.06	11.05	11.07±0.21
11.	*Citrullus colocynthis* (Crude)	8.50	8.09	8.30±0.19
12.	*Althaea rosea* (Crude)	10.51	9.07	9.79±0.20
13.	*Coriandrum sativum* (Crude)	9.47	10.04	9.76±0.19
14.	*Melia azedarach* (Crude)	9.30	7.09	8.20±0.17
15.	*Origanum vulgare* (Crude)	10.50	10.11	10.31±0.17
16.	*Urtica dioica* (Crude)	7.65	7.08	7.37±0.14
17.	*Equisetum arvense* (Crude)	9.89	9.45	9.67±0.18
18.	*Foeniculum vulgare* (Crude)	7.00	7.02	7.01±0.13
19.	*Nigella sativa* (Crude)	8.73	8.00	8.37±0.13
20.	*Ocimum basilicum* (Crude)	10.49	8.07	9.28±0.20
21.	Rifambin	10.50	10.08	10.29±0.19
22.	Cefotoxime	9.00	10.93	9.54±0.21
23.	Control	0.0	0.0	0.0

In *vitro* antimicrobial activity of plant extracts on *Enterococcus faecalis*

Diameter of zones of inhibition (mm) After 48 hr. for two repeated were (*Gramineae poaceae* (Crude) (8.06 and 9.05 mm), *Nerium olender* (Alkaloids) (9.27 and 9.06 mm), *Ricinus communis* (Alkaloids) (6.00 and 6.50 mm), *Linum usitatissimum* (Crude) (8.05 and 8.07 mm), *Anastatica hierochuntica* (7.00 and 7.09 mm), *Cassia angustifolia* (Crude) (11.90 and 9.06 mm), *Mentha viridis* (10.96 and 10.08 mm), *Artemisia annua* (Crude) (9.38 and 8.75 mm), *Quercus infectoria* (Crude) (11.06 and 11.05 mm), *Citrullus colocynthis* (Crude) (8.50 and 8.09 mm), *Althaea rosea* (Crude) (10.51 and 9.07 mm), *Coriandrum sativum* (Crude) (9.47 and 10.04 mm), *Melia azedarach* (Crude) (9.30 and 7.09 mm), *Origanum vulgare* (Crude) (10.50 and 10.11 mm), *Urtica dioica* (Crude) (7.65 and 7.08 mm), *Equisetum arvense* (Crude) (9.89 and 9.45 mm), *Foeniculum vulgare* (Crude) (7.00 and 7.02 mm), *Nigella sativa* (Crude) (8.73 and 8.00), and *Ocimum basilicum* (Crude) (10.49 and 8.07 mm) were effective against *Enterococcus faecalis*, Table 1. *Cassia angustifolia* (Crude) (Crude) (10.48±0.13) was very highly active against *Enterococcus faecalis*. The zinc metalloprotease (gelatinase) and Fsr (an Enterococcus faecalis regulator), a putative quorum-sensing system thought to be involved in gelatinase and/or serine protease regulation, are among the virulence-

related factors for Enterococcus faecalis that have been described. It is well known that enterococci are resistant to the majority of clinically relevant antibiotics. Enterococci that are multi-drug resistant and vancomycin resistant are frequently identified from humans [11], animal sources, aquatic habitats, foods, and agricultural run-off [12], demonstrating their potential to enter the human food chain. By combining cell-wall active medicines with an aminoglycoside, it is possible to overcome the tolerance that enterococci have developed [13,14]. It has been known for several decades that high-level aminoglycoside resistance, which cancels out the synergism between cell-wall active drugs and aminoglycosides, can occur in bacteria. Plants have long been a great source of natural remedies for human health, especially in the last ten years with more thorough research for alternative treatments [15-17]. Globally, the use of plant-based chemicals for medicinal reasons has grown progressively. In affluent nations, traditional medicine—which contains substances derived from medicinal plants—is used by about 80% of people. Therefore, more research into these plants is needed to better understand their characteristics, safety, and effectiveness [18].

Conclusion:

The GC-MS investigation of *Enterococcus faecalis* demonstrated the presence of thirty one bioactive chemical compounds. *Enterococcus faecalis* metabolites was very highly active against *Staphylococcus epidermidis* (9.92±0.05). *Enterococcus faecalis* metabolites was very highly active against *Alternaria alternate* (10.00 ± 0.07). *Cassia angustifolia* (Crude) (Crude) (10.48±0.13) was very highly active against *Enterococcus faecalis*. For along period of time, plants have been avaluable source of natural products form human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased worldwide. Therefore, such plants should be investigated to better understand their properties, safety and efficiency.

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