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RESEARCH ARTICLE

Chemical Constituents of *Moringa oleifera* Leaves of Ethanol Extract and its Cytotoxicity against *Enterococcus faecalis* of Root Canal Isolate

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ABSTRACT:

Moringa oleifera has been famous as a natural medicine due to its rich vitamins, minerals, and flavonoids. However, the study of its effect on *Enterococcus faecalis* (*E. faecalis*) is limited. This study analyzes the chemical constituents of the ethanol extract of Moringa leaves using GC-MS and assessing the toxicity against *E. faecalis*. *Moringa oleifera* leaves were extracted by ethanol, evaporated, and the concentrated extract was analyzed using GC-MS instruments. The effect of cytotoxic of *Moringa oleifera* against *E. faecalis* was investigated by morphological and coagulation cells; also, the toxicity area was evaluated by ImageJ software. The GC-MS Spectrum was confirmed by NIST databased resulted in 17 different compounds including Alpha-butyrolactone, 1,3-cyclopentanedione, Glycerin, Cis-1,2,6-trimethylpiperidine, 1,2-epoxy cyclohexane, benzeneacetaldehyde, Isobutyraldehyde, propylhydrazone, 2-pyrrolidinone, 2-butenamide,2-cyano-3-hydroxy, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 2-trideuteromethoxy-3-methyl pyrazine, Benzeneacetonitrile,4-hydroxy-, 1,2,3,3a,4,8b-hexahydrocyclopenta[b]indole, 1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid, Hexadecanoic acid, n-cbz-beta-alanine, and 3-(2,2-dimethyltetrahydrofuran-3-yl)phenol. These active compounds are involved in the cytotoxicity against *E. faecalis*. The *Moringa oleifera* leaves have better toxicity at lower concentrations (12.5% and 6.25%) with 24 hours of incubating.: At least 17 chemical components were detected in the ethanol extract of *Moringa oleifera* leaves with quinic acid, glycerol, and DDMP as the most abundant compound. They probably affect the toxicity of *E. faecalis* cells.

KEYWORDS: *Enterococcus faecalis,* Cytotoxicity, Root Canal, *Moringa oleifera*.

INTRODUCTION:

Most plants have been known to have an astonishing ability to produce a wide variety of secondary metabolites, such as flavonoids, polyphenol, alkaloids, glycosides, terpenoids, saponins, steroids, tannins, quinones, and coumarins[1]. Some plant-origin natural products are efficient in dealing with bacterial infections[2]. Moringa oleifera (M. oleifera) is one of the bioactive compounds rich plants. M. oleifera is a medicinal tree commonly referred to as the drumstick tree, the miracle tree, the ben oil tree, or the horseradish tree. Moringa is believed to be a natural medicinal ingredient since a few centuries ago because of its pharmacological properties such as antifungal, antiviral, antidepressant, and anti-inflammatory properties[3].

Moringa leaves were reported to have seven times more vitamin C than oranges, ten times more vitamin A than carrots, and even 25 times more iron than spinach. So, it is not surprising that M. oleifera is called the magic tree due to its ability to overcome malnutrition[4]. The diverse

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chemical compounds were beneficial to treat cancer, diabetes, and antioxidants with a wide range of applications[5,6]. Even the analysis of the content of several minerals, including Rb, Zn, Ce, La, Sm, Eu, and Sc, shows higher concentration levels compared to similar mineral content in peaches[7]. Several studies have shown that ethanol extracts from Moringa leaves contain various organic compounds, some of which are active compounds. The type of chemical content of moringa leaves is determined by the place to grow. For example, Moringa trees in China have a slightly different chemical range than those planted in India[8]. In Asia, such as India and Indonesia, the plant has a long usage history, either as nutrients, vitamins and minerals, or a traditional drug to prevent the infection. Moreover, the local people of Aceh believe that this plant's leaves could boost stamina due to its antioxidant and nutrient contents[9].

Besides minerals and vitamins, Moringa leaves also contain volatile organic compounds and secondary metabolic compounds. The GCMS analysis from several studies showed that Moringa leaves contain terpenoids, alkaloids, flavonoids, antioxidant compounds, and some aldehyde derivatives that possess antibacterial activity. Besides, the ethanol extract of Moringa leaves also contains unsaturated fatty acids, such as linoleic acid and oleic acid, that are useful as an anti-inflammatory[10].

Research on the use of *M. oleifera* to cure various diseases has been widely carried out, including diabetic treatment, Alcohol-induced Hepatotoxicity, Monosodium Glutamate-Induced Liver toxicity, Oxidative Stress, Genotoxicity, DNA Damage. Moringa's leaves were also reported to possess Cancer-Selective Antiproliferative Properties and biological activity against several pathogenic bacteria in oral infection[11]. Elgamily (2016) studied the effect of Moringa on oral pathogens. The research revealed that Moringa extract showed an antibacterial effect against *Staphylococcus aureus* and *Streptococcus mutans* growth. It showed a potential impact as an antimicrobial and antifungal agent[12]. Elgamily limited this study focus in inhibiting capacity without exploring other properties, like cytotoxicity effect on the oral bacteria, i.e., *E. faecalis*. Meanwhile, Sopandani (2020) recommended that Moringa be warranted to examine its toxicity[13].

Enterococcus is a Gram-positive commensal bacterium that acts by inhibiting the gastrointestinal tracts of humans and other mammals. Even though *E. faecalis* is usually found in healthy humans, but it also causes life-threatening infections, especially in the nosocomial environment[14]. The naturally high levels of antibiotic resistance are located in *E. faecalis*. It is also frequently found in root canal-treated teeth in prevalence values of 38% and 19% in saliva[15]. The level of *E. faecalis* in the dental root canal system ranges from 24% to 77% in most cases, and re-infected root canal-treated teeth are much more likely to be colonized by *E. faecalis* than cases of primary infections[16]. Therefore, in this study, we assessed the active components and cytotoxicity properties of *M. oleifera* against *E. faecalis* of the root canals in vitro and toxicity visualization of *E. faecalis* cell.

MATERIAL AND METHODS:

This study has received ethical clearance No. 126/KE/FKG.2019. The sensitivity of *M. oleifera* at various concentrations to *E. faecalis* in the root canals was investigated in this study. Identifying chemical compounds with antibacterial potential found in M. oleifera serves as a reference for determining M. oleifera toxicity against *E. faecalis* cells.

Plant Material:

The plant was collected from Aceh Besar District, Aceh Province, Indonesia (5.603444, 95.405863). The *M. oleifera was* extracted in the Chemical Laboratory, Faculty of Mathematics and Natural Science, Universitas Syiah Kuala, Darussalam Banda Aceh Indonesia. Voucher Number Co2021.3 Its assay material was collected in the Laboratory of Oral Biology, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Indonesia.

Plant Ethanol Extract:

The procedure extract refers to Yusuf (2021). The collected leaves of *Moringa oleifera* were washed with deionized water, dried at ambient temperature for three days, and grounded using the blender. The plant materials were then extracted using a reflux method. 100 g of grounded leaves sample was added to 500mL ethanol (Sigma-Aldrich, Singapore) and heated at a temperature between 60°C and 70°C for four hours, then evaporated using a rotary evaporator (Buchi, Switzerland) until concentrated extract was obtained. The concentrated extract was kept and stored in a refrigerator[17].

GC-MS Analysis:

GC-MS analysis of the ethanol extract of *M. oleifera* leaves was performed using Shimadzu Japan gas chromatography QP2010PLUS with a fused G.C column (2010) coated with polymethyl silicon (0.25nm x 50m). We set up the following conditions: The temperature of 80–200°C, and the flow rate was 5°C/min and 200°C for 20 min. The FID temperature was 300°C, the injection temperature was 220°C, and the nitrogen carrier gas was at a 1mL/min flow rate, split ratio 1:75. The pressure is at 116.9 kPa. The column length was 30m with a diameter of 0.25mm and a 50mL/min flow rate.

Toxicity Assay of *Enterococcus faecalis* in Root Canal:

Teeth that have been prepared for modeling the infection of the ducts were first sterilized. Then 100μL BHI medium was inserted into the root canal and incubated for 15 min, then discarded. Then each treatment and control group was injected 25μL *E. faecalis*. The treatment group consisted of concentrations of 75%, 50%, 25%, 12.5%, and 6.25%, Chlorhexidine 0.2% was used as a positive control. Furthermore, teeth with *E. faecalis* in the root canals of the teeth were incubated in an-aerobic atmosphere for 6 h. All teeth were given test material each of 75μL, which was drained slowly through the cavity edge area. All filled teeth were then blocked for 5 min at 200 rpm and incubated for 24 h and 48 h at 37°C. Subsequently, all groups were resuspended with PBS pH 7.0, and 30μL was taken to be cultured in the Nutrient Agar (NA) medium and then incubated in the an-aerobic atmosphere for 48 h at 37°C. Two *E. faecalis* colonies from the culture medium undergo gram staining[18]. Additionally, the area of *E. faecalis* cells undergoing coagulation was observed under the microscope as an indicator of the effect of toxicity caused by *Moringa oleifera*. The area of coagulation (toxicity) was quantified using ImageJ (μm)[19]. The value was then converted as a percentage (%) to measure each *Moringa oleifera* concentration's toxicity.

Visualization of Cell Toxicity of *E. Faecalis*:

Visualization studies of *E. faecalis* cells' toxicity began by dissolving 20µL *E. faecalis* ($1.5x10^8$) in 200µL *M. oleifera* extract with concentrations of 75, 50, 25, 15.5, and 6.25%. Then were adapted at room temperature and 500 rpm for 15 min. Subsequently, the samples were reincubated for 48 hours at 37°C, resuspended, and 50 µLof each sample cultured in nutrient media and incubated for 48 h. Three abnormal colonies were then undergoing Gram bacterial staining. Examination of *E. faecalis* cell toxicity was conducted by observing the morphological changes in cell colonies. The targeted colony was applied to the glass object's surface until homogeneously distributed in the first stage. Drops of absolute violet-purple dye are left for 1 min, then washed and dried. Then Iodine solution hatched for 1 min, then rinsed and dried. The bacterial isolate was dropped by 96% alcohol for 30 seconds, washed and dried, dropped by safranin for 30 seconds, and washed and dried with filter paper. The objects were then observed under a microscope (x400) assisted by optilab viewer (Miconos) software to enhance the visual quality.

Statistical Analysis:

Kruskal-Wallis was used to analyze the toxicity data of cell *E. faecalis* after interacting with *Moringa oleifera*. And T-test was used to analyze the difference between incubation times. $P < 0.05$ is significant.

RESULTS AND DISCUSSION:

Fig 1 is a G.C. spectrum showing several peaks of active organic compounds contained in the ethanol extract of *Moringa oleifera* leaves. Among dominant compounds are quinic acid, glycerol, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, as confirmed by Table 1, which listed 17 components based on GC-MS analysis of ethanol extract of Moringa leaves. Eight of them possess the cytotoxicity effect against various bacteria and cancer cells, including quinic acid[20],

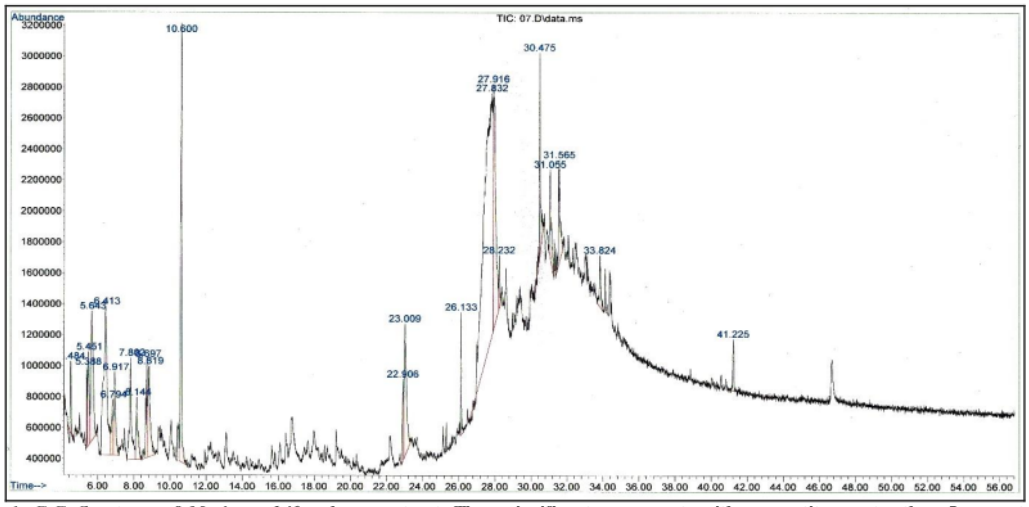

Fig. 1: G.C. Spectrum of *Moringa oleifera* leaves extract. Three significant components with a quantity greater than 5 percent are compound no.3, 10, and 14, as displayed in table 1, and quinic acid with retention time at 27.9 minutes being the most abundant compound.

Fig 1 shows the chromatogram of the *Moringa oleifera* leaves extract. The peaks started to show up at 4 minutes of retention until 41.225 minutes. The G.C. analysis was tandem with a mass spectrometer to obtain the relative mass of each compound. The data from the instrument was then compared with the NIST compound database. A total of 17 combinations from varied compound classes were detected and tabulated in Table 1.

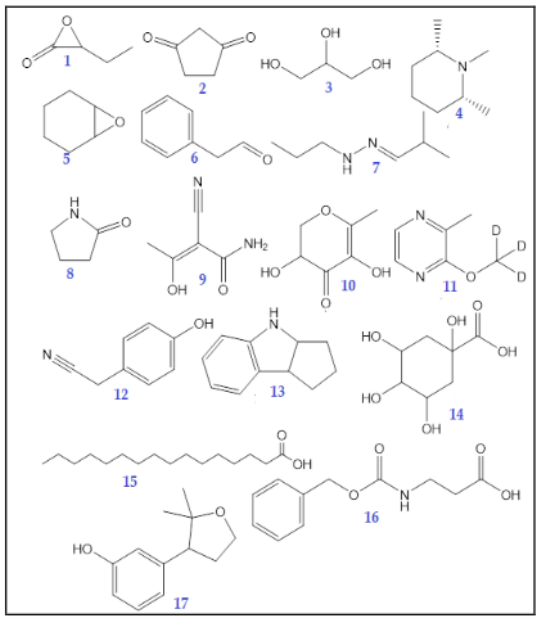

Fig. 2: Structure of chemical compounds contained in the ethanol extract of Moringa leaves analyzed using GC-MS instrument. Aromatic compounds (six different derivatives) were dominantly found in the extract, followed by nitrogen-containing compounds.

Fig 2 illustrates the organic compounds in the ethanol extract of moringa leaves. These structural formulas represent only volatile components as detected by the GC-MS instrument. However, the ethanol extract of moringa eleifera leaves also contains some secondary metabolites like phenolic and flavonoid compounds useful as antioxidants. Among the 17 compounds presented in the ethanol extract of Moringa leaves, compound number 14 with IUPAC name: 1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid referred to trivially as quinic acid, is the main component reaching 43.04 percent. Quinic acid is an essential compound in pharmacology, an especially antibacterial agent. This compound is the primary precursor of tannin produced in plants through gallic acid. Gallic acid binds to D-glucose and generates gallotannins and ellagitannins with the scheme shown in Fig 3.

Table 1: Chemical contents of Moringa leaf methanol extracts based on GC-MS spectra

S. No	Compounds	Retention time (min)	%
1	Alpha-butyrolactone	5.452	2.06
2	1,3-cyclopentanedione	5.645	4.90
3	Glycerol	6.411	8.48
4	Cis-1,2,6-trimethylpiperidine	6.797	1.33
5	1,2-epoxy cyclohexane	6.914	2.34
6	benzeneacetaldehyde	7.803	3.05
7	Isobutyraldehyde, propylhydrazone	8.141	1.83
8	2-pyrrolidinone	8.700	2.02
9	2-butenamide,2-cyano-3-hydroxy	8.817	3.20
10	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	10.603	8.00
11	2-trideuteromethoxy-3-methyl pyrazine	22.904	1.39
12	Benzeneacetonitrile,4-hydroxy-	23.008	4.87
13	1,2,3,3a,4,8b-hexahydrocyclopenta[b]indole	26.131	1.11
14	1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid	27.834	43.04
15	Hexadecanoic acid	30.475	3.04
16	n-cbz-beta-alanine	31.054	2.05
17	3-(2,2-dimethyltetrahydrofuran-3-yl)phenol	33.826	1.24

Table 1 shows the 17 compounds in the ethanol extract of Moringa leaves analyzed using GC-MS. Of the 17 compounds, 5 of them are alkanone class compounds (1, 2, 7, 8, and 10), two phenol and alcohol class compounds (3 and 17), two acid class compounds (14 and 15), and six aromatic compounds (6, 11-13, 16, and 17) as shown in Fig 2.

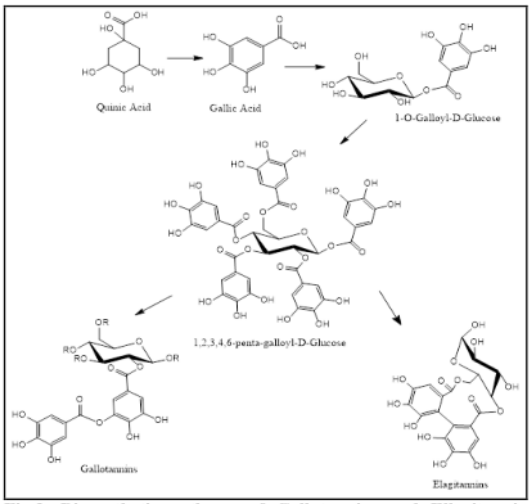

Fig.3: Biosynthetic pathway of Gallotannins and Ellagitannins from Quinic Acid. The quinic acid undergoes an additional reaction forming gallic acid, with a central of glucose. When more gallic acid molecules are attached, a complexed galloyl-glucose formed, which can either result in Gallotannins Ellagitannins as two crucial secondary metabolites.

Fig. 3 shows the biosynthesis of two different tannins from an essential precursor of quinic acid, the *Moringa oleifera* leaf extract's primary component. Tannins, biosynthetically derived from quinic acid, have been credited with essential properties such as antioxidant, anti-tumor, antiviral, and anti-mutagenicity. Due to the detecting limitation of GC-MS instrumentation, the complex compounds of secondary metabolite like tannins is not determined using this technique. Therefore, secondary metabolite determination is required to study *Moringa oleifera* leaf extract's biological activities comprehensively. However, even though we cannot firmly determine the chemical compounds responsible for the leaf extract's cytotoxicity, we can still evaluate the cytotoxicity of the extract using proper toxicity essay as what we conducted in this work.

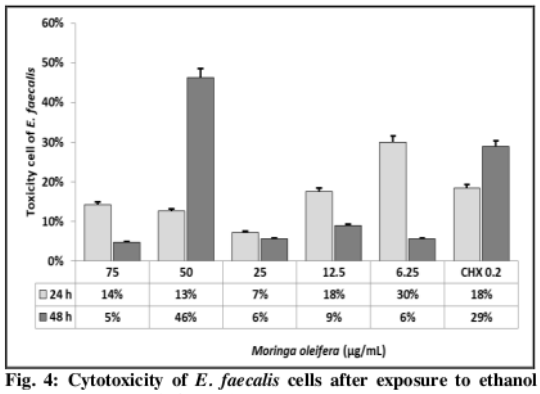

Fig. 4: Cytotoxicity of *E. faecalis* cells after exposure to ethanol extract of *M. oleifera*. At 24 hours incubation time, the concentration of 6.25% shows a better toxic power than other concentrations. Whereas at 48 hours incubation time, 50% concentration is more toxic than other concentrations at both incubation times. Bars (*E. faecalis* cell toxicity) and error bars (error with percentage)

Fig 4 shows that *Moringa oleifera* at all concentrations has a toxic effect against *E. faecalis* bacteria. The lowest concentrations (12.5% and 6.25%) at 24 hours have better toxicity. At the 48 hour incubation time, the toxic effect was better precisely at high concentrations (50%). While *Moringa oleifera* at other concentrations shows a decrease in toxicity compared to a 24-hour incubation period, chlorhexidine 0.2% was used as a control with good working power and increases with incubation time. Based on the Kruskal-Wallis analysis, there was no significant difference between concentrations ($p > 0.05$: 0.371). Whereas based on the T-test analysis between incubation times, there was no significant difference in the activity of the toxicity of *Moringa oleifera* against *E. faecalis* cells ($p > 0.05$; 0.476). These results imply that *Moringa oleifera* phytopharmacologically has an excellent ability to prevent *E. faecalis* bacteria's development.

Fig.5 shows that all concentrations have the same toxicity against *E. faecalis*. However, specific concentrations of 50% and 6.25% have shown a better toxicity profile than other concentrations. Generally, the lowest concentration (6.25%) possesses very good toxicity characterized by high toxicity and bacterial cell coagulation formation due to the penetration of active components into *E. faecalis* cells. At a concentration of 12.5%, many bacterial cells' residual growth was encountered an exudation process precisely due to the influence of test material.

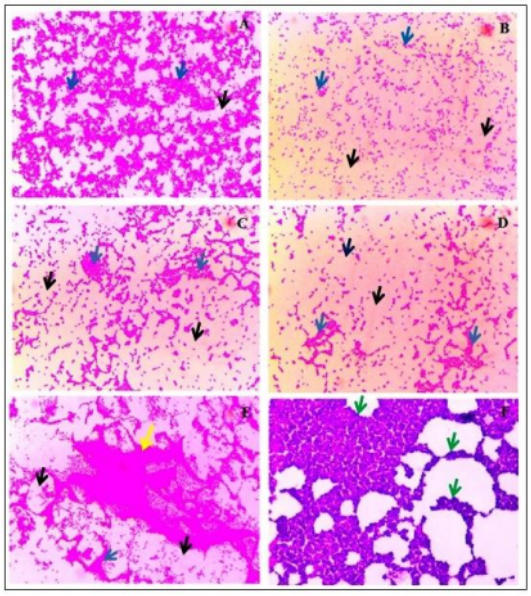

Fig.5: The profile of *E. faecalis* cells that being toxified after interacting with *Moringa oleifera* extract at varying concentrations. (A) 75%, (B) 50%, (C) 25%, (D) 12.5%, (E) 6.25%, and (F) a negative control (*E. faecalis*). Blue arrows (toxified *E. faecalis*), black arrows (The former area of *E. Faecalis* growing place), yellow arrows (*E. Faecalis* experiencing coagulation and lysis), and green arrows (normal *E. faecalis* cells). Magnification 400 x.

The volatile components of *Moringa oleifera* leaves were determined using GC-MS coupled instrument, and more than twenty peaks were observed on the chromatogram, as displayed by Fig 1. However, when the GC-MS peaks confirmed with NIST's internal instrument database (National Institute of Standard and Technology), only 17 components were confirmed. The structures of those chemicals are depicted in Fig 2. Among the detected volatile compounds, 1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid, or trivially named quinic acid, is the most abundant component (over 43%), followed by glycerol (8.48%) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (8.00%), as can be seen in Table 1.

Quinic acid is an excellent precursor of tannins, plays a significant role in binding with gallic acid to produce antioxidant compounds, as depicted by Fig 3. Gohari et al., 2010 showed that adding quinic acid in some antibiotics could increase the performance of Ampicillin and Vancomycin by 36 percent[21]. Quinic acid is also reported to be cytotoxic and capable of damaging Staphylococcus aureus bacterial cell membranes[22]. Comparative studies between the function of quinic acid and Shikimic Acid against cellular processes of the bacterium Staphylococcus aureus show remarkably reduce the DNA content of S. aureus and directly interact with genomic DNA[23]. Besides, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, another dominant component (8%), also plays a significant role as an antioxidant. The compound of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, usually abbreviated as DDMP, is contained in prune's extract and has shown a tremendous antioxidant effect when tested using DPPH assay[24].

The chemical content of a plant can be extracted using various solvents such as ethanol, methanol, chloroform, ethyl acetate, and n-hexane. Different solvents will extract different compounds depending on the characteristics of the substance. Substances with high polarity will dissolve in polar solvents such as ethanol, methanol, and ethyl acetate. In contrast, nonpolar substances tend to be more soluble in nonpolar solvents as chloroform, n-hexane, and petroleum ether. Besides the solvent effect, the types and amount of compounds identified from a natural source also depend on the method and instrument used. The GC-MS device can only identify low molecular weight volatile compounds. However, quantifying macromolecular compounds such as flavonoids, tannins, and polyphenols, the HPLC-MS[40], or the UPLC-QT-MS technique would give a better result[8].

In this study, only 17 compounds were identified, despite the ethanol extract of Moringa leaves known to contain alkaloids, flavonoids, and tannins[25], and polyphenol compounds[26]. Thus, the positive results shown by the Moringa leaf extract cytotoxicity test against *E.faecalis* in this study were not entirely affected by volatile content but were also influenced by secondary metabolites with large molecular masses not identified due to the limitations of the GC-MS instrument.

Fig 4 also illustrates that the ethanol extract of *Moringa oleifera* leaves can inhibit the development of *E. faecalis* through the agglutination mechanism. *Moringa oleifera* has several compounds, such as compound No. 1 and number 5 (Fig 1), which can interfere with the ROS mechanism or destroy the amount of phyli found on bacterial cells' surface communication device between bacteria on the pathogenesis of infection. Abdalla (2016) reported that most natural products such as *Moringa oleifera* have bactericidal and bacteriostatic properties [27]. These antibacterial properties are shown based on concentration incubation time, as shown in this study's results. Arévalo-Híjar (2018) Adi proved that at a 75 mg/ml concentration, *Moringa oleifera* leaves possess antibacterial and bactericidal properties[28]. It was also confirmed that the concentrations of 75%, 50%, and 25% show bactericidal properties and antibacterial effects that work against *E. faecalis* for the first 24 and 48 hours. The results showed that 50% *Moringa oleifera* at 48 hours incubation time shows higher toxicity against *E. faecalis,* so that this substantial toxicity confirmed the bactericidal properties of *Moringa oleifera* leaves extract.

The bactericidal nature of many bacteria generally occurs by damaging the interaction mechanism between natural active compounds with bacterial cell surface lipids[29]. The lipopolysaccharide component causes structural changes through phospholipid exchange, leads to an osmotic imbalance, and eventually, rapid mortality of bacteria[30]. Destruction of cytoplasmic membranes and cellular energy metabolism can cause loss of permeability, leakage of intracellular constituents, and coagulated cytoplasm[31]. *Moringa oleifera* leaves contain several antibacterial agents, especially nitrogen-containing compounds such as Cis-1,2,6-trimethylpiperidine, Isobutyraldehyde propylhydrazone, 2-pyrrolidinone, 2-butenamide, 2-cyano-3-hydroxy, 2 -trideuteromethoxy-3-methyl pyrazine, Benzeneacetonitrile, 4-hydroxy, 1,2,3,3a, 4,8b-hexahydrocyclopenta [b] indole, and n-cbz-beta-alanine (Compounds structures can be seen in fig 1) that can be toxic against a wide range of bacteria[32].

The mechanism of action of chemical compounds as antibacterial can cause bacterial cell death by forming complex compounds with extracellular proteins and dissolved to damage the bacterial cell membrane, followed by the release of intracellular compounds[33]. Compounds such as saponins and phenylacetaldehyde have antibacterial properties that lead to cell leakage by decreasing surface tension, resulting in increased permeability and intracellular compound excretion[34]. David et al. reported that the phenylacetaldehydes, isobutyraldehyde, 3-methyl butyraldehyde, 2-ethyl butyraldehyde, 2-phenyl-propionaldehyde cytotoxic against tumor cells[35].

Antibacterial compounds can inhibit the enzyme reverse transcriptase and DNA topoisomerase so that the formation of bacterial cells can be prevented[36]. The mechanism of phenol compounds and their derivatives, such as compounds 11 and 17 as antibacterial, can

promote cells' lysis by denaturing cell proteins. The hydrogen bonds formed between phenol and protein cause the damage of protein structure, thus affecting the permeability of the cell wall and cytoplasmic, leading to macromolecules and ions unbalancing[37]. As an antibacterial, alkaloids can disrupt the chemical constituent of peptidoglycan in bacterial cells so that the cell wall layers cannot be generated, thus causing cell death. Steroids can interact with cell phospholipid membranes permeable to lipophilic compounds as an antibacterial agent, causing membrane integrity to decrease and changes in cell membrane morphology, which causes brittle cells and lysis[38].

Based on the results displayed in Fig 5, it can be deduced that *Moringa oleifera* can cause toxicity to *E. faecalis* cells, which is indicated by the occurrence of morphological changes (irregular coccus). In addition, cells undergo lysis characterized by coagulation between damaged cells. The cell's purple color indicates redox reactions between the enzymes succinate dehydrogenase in the mitochondria of living cells and the active compound possessed by *Moringa oleifera* occurred[39]. This active compound will lyse the cell membrane so that formazan salts in the cell mitochondria can get out, more and more cells with an indication of high purple crystals. The results of this study can be assumed that there has been a redox reaction during the interaction between *E. faecalis* and Moringa oleifera. The bacterial cells observed by microscopy show a contrasting purple color in the area of the cell that is experiencing toxicity.

CONCLUSION:

As we report in this study, Moringa leaves contain very diverse compounds, ranging from volatile compounds to secondary metabolites such as flavonoids, tannins, and polyphenols. The most abundant chemical found in the ethanol extract of *Moringa oleifera* leaves 1, 3, 4, 5-tetrahydroxy-cyclohexane carboxylic acid (quinic acid) contributes to the cytotoxicity of the extract against *E. faecalis*. Furthermore, Moringa leaf extract has a high inhibitory power against *E.feicalis* bacteria at low doses. Thus, it can be used as a natural antibacterial agent with minimal side effects.

CONFLICT OF INTEREST:

The authors declare no conflicts of interest.

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