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QUALITATIVE PHYTOCHEMICAL CHARACTERIZATION OF AQUEOUS AND ETHANOLIC EXTRACT OF *ZIZIPHUS JUJUBA* LEAVES AND ANTIBACTERIAL ACTIVITY AGAINST *ENTEROCOCCUS FAECALIS*

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Abstract

Due to the high expense of synthetic medications and the emergence of synthetic antibiotic resistance, many diseases have emerged. *Ziziphus jujuba* leaf extracts, both aqueous and ethanolic, have demonstrated antibacterial efficacy against *Enterococcus faecalis* bacterium. Separate ethanol and aqueous extracts were obtained from leaves for demonstration of phytochemical examination of *Z. jujuba* leaves by using the standard methodology for qualitative analysis of active components and it shows highly significant results. The presence of active components was quantitatively examined using GC/MS analysis. Using the agar well diffusion method, the zone of inhibition was examined using a pure culture of *Enterococcus faecalis*. The Minimum Inhibitory Concentration was obtained using the Broth Dilution Method. Every analysis was performed three times, and the means of the results were displayed. Natural plant *(Ziziphus jujuba)* leaf extracts are beneficial against bacterial infections which caused by *Enterococcus faecalis.*

Keywords: *Ziziphus jujube,* Antibiotics, Antimicrobial, Antioxidant.

1. INTRODUCTION

Evidence of the usage of herbal remedies dates back 60,000 years in both industrialized and developing nations. The resurgence of interest in medicinal plant usage has also led to the development of so-called "Nutraceutical" therapeutic medicines. Nutraceuticals can be foods or dietary components that provide health advantages as well as illness prevention and therapy.

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Food is consumed to obtain nutrition and prevent illness, hence nutraceuticals cover all forms of food [1]. Almost 35 thousand plant species from various locations are frequently utilized as medicines. Due to the fact that plants have a variety of phyto-components that are effective in treating ailments [2].

Due to the bioactive components that plants contain, it has been discovered that they have antibacterial characteristics. Plants emit a lot of oxygen and secondary metabolites when they receive sunlight [3] The traditional medicines are used by approximately 60% population of the world. The traditional medicines are prepared from plants, minerals and organic matter. The herbal medicine are only prepared from plants [4].

There are different plants which are used in medical field and one of them is *Ziziphus Jujuba.* It has antibacterial, anticancer, antifungal and antiulcer properties [5].

The *Ziziphus jujuba* belongs to Rhamnaceae family is prevalence in all over the world especially in Asia. It has been cultivated from 4000 years in china. The fruit of this plant has gained very importance in herbal medicine field due to anticancer and antifungal properties. [6].

Numerous illnesses, including diabetes, diarrhea, skin infections, liver complaints, urinary disorders, obesity, fever, pharyngitis, bronchitis, anemia, insomnia, cancer, and blood purification and gastrointestinal tract tonification, can be treated with various parts of *Z. jujube* [7] . Additionally, it has been utilized as a treatment for hypotension, cardiotonic antinephritis, antioxidants, immunostimulants, antispasticity, antifertility/contraception, wound healing, and chronic constipation [8].

Saponin, glycosides, alkaloids, steroids, polysaccharides, and terpenoids are the primary components of *Z. jujuba,* and they play a dynamic role in a variety of actions, including those that are hypoglycemic, hypolipidemic, antioxidant, antibacterial, and that increase permeability. *Z. jujuba* leaves were used to create the alkaloids Coclaurine, Isoboldine, Norisoboldine, Asimilobine, Iusiphine, and Iusirine.[9].The leaves are said to stimulate hair growth and are febrifuge and astringent.

They are used to create a plaster for the treatment of strangulation. *Ziziphus jujuba* extract was tested for its antibacterial and antifungal properties using the disc diffusion method [10]. Many animal species, from humans to flies, have enterococcal species as essential members of their intestinal flora. Over the past few decades, enterococci have become well-known as frequent causes of due to their ability to spread antibiotic resistance to other microbes and their prevalence in hospital-acquired bloodstream, urinary tract, and surgical wound infections that are resistant to multiple antibiotics.

Although more than a dozen different enterococcal species have been linked to human illness, Enterococcus faecalis is the species that causes the majority of human enterococcal infections.

E. faecalis isolates' propensity to produce severe infections has been related to the bacterium's inherent toughness, which enables the organism to survive in the hospital environment and withstand several host defenses, which is made more difficult by the

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acquisition of numerous variable virulence characteristics by horizontal transfer from other species[11]. As typical commensals, enterococci live in the human gastrointestinal tract, oral cavity, and vagina.

They can infect the urinary tract, circulation, endocardium, abdomen, biliary tract, burn sites, and indwelling foreign devices in people, resulting in a wide range of illnesses [12]. The aim of the present study was determination of qualitative phytochemical characterization of *Ziziphus jujube* leaves extract and check its Antibacterial activity effect against *Enterococcus Faecalis*

2. MATERIALS AND METHODS

2.1 Collection of Leaves

Z. jujuba fresh leaves were collected from Punjab University in Lahore. Leaves were identified by using the local image processing method [13].

2.2 Preparation of Sample

The samples were cleaned with running water to remove the contaminants and allowed to air dry for a week at room temperature in the shade. Then, powder of dried leaves were prepared using an electric grinder [9].

Figure 1: *Ziziphus jujube* leaves and its powder

2.3 Preparation of ethanolic extract

Ethanolic extract was prepared using the prescribed method of Naqvi et al., (2011). In this method, 35g of leaf powder was soaked in 350ml of ethanol in glass bottle. It was then kept in a shaking incubator for 48 hours at room temperature while shaking nonstop at 150 rpm. The mixture was filtered via Whatman No. 1 filter paper after two days. A hot air oven was used to evaporate the solvent. The evaporation time of ethanol was shorter; ethanol evaporates five time quick than water. Reserve extract stored for later use in an airtight receptacle [14].

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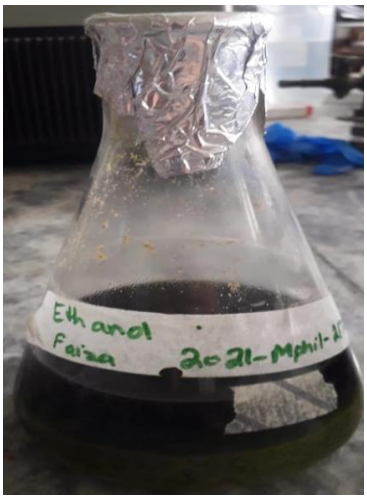
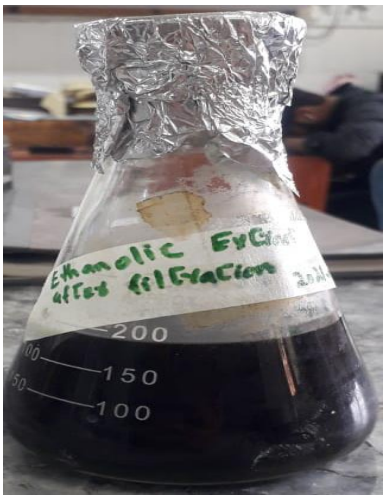
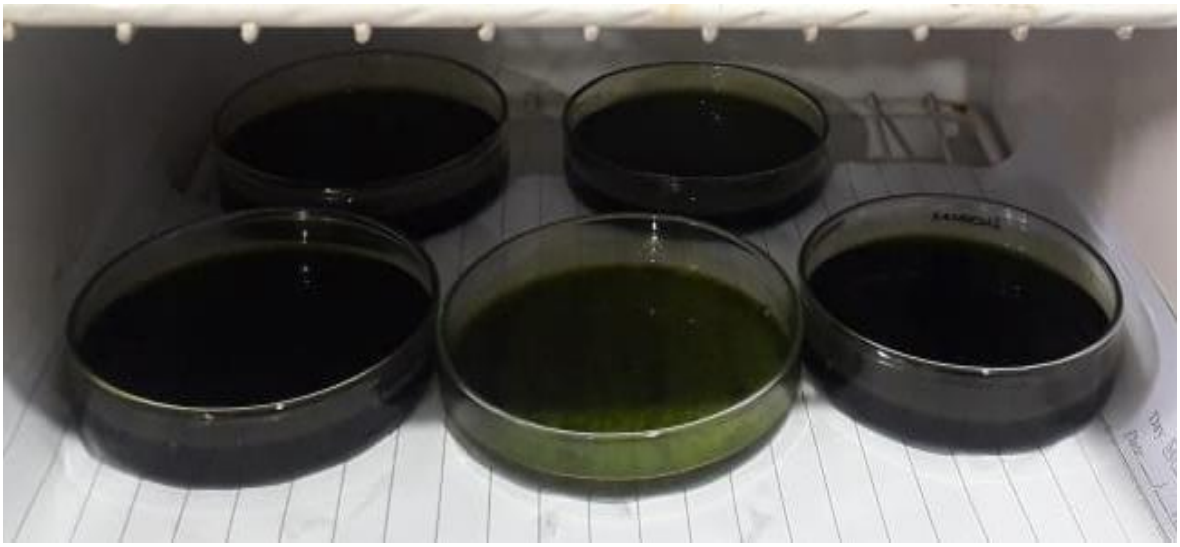


Soaking of powder in ethanol

Filteration of Extract



Ethanolic Extact after filteration



Pouring of filtrate in plates for evaporating in Hot air oven

Figure 2: Preparation of Ethanolic Extract

2.4 Preparation of *Ziziphus jujube* leaves aqueous extract

350ml of water were mixed with 35g of powder. After that, it was kept at ambient temperature in a shaking incubator. Shaking persistently helps prevent fungal infection. After two days, the mixture was filtered using Whatman No. 1 filter paper. The hot air oven was used to dry the filtered mixture. Comparatively speaking, ethanolic extract evaporated more quickly than aqueous extract. Eppendorf tubes containing the dried extract were kept for later examination [14].

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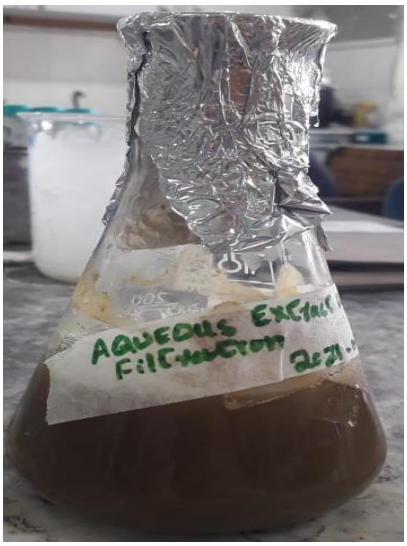
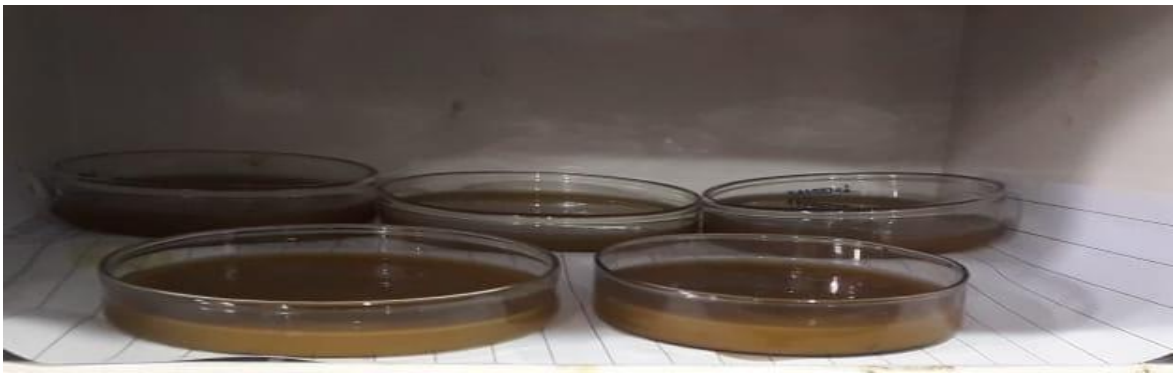
Soaking of powder in aqueous solution

Filteration of Extract



Aqueous extact after filteration



Pouring of aqueous filtrate in plates for evaporating in Hot air oven

Figure 3: Preparation of Aqueous Extract

2.5 Phytochemical Characterization

The extract was undergoing a phytochemical screening to identify the unique components. For this, the standard process was used. Steroids, alkaloids, saponins, phenol, glycosides, terpenoids, flavonoids and tannins was identified [15].

2.5.1 Test for Alkaloids

Wagner's reagent was prepared by adding 2g of iodine and 6g of potassium iodide to a beaker, and then adding distilled water to get the desired final volume of 100ml. Alkaloids are detected with its help. After taking two millilitres of plant extract, a few drops of HCl were added. The test tube received a gentle heating. The Wagner's reagent was then added in a few drops. Presence of white or yellowish precipitate and green color shows the presence of alkaloids [16].

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2.5.2 Test for Saponins

4ml of distilled water were added to 1 mL of plant extract, and the mixture was agitated in a graduated cylinder for 10 minutes lengthwise. The presence of saponins is indicated by the formation of a 1 cm layer of foam [16].

2.5.3 Test for Tannin:

Four drops of 2% $FeCl_3$ were added to a test tube containing 2 mL of extract and greenish black coloration indicates the presence of tannin and which color was appeared which indicate the presence of tannin [16].

2.5.4 Test for Flavonoids

Two milliliter's of the extract and two milliliters of a 2% NaOH solution were combined in a test tube. A few drops of diluted sulphuric acid were added, and the golden color changed to a colorless state [16].

2.5.5 Test for Phenol

2ml each of extract and 2mL of distilled water and followed by few drops of 2% $FeCl_3$ solution were combined. Appearance of Blue or greenish color shows presence of phenol [16].

2.5.6 Test for Terpenoids:

We took 2mL of extract in a test tube. After adding 2mLof chloroform, it was evaporated with a burner. The test tube was then heated after adding 2mL of HCl. Reddish brown color appear it shows the presence of terpenoids [16].

2.5.7 Test for Carbohydrates:

In a test tube, 2mL of extract and 1mL of Molish's reagent were added. Next, 2mL of concentrated H_2SO_4 were poured along the test tube walls. A violet ring that forms signifies the presence of carbohydrates [16].

2.6 Gas chromatography/Mass spectrometry (GC/MS)

To determine whether the bioactive ingredients in the Z. jujuba leaf extracts were present, GC/MS analysis was performed. The column was loaded with fresh ethanolic leaf extract from Z. jujuba mill. Utilizing helium gas (99.999%) at a steady flow rate of 1 ml/min and an injection volume of 0.5 µL (split ratio of 10:1), the injector temperature was 250 ºC, while the ion source temperature was 280 ºC. The oven was set to start at 110 ℃ (isothermal for 2 minutes), then grow by 10 ℃/min to 200 ℃, then by 5 ℃/min to 280 ℃, and finally by 9 ℃ at 280 ℃. Mass spectra were obtained at 70 eV with pieces ranging from 40 to 450 Da and a scan interval of 0.5 seconds. The GC takes 36 minutes to complete. By comparing the average peak area of each component to the total areas, the relative percentage amount was determined. Turbo Mass Ver 5.2.0 software was used to manage chromatograms and mass spectra [17].

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2.7 Antibacterial Screening

Already characterized lyophilized isolates of *E. faecalis* were revived on selective medium that inhibit the growth of any impurity. Slantz and bartly medium was used as a selective medium for the growth of *E. faecali*s. Wikler (2006) described making a 0.5 McFarland standard to control the turbidity of bacterial samples. Sulfuric acid and barium chloride dehydrate were synthesized at 1.175% and 1%, respectively. The suspension was made by adding 0.5 mL of 1% $BaCl_2$ to 99.5 mL of 1% sulfuric acid while continuously stirring. The spectrophotometer's wave length was set at 625, and an absorbance of 0.08–0.13 was deemed suitable. The antibacterial activity of two extracts i.e., *Ziziphus jujube* was determined against the resistant isolates of *E. faecalis* through well diffusion assay.

2.7.1 Agar well diffusion Assay

Muller hinton agar medium was produced and added to pre-sterilized petri plates in a sterilized environment in order to execute the diffusion assay well. Then media was allowed to solidify. After standardizing the inoculum using the 0.5 McFarland standards, 100 µL was swabbed onto MHA medium plates. Using a sterile well borer, wells were created, and the corresponding remaining molten agar medium was used to seal the wells. 0.5 McFarland standard was swabbed on the agar plates. 100µL of the extract was poured in the respective well. Plates were incubated at 37°C for 24 hrs.

2.8 Minimum Inhibitory Concentration (MIC)

For plant extract, a 96-well flat bottom microtiter plate was used to compute the MIC. Using a micropipette, 100 µL of sterile MHB broth was applied to the 12th well of a microtiter plate. Next, a two-fold serial dilution was prepared by transferring 100 µL of liquid from the first well to the second and all the way up to the tenth well. This was achieved by adding 100 µL of plant extract from the produced stock to the first well. The liquid in the tenth well (100 µL) was thrown away.

Next, up to the eleventh well, 100 µL of bacterial suspension with turbidity equal to 0.5 McFarland was added. The well numbered eleven served as a positive control for growth, while the well numbered twelve served as a negative control for sterility. Using an ELISA reader, the optical density was measured at 600 nm at 0-hour. Microtiter plates were incubated in an aerobic environment for 24 hours at 37 °C. The optical density was measured at 600 nm following incubation. The OD was computed as follows:

$$\textbf{OD = Final OD (after 24 h) − Initial OD (at 0 h)}$$

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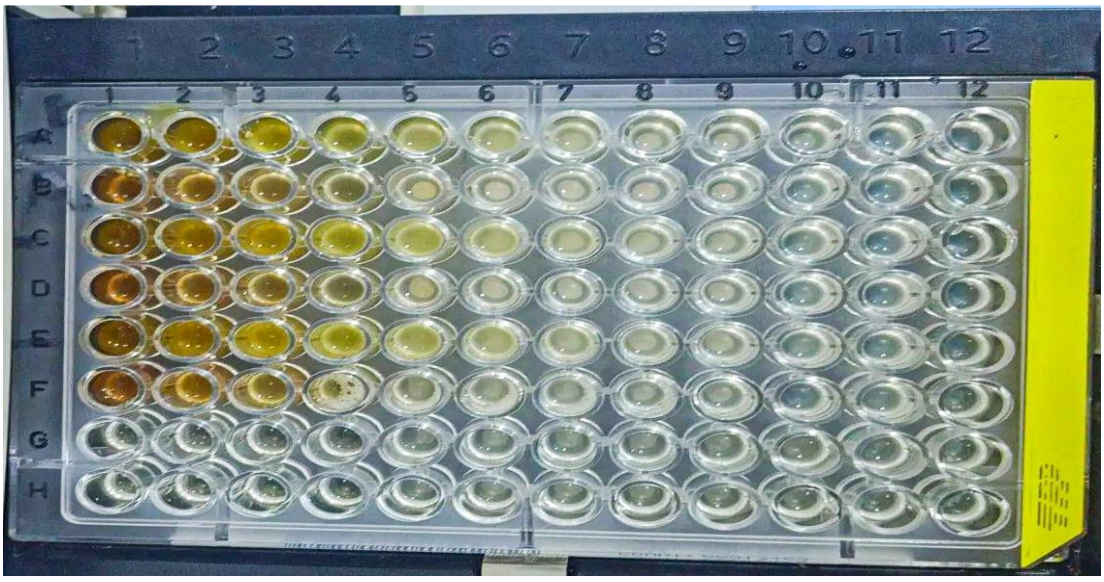

Figure 4: MIC of Ethanolic Leaf Extract

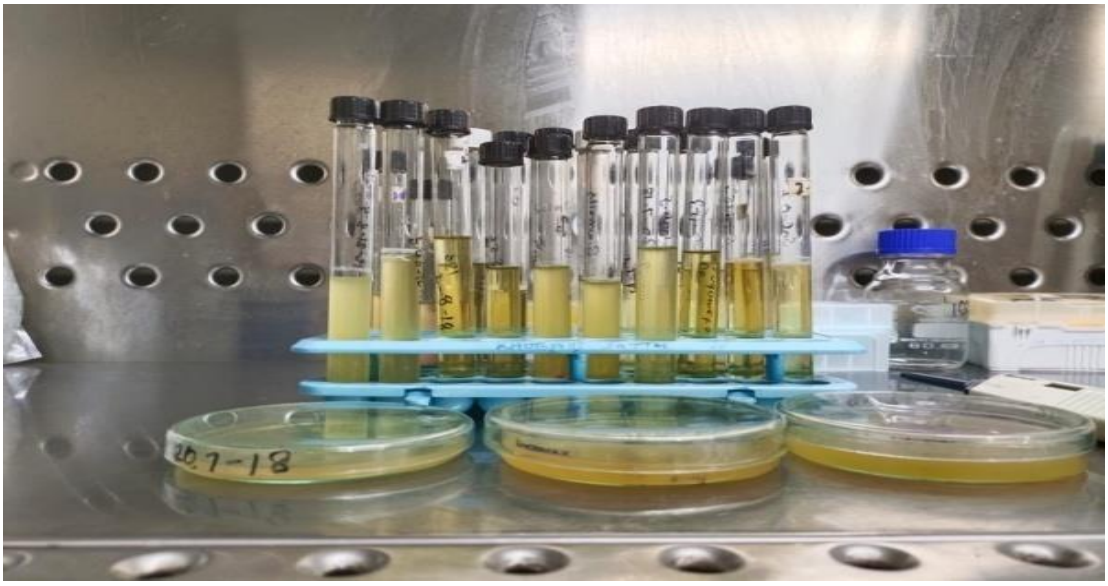

Figure 5: Dilutions of Plant Extract

2.9 Statistical Approach

All the experiment was performed in the triplicates. Microsoft Excel 2010 was utilized to analyze the statistical data [9].

3. RESULTS AND DISCUSSION

Z. jujuba extracts have been shown to have strong pharmacological action and are widely utilized as an alternative medication to treat a variety of illnesses and conditions [18]. The ground sample mix with solvent and got extract for detection of phytochemicals these

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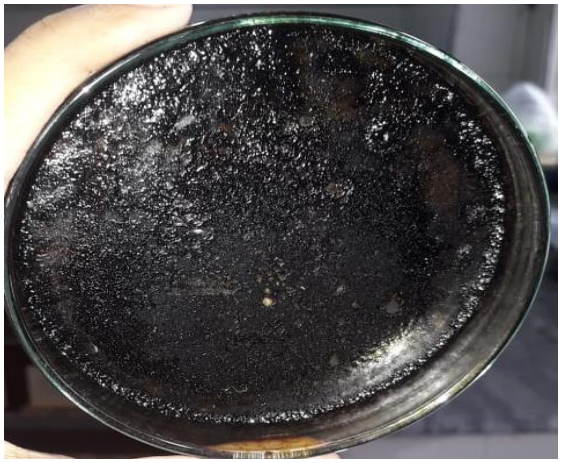
results are lined with Banu *et al*. (2015) who reported that ethanolic, methanolic and aqueous extracts are used to studied phytochemicals by using maceration method. Studies have demonstrated that Chinese plants can defend against illnesses by controlling immunological and endocrine systems [19]. Numerous *Ziziphus* species' seeds and leaves extracts have been shown to exhibit hypnotic-sedative and anxiolytic properties. They have a reputation for lowering CNS activity, which lowers anxiety and promotes sleep. It was discovered to induce sleep but not to have any anticonvulsant or muscle relaxant properties [20].



Ethanolic Extract after evaporation

Aqueous extract after evaporation

Storage in Eppendorf tubes

Figure 6: Z. *jujuba* extracts after evaporation and storage

3.1 Qualitative Analysis

The naturally occurring substances found in plants are called phytochemicals. These phytochemicals are becoming more well-known these days because of all of their therapeutic applications [15]. Phytochemical analysis was done for both aqueous and ethanolic extracts of *Z. jujuba* plant leaves by using maceration method. Phytochemicals which were determined are presented in Figure 6 and 7.

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A preliminary phytochemical screening of *Z. jujuba* leaf extracts (Table 1) identified a number of chemical compounds, some of which have been linked to pharmacological activity in the past Abd-Alrahman, (2013) including alkaloids, saponins, flavonoids, phenols, carbohydrates, tarponoids, and tannins.

The leaf extracts were subjected to phytochemical screening to ascertain the presence of various secondary metabolites [21]. According to the results of the phytochemical screening, *Z. jujuba's* ethanolic and aqueous extracts contain every evaluated secondary metabolite that Ziziphus jujube contains alkaloids, tannins, flavonoids, saponins, triterpenes, and carbohydrates [22].

Figure 7: Qualitative Analysis for Aqueous Leaf Extract

1. Control
2. No greenish color or white precipitate – alkaloid absent
3. Violet ring appears- carbohydrate present
4. Yellow color disappear- flavonoids present
5. Blue coloration- phenol present
6. Foam occur- saponin present
7. No Reddish brown color appear- terpenoid absent
8. greenish-black coloration- Tannins present

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Figure 8: Qualitative Analysis for Ethanolic Extract

1. Control
2. Greenish color and white ppt form- alkaloid present
3. Yellow color disappear- flavonoid present
4. Blue coloration- phenol present
5. No Foam formation occur- saponins absent
6. reddish-brown color- terpenoids present
7. greenish- black coloration- tannins present
8. Violet ring appear – carbohydrates present

3.2 Phytochemical Screening of the *Ziziphus jujube* leaves

Phytochemical characterization of *Ziziphus jujube* leaves is presented in Table 1. Results of qualitative phytochemicals characterization of aqueous extracts indicates that all phytochemicals (carbohydrates, flavonoids, phenols, tannins) are present in it except alkaloid and terpenoids. Similarly, result of ethanolic extracts showed that all phytochemicals (alkaloids, carbohydrates, flavonoids, phenols, terpenoids and tannins) are present in the extract. However, saponins was absent in the extract.

Table 1: Phytochemicals in Aqueous and ethanolic extracts of *Z. jujuba* leaves

Sr. No	Phytochemicals	Aqueous extract	Ethanolic extract
1.	Alkaloid	_	+
2.	Carbohydrates	+	+
3.	Flavonoid	+	+
4.	Phenol	+	+
5.	Saponins	+	_
6.	Terpenoids	_	+
7.	Tannins	+	+

+ = Presence of phytochemical - = Absence of phytochemical

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3.3 Gas Chromatography/Mass Spectrometry

Figure 9, shows the graphical depiction of GC/MS of the extract. Results indicates that different peaks was based on the retention time of various peaks.

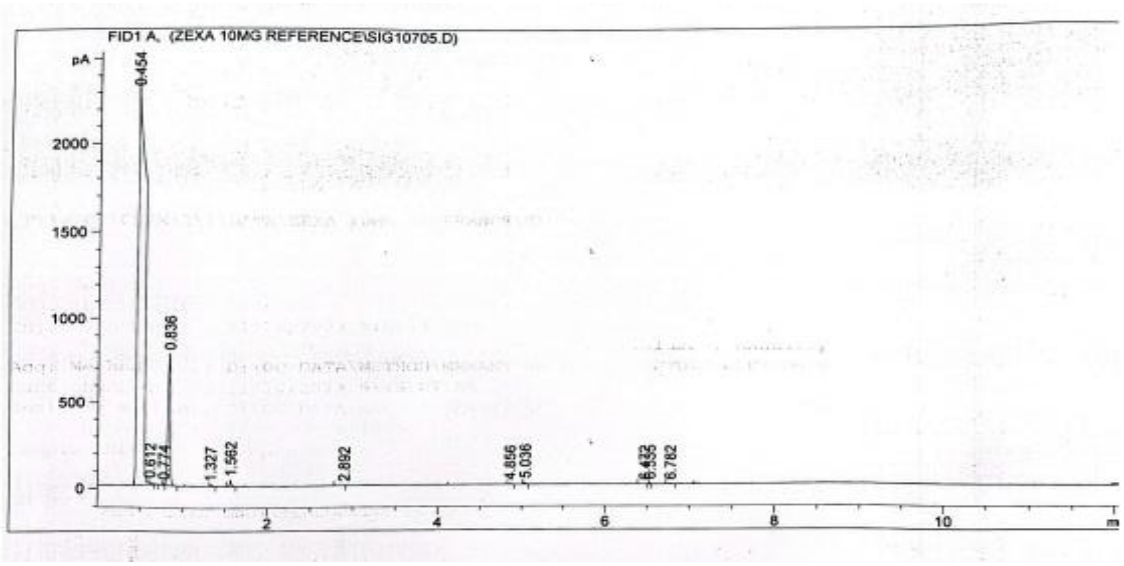

Figure 9: Graphical representation of GC/MS of extract

In the ethanolic extract of Ziziphus jujuba, the GC-MS spectra formed a total of 11 peaks. Seven main bioactive components were found in ethanolic leaf extracts of Ziziphus jujuba by GC-MS analysis. 2-hexadecen-1-ol, tetramethyl, 3, 7, 11, 15, isoethnol, squalene, 9 octadecenoic acid, Thymine, Levetiracetam and maltol.

According to Patil. T (2021) 2-hexadecen-1-ol, 3, 7, 11, 15-tetramethyl belongs to phytochemical phenol. Damage to the bacterial membrane, inhibition of virulence factors including enzymes and toxins, and suppression of bacterial biofilm formation have all been proposed as part of the mechanisms of action of phenolic compounds on bacteria.

Isomenthol belongs to steroids family, biological actions of isomenthol include antibacterial, anti-inflammatory, and antioxidant properties. One naturally occurring unsaturated hydrocarbon that is crucial to human health is squalene. It has been discovered that 9 octadecenoic acid (z)/phenylmethyl ester has antibacterial activity, especially in preventing the growth of certain Gram-positive bacterial species. Thymine exhibits greater antibacterial efficacy when it comes to G+ bacteria.

3.4 Preliminary Identification of *E.faecalis* Isolates

By streaking, the bacterial isolates were revived from microbeads on slanetz and bartley agar plates. After 24 to 48 hrs, the distinctive growth pattern was observed. By using microscopy and observing the cultural traits of the colonies, the isolates were first identified as reported by Tajabadi, (2011) five isolates of enterococcus faecalis was identified by using selective agar plates.

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3.5 Colony characteristics and microscopy of *E.faecalis*

Small pin point colonies of pink colour were observed on slantez and bartely agar plates. They showed characteristic purple color cocci like appearance when observed at 100X magnification. A type of enterococci bacteria known as *(E. faecalis)* is typically found in the gastrointestinal (GI) tract, although it can also be found in the oral cavity and vaginal tract. It is gram positive bacteria, While *E. faecalis* often poses little threat to human health, in cases where an individual's immune system is compromised, it may transform into an opportunistic pathogen and cause illness. *E. faecalis* is regarded as an opportunistic pathogen as a result. It is the most frequent enterococci species that cause urinary tract infections [23].

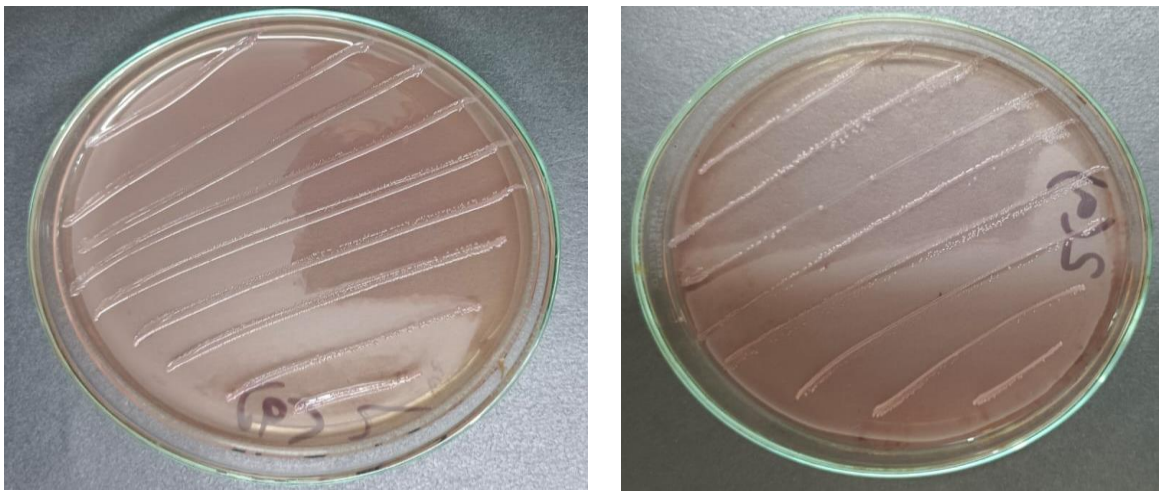

Figure 10: Strain of *E. faecalis*

3.6 Antibacterial Activity of plant extract

In antibacterial activity all the isolates of *E. faecalis* were treated with ethanolic and aqueous extract of *Z. jujuba* plant. The activity of both the extracts were noted which showed that ethanolic extract is more effective against all the isolates of *E. faecalis.* The higher activity of ethanolic extract was against isolate 1 and lowest zone was observed isolate 5. The zone against isolate 1 was recorded as 11mm. the collective response was noted as **9.00±1.37.** The activity of aqueous extract was comparatively less than the ethanolic extract of *Z. jujuba* plant. The higher activity was recorded against isolate 2 and lowest was observed against isolate 3 of *E. faecalis*. The whole response of aqueous extract against all tested isolates was recorded as **4.60±2.63.** The activity of negative control was recorded as **0.00±0.** Most of the *E. faecalis* isolates are resistant to a wide range of antibiotics. A study conducted by I.U. Rathnayake in 2012 showed that the 71.2% clinical isolates of *E. faecalis* were resistant to a wide range of antibiotic [24] The ethanolic leaves extract of *Ziziphus* exhibited varying degrees of antibacterial activity on the Gram-positive bacteria, according to the results of an Agar-well diffusion test [25]. *Ziziphus jujuba* is a valuable and significant medicinal herb. The crude methanol extract of the Z. jujuba plant was found to have low activity against *S. typhi, S. epidermidis, S. pneumoniae, S. aureus,* and *K. pneumoniae* with 37.03, 34.61, 31.03, 30.76, and 28.57%

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inhibition, respectively, and moderate activity against *P. aeruginosa, B. pumalis*, and *E. aerogens* with 55.55, 52, and 41.37%, and 41.37%, respectively [26].The blue bar in the graph reveal the activity of ethanolic extract and red bar manifest the activity of aqueous extract.

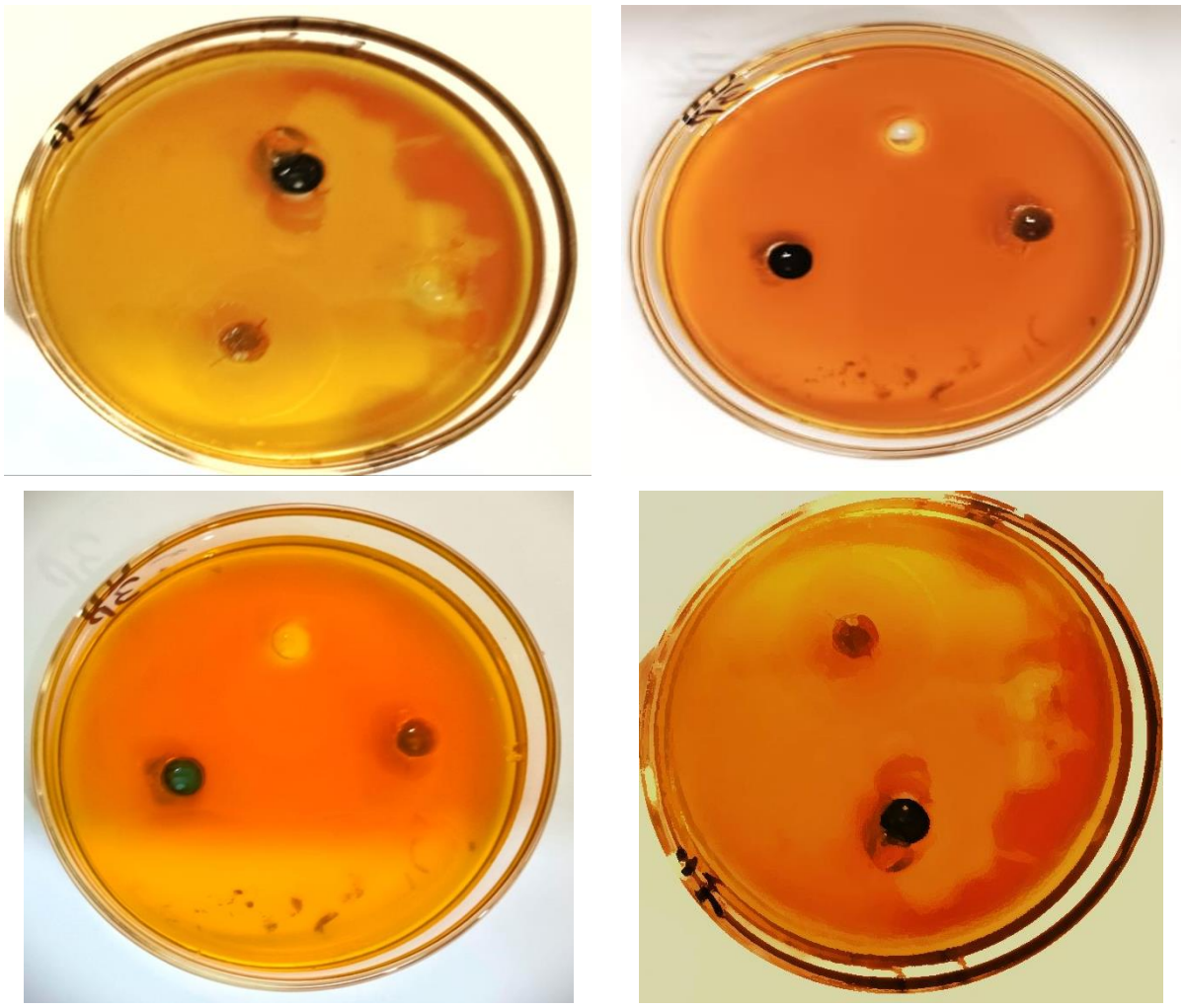

Figure 11: Antimicrobial activity of *Z. jujuba* plant ethanolic and aqueous extracts against *E. faecalis*

Table 2: Antimicrobial activity of *Z. jujuba* plant extracts against *E. faecalis*

Sr No.	Plant extracts	Diameter of zone of inhibition (ZOI) (mm)					Mean ± S.D
		Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	
1.	Ethanol	11	9	8	9.5	7.5	9.00±1.37
2.	Aqueous	6	6.5	0	5	5.5	4.60±2.63
3.	-ve Control	0	0	0	0	0	0.00±0

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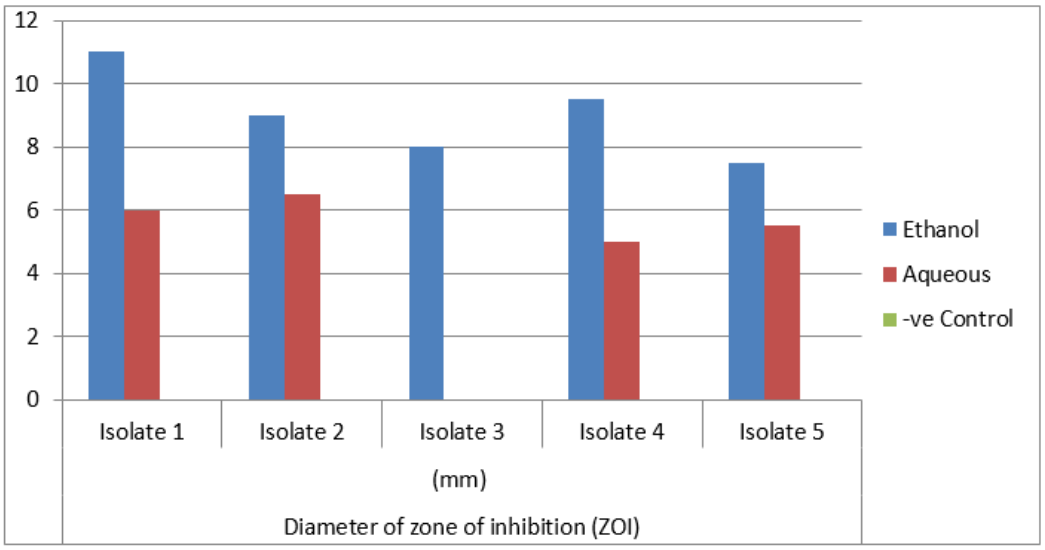

Figure 12: Antibacterial activity of plant extract against *E. faecalis* isolates

3.7 Minimum inhibitory concentration (MIC) of *Z. jujuba* against *E. faecalis*

MIC Figure 13 showed that the components that are derived from plants that have bactericidal properties target microorganisms directly. Direct antibacterial effect against *E. faecalis* was attributed to *Z. jujuba*. Antibacterial characteristics are exhibited by active ingredients found in plants [9].

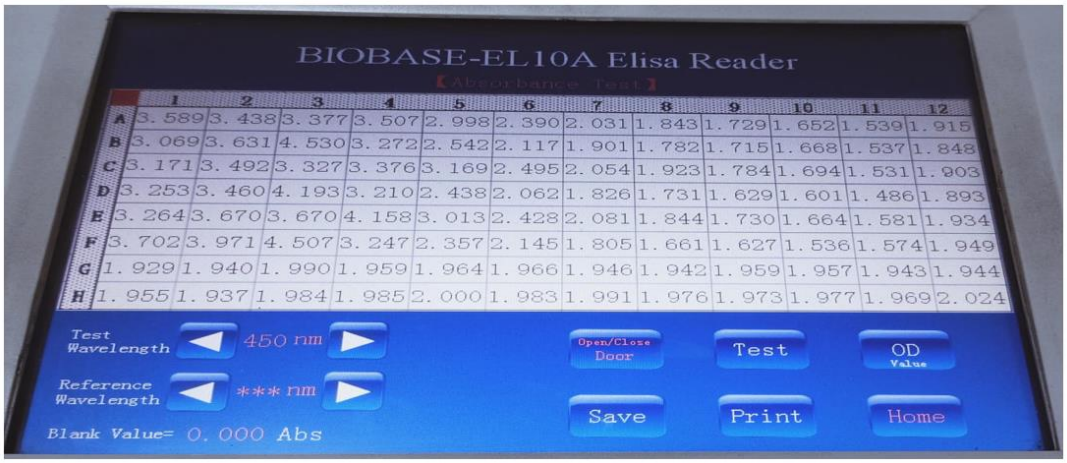

Figure 13: Minimum inhibitory concentration (MIC) of *Z.jujuba* against *E. faecalis*

Table 3: MIC of Z. jujuba against E. faecalis

Sr. No.	Plant extract	MIC					Mean±SD
		Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	
1	Methanol	50	50	50	25	50	45±11.18[a]
2	Aqueous	25	50	25	25	50	45±11.18[a]

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4.0 CONCLUSIONS

According to this study, *Ziziphus jujuba* is an effective antibacterial agent that can combat a variety of pathogenic agents. It is possible that the plant's antibacterial activity is being enhanced by synthetic means. In order to replace the plant with a highly helpful antibacterial medication and strengthen its effects, more research is needed in this area. The market's synthetic medications have a number of adverse effects. Because antibiotics are used so frequently, bacteria have become resistant to them. This study was conducted to see whether any natural medicinal plants have antibacterial potential. The results showed that the ethanolic extract of *Z. jujuba* leaves had the most potential against the chosen strain, which is Gram positive *E. faecalis.* However, *Z. jujuba's* ethanolic leaf extract had greater efficacy than its aqueous counterpart. The findings of the phytochemical research demonstrated that the leaf extracts contained active ingredients. According to the results of the qualitative phytochemical screening of the plant, which included saponin, tannin, flavonoid, alkaloid, phenol, carbohydrates, and terpenoid, *Ziziphus jujube* has antibacterial properties against *E. faecalis.* It would be preferable to use inexpensive plant components to create herbal remedies that have fewer side effects and are helpful against a variety of bacterial illnesses, including stomach issues and diarrhea. On the other hand, more vigorous compound extraction can result in a major advancement in the development of effective antibacterial medications.

References

1) Gossell-Williams, M., R. Simon, and M.J. West, The past and present use of plants for medicines. West Indian Med Journal 2006. **55**(4): p. 217-218.

2) Abidullah, S., et al., A comprehensive review on distribution, paharmacological uses and biological activities of Argyrolobium roseum (Cambess.) Jaub. & Spach. Acta Ecologica Sinica 2022. **42**(3): p. 198-205.

3) Dilshad, R. and R.J. Batool, Antibacterial and Antioxidant Potential of Ziziphus jujube, Fagonia Arabica, Mallotus phillipensis and Hemidesmus Indicus. 2022. Jordan Journal of Pharmaceutical Sciences. **15**(3): p. 413-427.

4) Wachtel-Galor, S. and I.F. Benzie, Ph. D., Herbal medicine. Journal of Pharmacognosy and Phytochemistry. 2011: p. 1.

5) Lu, Y., et al., Research advances in bioactive components and health benefits of jujube (Ziziphus jujuba Mill.) fruit. Journal of Zhejiang University-SCIENCE B. 2021. **22**(6): p. 431-449.

6) Chen, J., et al., A review of dietary Ziziphus jujuba fruit (Jujube): Developing health food supplements for brain protection. Evidence-Based Complementary and Alternative Medicine. 2017. **2017**.

7) Tahergorabi, Z., et al., "Ziziphus jujuba": A red fruit with promising anticancer activities. Pharmacognosy reviews. 2015. **9**(18): p. 99.

8) Al-Saeedi, A.H., et al., Comparative evaluation of total phenols, flavonoids content and antioxidant potential of leaf and fruit extracts of Omani Ziziphus jujuba L. Pacific Science Review A: Natural Science and Engineering. 2016. **18**(1): p. 78-83.

9) Bibi, J., B.N. Mahnoor, In-Vitro efficacy of crude extract of Zizipus Jujuba against selected bacterial strains. International Journal of Scientific and Research Publications. 2014. **4**(2): p. 514.

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10) Venkatachalam, D., et al., Evaluation of antibacterial activity of Zizyphus jujuba. Journal of Pharmacognosy and Phytochemistry. 2020. **9**(3): p. 1510-1513.

11) McBride, S.M., et al., Genetic diversity among Enterococcus faecalis. PloS one. 2007. **2**(7): p. e582.

12) Kayaoglu, G., D. Ørstavik, Virulence factors of Enterococcus faecalis: relationship to endodontic disease. Critical Reviews in Oral Biology & Medicin. 2004. **15**(5): p. 308-320.

13) Thyagharajan, K. K., & Kiruba Raji, I.. A review of visual descriptors and classification techniques used in leaf species identification. Archives of Computational Methods in Engineering.2019. 26(4): p 933-960

14) Naqvi, S.H., et al., Anti-microbial efficacy and biochemical analysis from different parts of Acacia nilotica L. and Ricinus communis L. extracts. Journal of medicinal plants Research. 2011. **5**(27): p. 6299-6308.

15) Banu, K.S. and Cathrine, General techniques involved in phytochemical analysis. International journal of advanced research in chemical science.2015. **2**(4): p. 25-32.

16) Abd-Alrahman.et al. Phytochemical screening and antimicrobial activity of EthOH/water Ziziphus jujuba seeds extracts. Journal of Pure and Applied Microbiology,2013. **7**: p. 823-828.

17) Patil, T., & Khan, S. Phytochemical Screening And Determination of Bioactive Constituents of Methanolic Leaf Extract of Ziziphus jujuba (Mill.). Indian Journal of Pure and Applied Biosciences.2021. **9** (2): p.34-40.

18) Hossain, M.A., A phytopharmacological review on the Omani medicinal plant: Ziziphus jujube. Journal of King Saud University-Science.2019. **31**(4): p. 1352-1357.

19) Cheng, G., et al., Flavonoids from Ziziphus jujuba Mill var. spinosa. Tetrahedron. 2000. **56**(45): p. 8915-8920.

20) Erenmemisoglu, A., et al., Hypoglycaemic effect of Zizyphus jujuba leaves. J Pharm Pharmacol. 1995. **47**(1): p. 72-74.

21) Tiwari, P., et al., Phytochemical screening and extraction: a review. Internationale pharmaceutica sciencia. 2011. **1**(1): p. 98-106.

22) Alhassan, K.A., et al., Phytochemical analysis, proximate composition and antibacterial activities of Ziziphus species (Z. jujube and Z. spina-christi). Journal of Applied and Advanced Research. 2019. **4**(1): p. 42-46.

23) Švec, P., & Franz, C. M. The genus Enterococcus. Lactic acid bacteria: biodiversity and taxonomy. 2014. p: 175-211.

24) Rathnayake, I., et al., Antibiotic resistance and virulence traits in clinical and environmental Enterococcus faecalis and Enterococcus faecium isolates. Systematic and applied microbiolog. 2012. **35**(5): p. 326-333.

25) Abdallah, E.M. Antibacterial activity of fruit methanol extract of Ziziphus spina-christi from Sudan. Journal of Current Microbiology and Applied Sciences. 2017. **6**(5): p. 38-44.

26) Ahmad, B., et al., Screening of Zizyphus jujuba for antibacterial, phytotoxic and haemagglutination activitie. African Journal of Biotechnology. 2011. **10**(13): p. 2514-2519.