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## Analysis of Antimicrobial Compound in *Ziziphus Mauritiana* Extract using Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR)

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**Abstract**—*Ziziphus mauritiana* is locally known as *Bidara* that belongs to *Rhamnaceae* family which is a herbal plant that believed originated from Indo-Malaysian region of South-East Asia. It is commonly known for its various benefits as pharmacological such as antioxidants and antimicrobial. In order to prove its efficacy, antimicrobial compound analysis of *Ziziphus mauritiana* leaf extract was performed using Attenuated total reflection-fourier transform infrared (ATR-FTIR). The spectrum and wavelength obtained can be used to identify the functional group and thus, the antimicrobial compound was discovered present in the plant. A liquid-liquid extraction had been carried out to obtain the extract of *Ziziphus mauritiana* leaf sample and rotary evaporator is used to remove the solvent of ethyl acetate. Based on this study, *Ziziphus mauritiana* leaves do have antimicrobial compounds such as eugenol and vanillin which are one of the antimicrobial agents.

**Keywords**— *Ziziphus mauritiana*; ATR-FTIR; liquid-liquid extraction; antimicrobial compound.

### I. INTRODUCTION

In the tropical and subtropical regions of Malaysia, *Ziziphus mauritiana* or known as *Bidara* grow in forests, thickets along riverbanks, hills and slopes in its habitats. It grows in average annual temperature areas as minimum of 7-13° C to maximum 37-48 ° C, mean annual precipitation of 15-225 mm from sea level to 1,500 m elevation. Trees do not perform well above the 1.000 m plateau, and cultivation becomes less economical [1]

The leaves are about 2.5 to 3.2 cm long with a fine toothed at the edge and 1.8 to 3.8 cm wide. The foliage of *Ziziphus mauritiana* can be evergreen or deciduous, contingent upon the atmosphere. The leaves, with 3 discouraged longitudinal veins at the base, are substitute, applaud or elongated elliptic with an adjusted peak. The flesh is crisp and clean. This fruit is a little juicy when slightly underripe and has a good fragrance. The skin of its fruit is smooth and shiny, thin yet tight. In the tropical and subtropical areas, where it is most frequently found [2].



Fig 1. Round leaves of *Ziziphus mauritiana*

Plants as *Ziziphus mauritiana* or known as *Bidara* are known to blend an assortment of gatherings of bioactive mixes in plant tissues as auxiliary metabolites with antifungal action to stop or hinder mycelial development creation, restrain germination or abatement contagious microbes sporulation as its variable components reacts as bioactive phytochemicals along with antifungal microorganisms. From that point onward, its antifungal hinders the development of contagious microorganisms.

Then, regarding the appliance used in this research which is the Fourier-change Infrared Spectroscopy (FT-IR), it is an instrument to measure an infrared range of assimilation or discharge of a fluid, gas, or solid. This provided a crucial data about spectra, which can gauge the power of spectra over a wide scope of all frequencies at once. In addition, an (FT-IR) spectrometer simultaneously gathers a high ghastrly goal information over a wide scope of phantom. The term (FT-IR)

spectroscopy has been taken from the fact that a Fourier transform (a mathematical process) changes the raw data into the real spectrum or real data [3],[4].

Besides that, FT-IR is a technique that is used nowadays for measuring and calculating the intensity of infrared radiation as a function of frequency or wavelength. [5]. The term “infrared” usually refers to any electromagnetic radiation which lies in the region from 0.7 mm to 1000 mm. But, the region between 2.5-25 mm (4000 to 400 cm) is most suitable term for biochemical examination.

In addition, these plants act as bioactive mixes that are normally created as optional metabolites, with terpenes, tannins, flavonoids, basic oil, alkaloids, lecithin and polypeptides as the principle bunches with antifungal action. These fundamental for the physiology of plants that give protection from microorganisms and different life forms and help keep up with ceaseless ecological stressors, for example, bright radiation, high temperatures or drying out [6].

To be detailed, some of the bioactive compounds were studied to give description in relating the antimicrobial agents with *Ziziphus mauritiana*. It is because it does contain bioactive compounds of antimicrobial compounds. Yet, there are minimal studies done on verifying it in relation to traditional medicine. Thus, this research is focused on analysing the antimicrobial compound in *Ziziphus mauritiana* extract to be relate with the antimicrobial agent found in this extract.

Some of the bioactive compounds studied were tannins, polyphenols and alkaloids. Tannins was reported to has antimicrobial activity [7],[8]. This was proven by Abdallah et al., (2016) that stated it is due to its ability to damage the bacterial cell wall. Tannins are a polymeric phenolic group. They are part into two significant classes: tannins that are hydrolyzable and consolidated. The wellspring of hydrolyzable tannins, ordinarily esterified at a few areas with D-glucose, is gallic corrosive. Consolidated tannins are more plentiful; they can be alluded to as proanthocyanidins and are gotten from flavonoid monomers. The organic conduct of tannins might be connected to oxidation and polymerization designs. By complexing with proteins through both covalent and non-covalent associations, tannins apply their antimicrobial impact. They can likewise be compounded with polysaccharides. There is additionally proof for the immediate inactivation of microorganisms; low tannin fixations have been appeared to change the morphology of the *Crinipellis perniciosa* germ tubes. They have additionally been demonstrated to be equipped for repressing development, restricting the cell dividers of ruminal microorganisms, and protease action on account of dense tannins.

For polyphenols, according to Abalaka et al. (2010), this bioactive compound was found in *Ziziphus mauritiana* and variously been reported to has antimicrobial activity. The bioavailability of polyphenols, or the number of polyphenols that are devoured unaltered, identifies their organic capacity. Subsequent to being assimilated, polyphenols could likewise go through the gastrointestinal framework, consequently

influencing the intestinal microbiota. This can have two ramifications: first, the dynamic kind of polyphenols is changed; second, the composition of the intestinal microbiota is modified, potentially repressing pathogenic microscopic organisms and advancing helpful microorganisms. Polyphenols accordingly have a significant effect on the wellbeing of the human host [10].

Lastly, for bioactive compound studied which is alkaloids, from phytochemical screening by Jain et al. (2019), the presence of alkaloids was detected. This compound was believed to attributed antimicrobial activity of *Ziziphus mauritiana*. Alkaloids are natural mixes of heterocyclic nitrogen which are water-solvent salts that are essential shaping. They contain nitrogen, which is typically created by amino acids. Phenylalkylamines, pyrrolidines, tropane alkaloids, pyrrolizidines, and purine alkaloids incorporate numerous sorts of flavonoids. Contingent upon the atom's antecedents and last structure, these metabolic mixes are ordered into three gatherings. The gatherings include: (1) genuine alkaloids that are essential and contain nitrogen in a heterocyclic ring-including nicotine, (2) pseudo-alkaloids that are additionally fundamental yet not got from amino acids-including caffeine, and (3) fundamental proto-alkaloids that are amino corrosive subordinates, however nitrogen not in a heterocyclic ring-including alkaloids got from phenylethylamine, including mescaline. Moreover, it has been demonstrated that alkaloids have pain relieving impacts; morphine alkaloids are torment relievers utilised as opiates [11].

## II. MATERIAL AND METHOD

### A. Material

The materials used for this study were *Ziziphus mauritiana* leaves which was bought at a market in Selangor, Malaysia. Then, ethyl acetate made in United states that had been used as the solvent. Other than that, materials used were distilled water and filter paper of Whatman's No.1 Filter Paper brand.

### B. Preparation of materials

The *Ziziphus mauritiana* leaves was firstly being dried in an oven under temperature of 60°C for 15 minutes in an oven. The leaves was then grind using pestle and mortar and inserted into beaker. 2.0 g of the *Ziziphus mauritiana* was weighed and put into two conical flasks. While for the solvent, Ethyl acetate originated in United States was chosen.



Fig 2. *Ziziphus mauritiana* which has been grinded

### C. Extraction

40ml of ethyl acetate was poured into two conical flasks which contain the *Ziziphus mauritiana* leaves. Both of the conical flasks were covered immediately by aluminium foil. The samples were then left for 24h in a room temperature. Moreover, the sample was filtered into another conical flask using a Whatman's filter paper. Thus, liquid-liquid extraction was carried out. Distilled water and ethyl acetate were poured into a filter funnel and followed by the sample solution. The filter funnel was then shaken lightly, and these steps were repeated for three times. The solvent was then removed using rotary evaporator in which the temperature was set as 76°C to get the samples' extract.



Fig 3. Liquid-liquid extraction to obtain *Ziziphus mauritiana*'s extract

#### D. Characterization

The samples of *Ziziphus mauritiana* were subjected to FT-IR analysis. The ethyl acetate extract was mounted directly on the diamond window of the leaf portion of *Ziziphus mauritiana*'s leaf samples. In the north-south configuration, all samples were positioned and aligned with the probing beam in order to eliminate spectral variations due to the sample location. The study was performed using the FT-IR (Agilent Technologies, USA) spectrometer and the frequency ranges were determined according to Bunawan's study (2015). Four scans spectrum of wave frequency from 3800  $\text{cm}^{-1}$  to 800  $\text{cm}^{-1}$  ranges were recorded. The average of the spectral was used for further study. Normalization and correction of the background interference was performed on the spectrum. Spectrum transmission was obtained by using MicroLab software (Agilent Technologies, USA). Transmitted peak in ranges of 3800  $\text{cm}^{-1}$  to 800  $\text{cm}^{-1}$  was generated in Microsoft Excel format (.xls) to facilitate the construction of graphs for further analysis.

### III. RESULTS AND DISCUSSION

Using ATR-FTIR, the functional groups of compound present in the *Ziziphus mauritiana* leaves were identified. From the spectrum graph in Figure 4, the frequency of peaks was obtained. The frequencies were used to identify the functional groups of the plant in the Table 1.

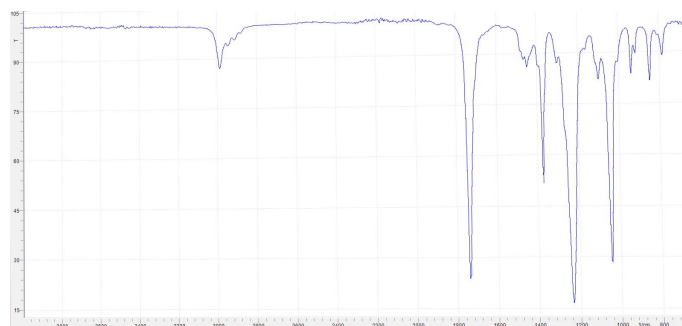


Fig. 4. A spectrum graph of *Ziziphus mauritiana* obtained from the ATR-FTIR

TABLE I

Frequency ( $\text{cm}^{-1}$ )	Transmitters (%)	Functional group
2988.09	87.43	C-H stretch alkanes
1741.05	23.99	C=O stretch carbonyls
1449.64	86.25	C-C stretch (in-ring) aromatics
1235.37	17.18	C-N stretch carboxylic acids, C-O stretch alcohols, carboxylic acids, esters, ethers
1044.67	28.98	C-N stretch aliphatic amines
939.68	83.89	O-H bend carboxylic acids
849.69	82.18	C-Cl stretch alkyl halides

Table 1 shows the functional groups and compounds identified from each sample.

From the spectrum graph, *Ziziphus mauritiana* has functional groups which are the C-H stretch alkanes, C=O stretch carbonyls, C-C stretch (in-ring) aromatics, C-N stretch carboxylic acids, C-N stretch aliphatic amines, O-H bend carboxylic acids and C-Cl stretch alkyl halides.

The transmission measurements observed at 2988.09  $\text{cm}^{-1}$  are attributed to C-H stretch alkanes, 1741.05  $\text{cm}^{-1}$  attributed to C=O stretch carbonyls, while, 1449.64  $\text{cm}^{-1}$  attributed to C-C stretch (in-ring) aromatics. Furthermore, 1235.37  $\text{cm}^{-1}$  attributed C-N stretch carboxylic acids and C-O stretch alcohols, carboxylic acids, esters and ethers. The transmission measurement observed at 1044.67  $\text{cm}^{-1}$  attributed to C-N stretch aliphatic amines, while, 939.68  $\text{cm}^{-1}$  attributed to O-H bend carboxylic acids. Other than that, transmission measurement of 849.69  $\text{cm}^{-1}$  attributed C-Cl stretch alkyl halides. It was discovered through the spectrum and wavelength released by the ATR-FTIR.

From these functional group collected, functional group of vanillin and eugenol was identified as well. It shows that all those functional groups were found in *Ziziphus mauritiana*. Then, at this point, it proves that *Ziziphus mauritiana* do has antimicrobial activity. This is in line with the previous studies as to prove the effectiveness of this plant, *Ziziphus mauritiana* as an antimicrobial, which bioactive

compound was found in the plant to relate with the antimicrobial agent suggested.

In comparison to previous study done, there were compounds found on *Ziziphus mauritiana* leaves such as tannins, saponins, flavonoids and phenols, based on phytochemical analysis of the leaves of *Ziziphus mauritiana* [8]. The results of qualitative identification of *Ziziphus mauritiana* leaf herbal drinks also showed the presence of flavonoids, polyphenols and tannins characterised by a change in colour when added by reagents [13]. This is supported by Boulogne et.al (2012), as terpenoids, alkaloids, and phenolic compounds are compound produced by plants with anti-fungal property. Some of the organic compounds were documented to have anti-microbial activity in different ways and may be the explanation for the activities recorded against these test organisms [15].

Meanwhile, *Ziziphus* leaves are reported by Masullo et al (2019) to contain dammarane-type tri-terpene saponins. Flavonoids occurring in the leaves of *Ziziphus* were O-diglycosylated derivatives of quercetin and kaempferol. The bioactivity of *Ziziphus sp.* leaves might be caused by their antimicrobial impacts in the rough structure particularly toward *Candida* yeasts since *Ziziphus* plant leaves have phenols, saponins, and alkaloids. This is similar study done by Sheba et al (2019) which terpenoids, coumarins, alkaloids, anthraquinones, phenols, flavonoids, tannins, quinones and steroids were screened on *Ziziphus mauritiana* as well. It was measured using visible spectrophotometry for the presence of phenolic compounds as alike method that done by Samirana et al (2017) research previously, that the levels of phenolics and flavonoids content have the ability to donate radical hydrogen. It will show the presence of phenolics and flavonoids compound. Also, among 38 fungal isolates 16 crude extracts showed significant phytochemical constituents.

Furthermore, in comparison to previous study done, according to Al-Bahrani et al. (2018), the LC-ESI-MS investigation of the species demonstrated that concentrates of *Ziziphus mauritiana* leaves contained the biggest centralizations of phenolic mixes. The biggest number of individual phenolic mixes were introduced by *Ziziphus mauritiana* leaves separate with nineteen revealed molecules. In the current investigation, the quantity of phenolic exacerbates distinguished is more prominent than those recently recognized in the variety *Ziziphus* [20]. Moreover, according to Mehmood et al (2020), *Ziziphus mauritiana* contained lignin, phenol, glycoside, saponin and tannin because it shown positive response to all of the compound. Similarly, reported by Asha and Thirunavukkarasu (2019) *Ziziphus mauritiana* plant aqueous extract, alkaloids, steroids, saponins, tannins and phenols compounds are present. This showed that the antimicrobial compound exists in *Ziziphus mauritiana* plants as this study discovered as well. Besides that, phytochemical experiments performed on *Ziziphus mauritiana* leaves showed that cyclopeptide alkaloids, sterols, triterpene saponins and flavonoids were found in the plant. It was then shown from recent study too that this leaf extract of *Ziziphus mauritiana* has antimicrobial and antioxidant activities [23].

Moreover, as, shown in phytochemical screening, the antibacterial action reacts as of the various bioactive mixes, which are alkaloids, saponins, and tannins. The antimicrobial impacts of ethanol separate against *E. coli*, *S. Aureus* and *Streptococcus pyogenes* of the family *Ziziphus* leaves that have been distinguished, somewhere in the range of 0 and 26 mm. The activity against *S. pyogenes* discovered was a bacterium which is gram-positive. This is because plant oftentimes expressed to be more dynamic against gram-positive microbes than against gram-negative microorganisms. Gram-negative microbes were the most dynamic and were discovered to be more compelling than gram-positive microscopic organisms against gram-negative microorganisms [8].

In comparison to a recent study, based on Prakash et al (2020), the examination noticed that *Ziziphus mauritiana* leaves and organic products are a rich wellspring of bioactive flavonoids. The flavonoids distinguished were different mixes. Some of it are called quercetin 3-O-rutinoside, quercetin 3-O-robinobioside and quercetin 3-O-galactoside. At that point, nine phenolic acids were likewise depicted and evaluated from the leaves of the *Ziziphus* family. Protocatechuic corrosive, p-hydroxybenzoic corrosive and ferulic corrosive are also found from the leaves. Then, the results of qualitative phytoconstituents analysis of aqueous *Ziziphus mauritiana L.*'s leaf extract revealed the presence of glycosides, saponins, tannins, coumarins, and flavonoids.

Also, the plant 's antibacterial test was performed with a gram positive antibiotic resistant effect. The plant 's antibacterial measure was done against anti-microbial resistant gram-positive strains of microscopic organisms. For example, *S. aureus*, *B. Subtilis*, and bacterial gram-negative strains, as *P. aeruginosa*, and *E. coli* utilizing the disk diffusion method. Streptomycin was likewise utilized as a control antimicrobial, and the significance of the hindrance zone was resolved for various plant fixations. For instance, for various *Ziziphus mauritiana* focuses, a restraint estimation of 7  $\mu$ L, 14  $\mu$ L, 28  $\mu$ L, and 28  $\mu$ L (100  $\mu$ g/mL) was estimated. From the outcomes acquired in this investigation, it tends to be seen that on account of biosynthesized AgNPs at a weakening of 28  $\mu$ g/mL against *S. aureus*, a gram-positive strain of microorganisms is a lot higher than the hindrance zone got with Streptomycin guideline as the most elevated restraint zone of around 28 mm was accounted for. In addition, as the grouping of *Ziziphus mauritiana*-AgNPs diminished, the restraint zone is diminished as well. Moreover, on the activity against *B. subtilis*, a gram-positive bacterial strain, was discovered to be similar to the one gotten utilizing the control. At the point when the examination was applied to gram negative strains of microbes including *P. aeruginosa* and *E. coli*. The restraint zone got was discovered to be 21 mm and 22 mm separately, which is a lot more modest than that acquired by the screen, 26 mm and 28 mm individually. It very well may be induced from the outcomes over that the *Ziziphus mauritiana*-AgNPs show specific movement against *S. Auroraes* [25].

Moreover, according to Yahaya et al (2019), The results of the quantitative analysis of *Ziziphus mauritiana* plant extracts are that *Ziziphus mauritiana* has 7.8 percent of the highest concentration of alkaloids, followed by other plants studied. With 6.4 percent, and the least was *Cassia occidentalis*. With 6.0%, *Guiera senegalensis*. In *Ziziphus mauritiana*, the tannin level was estimated at 0.6 percent and the saponin concentration in *Ziziphus mauritiana* was 1.9 percent. In the meantime, the amount of flavonoids present in *Ziziphus mauritiana* is 0.66 percent, while the value of glycosides was 2.9 percent in *Ziziphus mauritiana*.

A fundamental phytochemical investigation of the equivalent uncovered that *Ziziphus mauritiana* methanol extract contains phenols, flavonoids, tannins, saponins and alkaloids, based on a previous analysis by Kushwaha et al. (2019) viable customary hot soxhlet extraction of the *Ziziphus mauritiana*'s fruit pulp. In the methanol concentrated of *Ziziphus mauritiana*, the presence of various bio-dynamic mixes legitimized the fruit pulp as a generally excellent wellspring of helpful operators for different sicknesses, for example, malignant growth, epilepsy, Alzheimer's illness, Parkinson's illness, amyotrophic lateral sclerosis (ALS), bacterial and parasitic contaminations.

Besides, with a high-performance liquid chromatography combined with evaporative light scattering on (HPLC-ELSD) measure, two flavonoids and three triterpenoids were broke down, which was approved by linearity, breaking point of location (LOD) and amount (LOQ), intra-day and between day accuracy, dependability and exactness assurance. The normal spinosin content in *Ziziphus mauritiana* (0.95 mg/g) was demonstrated to be higher for the flavonoids, while no criticalness was found for 6-feruloylspinosin. The normal substance of *Ziziphus mauritiana* seed A and *Ziziphus mauritiana* seed B in the plant was 0.79 and 0.18 mg/g, separately, for triterpenoids [27]. Thus, based on previous research, *Ziziphus mauritiana* do contain antimicrobial compound which are the flavonoids, alkaloids, tannins, polyphenols and phenolics. From that point, antimicrobial agents such vanillin and eugenol are possible to be consisted in *Ziziphus mauritiana* plant.

#### IV. CONCLUSIONS

Due to the identification of functional group of *Ziziphus mauritiana*, it is proven that the plant contains antimicrobial agent such as vanillin and eugenol. It also contain antimicrobial compound which support the statement of this plant do has antimicrobial activity.

Besides that, this study is beneficial because the value of this plant contains antimicrobial property that can be shown with strong evidence of scientific research. Community can use it as one of the traditional medicine to inhibit microbes. This study can also contribute as much as to the country's economy as this nutritious plant can be commercialized as traditional medicine with antimicrobial property.

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