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

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Abstract. The usage of herbal medicinal is widely used among society today in daily lives. *Ziziphus mauritiana* locally known as Bidara belonging to family *Rhamnaceae* is a herbal plant that believed originated from Indo-Malaysian region of South-East Asia. It is commonly known for its various added values in term of pharmacological such as antioxidants and antimicrobial. 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 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.

Keyword: *Ziziphus mauritiana; ATR-FTIR; liquid-liquid extraction; antimicrobial compounds*

Introduction

Ziziphus mauritiana

Local people in Malaysia generally know it as *bidara.* In the tropical and subtropical regions, *Ziziphus mauritiana* occurs in forests, thickets along river banks, hills and slopes in its native habitats. It grows in average annual temperature areas: minimum 7-13 to maximum 37-48 ° C.The leaves are about 2.5 to 3.2 cm long with a fine tooth at the edge and 1.8 to 3.8 cm wide. 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 the fruit is smooth and shiny, thin yet tight. In the tropical and sub-tropical areas, it is most frequently found [1].

A Fourier-change infrared spectroscopy (FT-IR)

Fourier-change infrared spectroscopy (FT-IR) is an instrument used to take an infrared range of assimilation or discharge of a fluid, gas, or solid. This give a crucial data about spectra, which can gauge

the power of spectra over a wide scope of frequencies all at once. The electromagnetic radiation which lies in the the region between 2.5-25 mm (4000 to 400 cm) is the most suitable for biochemical examination. Then, FT-IR is a technique that is use now a days for measuring and calculating the intensity of infrared radiation as a function of frequency or wavelength [2].

Antimicrobial agents

A few studies have focused on eugenol as a contagious operator for the treatment of infections. One of it is oral candidiasis, as a clarification of how it responds with *Candida sp*. The most grounded hostile to bacterial action against *S. gordonii* and *Porphyromonas gingivalis* was shown by Eugenol. Then, for Vanillin, it has antimicrobial properties and has been used in dermatitis treatment formulations as a consequence. It has been documented that vanillin and, to a lesser degree, ethyl vanillin have shown strong antimicrobial effects at dosages between 500 and 1000 ppm. Against fungi and non-lactic gramme-positives, both compounds are more effective than against gram-negative bacteria.

Antimicrobial compound
In plants, these bioactive mixes are normally created as optional metabolites, with terpenes, tannins, flavonoids, basic oil, alkaloids, lecithin and polypeptides as the principle bunches with antifungal action. Firstly, tannins. It has 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 restricting the cell dividers of ruminal microorganisms, and protease action on account of dense tannins. Secondly, polyphenols which has a ramification that the composition of the intestinal microbiota is modified, potentially repressing pathogenic microscopic organisms and advancing helpful microorganisms. Thirdly, alkaloids. Phenylalkylamines, pyrrolidines, tropane alkaloids, pyrrolizidines, and purine alkaloids incorporate numerous sorts of flavonoids. It has been demonstrated that alkaloids have pain relieving impacts; morphine alkaloids are torment relievers utilized as opiates [4].

Thus, this research is focused on analysing the antimicrobial compound in *Ziziphus mauritiana* extract from the antimicrobial agent found in this extract. This is because in certain studies, the antimicrobial compound are studied but did not well related with traditional medicine such *Ziziphus mauritiana.*

Material and methods

Preparation of materials

Ziziphus mauritiana leaves was bought at market in Selangor, Malaysia. It was firstly being dried in an oven under temperature of 60ºC 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. The *Ziziphus mauritiana* leave was dried in an oven under temperature of 60ºC for 15 minutes in an oven. As the solvent, 40ml of ethyl acetate was poured into a conical flasks which contain the *Ziziphus mauritiana* leaves. Both of the conical flasks were covered immediately by aluminium foil. The samples was 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 76ºC to get the samples' extract.

Characterisation

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. The frequency ranges were determined according to Nurdalila A.A. (2016). Four scans spectrum of wave frequency from 3800 cm-1 to 80p cm-1 ranges were recorded. The average of the spectral was used for further study.

Results and discussions

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

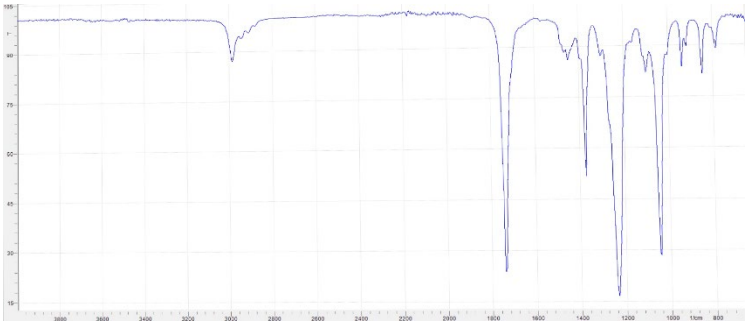

Figure 1. A spectrum graph of *Ziziphus mauritiana* obtained from the ATR-FTIR

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

Frequency (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

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

From these functional group collected, functional group of vanillin and eugenol was identified. It shows that all those functional groups were determined in *Ziziphus mauritiana*. Then, at this point, it proves that *Ziziphus mauritiana* do has antimicrobial activity. Then, to prove in further the effectiveness of this plant, *Ziziphus mauritiana* as an antimicrobial plant with stating the bioactive compound found in the plant to relate with the antimicrobial agent suggested.

Conclusions

Due to the identification of functional group of *Ziziphus mauritiana*, it is proven that the plant contain antimicrobial agent such 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 which it contains antimicrobial property can be shown with strong evidence of scientific research. Then, 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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