Indian Journal of Ecology (2020) 47(2): 493-496

Manuscript Number: 3030
NAAS Rating: 4.96

Phytochemical Screening and Characterization of *Meliadubia* Leaves Extract for Antimicrobial Activity against *Escherichia coli* and *Staphylococcus aureus*

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Abstract: Abstract*:* In this study, the leaves of *Meliadubia* were extracted through the hot-extraction method using distilled water as a solvent. The crude extract of *M. dubia* was evaluated for their chemical group compounds using phytochemical screening for the chemical group compounds. Meanwhile, Gas chromatography (GC), Energy-dispersive X-ray spectroscopy (EDX) and Fourier-transform infrared spectroscopy (FTIR) were used to conform to the fictional groups in the leave of *M. dubia*. Phytochemical screening analysis was done using common procedures and had shown the presence of alkaloids, carbohydrates, tannins, steroids, and flavonoids. Twenty-two compounds were identified in the GC-Mass spectrometry. The highest compounds were Pyridine, 2,3,4,5-tetrahydro-3-methyl (17.03 %), 1-azabicyclo (3.1.0) hexane (12.16 %), and 2-Undecanol (7.63 %), while the lowest compounds were Heptafluorobutyric acid, n-tetradecyl ester (0.79 %) and 4-Methyl-3-pentenal (0.79 %). The EDX analysis presented two elements, which were carbon (53 %) and oxygen (46 %). The *M. dubia* band at 1668 cm^{-1} refers to the amide I C=O stretching, and the peak at 2140 cm^{-1} is associated to the alkyne group that exists in the phyto-constituents of *M. dubia* extract. Meanwhile, the peak that was monitored at 3301 cm^{-1} corresponds to the amide A (N-H). The observed peaks are mainly discovered as flavanoids and terpenoids that exist significantly in the plant extract. The antibacterial activities investigated against gram-negative bacteria, *Escherichia coli* (*E.coli*) and gram-positive bacteria, *Staphylococcus aureus* (S. *aureus*). The results had presented the factional activity for *M. dubia* against both pathogens.

Keywords: *Melia dubia*, Phytochemical screening, Antimicrobial activity, GC-mass

The use of medicinal plants in the development of a drug is crucial to the human as they are being used to treat various kinds of diseases (Mustafa et al 2018). Traditional treatment from the wild plants had been always referred to guide the researcher to discover the best medications to create a healthy life for humans and animals (Ahmadipour et al 2016). However, there are still a few more medicinal plants that are still hidden and undiscovered, which requires further scientific evaluation (Mudhafar and Ismail 2019). *Melia dubia* belongs to the Meliaceae family. It is significantly distributed in India, Iran, Pakistan, Argentina, Brazil, Bermuda, China, Australia, and Malaysia (Saravanan et al 2013, Ram et al 2014, Parthiban et al 2019). Traditionally, *M. dubia* leaves were used as a medicine for various kinds of treatment such as insect pests, wound healing (Koul et al 2000). Different parts of *Melia dubia* have been extracted and utilized for any kinds of skin infections such as the microbial and gastrointestinal tract (Purushothaman et al 1984), hypoglycaemic, and antidiabetic (Susheela et al 2002). *M. dubia* has many properties that have been investigated in the previous studies (Mudhafar et al 2019), however, there is no study about a phytochemical screening leaves to identify the chemical components of the extract. In the present study phytochemical screening of *M. dubia* leaves have been done via using standard procedures along with investigated the biological properties of the leaves via identified its antibacterial activity against gram-negative bacteria, such as *Escherichia coli* and gram-positive bacteria, such as *Staphylococcus aureus.*

MATERIAL AND METHODS

Washed many times by distilled water to remove all of the dust and fungus and were. The sun-dried 7 days and were cut down to small pieces and keep it in future work. 25 g of the leaves were extracted with 200 ml of distilled water in 250 ml conical flask. The leaves were boiled for 20 minutes and filtered to get the crude extract. The extract of the leaves was stored at 4°C for future work (Vijayan et al 2019).

The phytochemical screening was carried out for *Melia dubia* leaves Any difference in hues or the appearance of

precipitate in solution was utilized as demonstrative of positive reaction to these tests. Common procedures have been used for alkaloids, saponins, carbohydrates, cardiac glycosides, tannins, steroids, terpenoids flavonoids and coumarins.

The leaves of were characterized by using several equipment such as Gas chromatography-Mass spectrometry (GC-MS, Shimadzu GC-14B) analyzer, FTIR (Fourier-transform infrared spectroscopy) by Termo Scientific Perkin Elmer Model: Spectrum 100 Spectrometers, and EDX (Energy-dispersive X-ray spectroscopy) using FESEM instrument used was Hitachi SU8020.

Antibacterial activities: Agar Preparation: An amount of 8 g nutrient broth 20 g agar powder was dissolved in 1000 mL distilled water, then sterilized by an autoclave for 20 min at 121°C. Then, it was cooled to 55°C and 25 ml of cooled media was added to the plate and left for some time to solidify, and stored at 4 °C in the dark for further experimentation.

Antimicrobial test: The test bacterial strains were transferred from the stock cultures as streaked on nutrient agar (NA) plates and incubated for 24 h. Well, separated bacterial colonies were then used as inoculums. Bacteria were transferred using a bacteriological loop to autoclaved nutrient agar that was cooled to about 45°C in a water bath mixed by gently swirling the flasks. The medium was then poured to sterile Petri plates, allowed to solidify and used for the biotest (Jain et al 2009). A fresh culture of inoculums of each culture was streaked on nutrient agar media in a petri dish. Different concentration of crude extract has been used to investigate the antibacterial activity. Filter paper discs (about 6 mm in diameter), containing the test compound at the desired concentrations, were placed on the agar surface. The petri dishes were incubated in 37°C conditions. Antibacterial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured. In this test, the distilled water was used as a negative control, while the ampicillin was used as a positive control. 5, 10, 20, 30 and 50 μgml^{-1} concentrations of *M. dubia* crude were mixed with the distilled water to dissolve. Then, the mixtures were poured onto a filter paper of 6 mm and placed in the petri dish. As mentioned previously, the antibacterial activities of the *M. dubia* crude was affirmed based on the method of disk diffusion. A ruler was used to measure the ability of *M. dubia* crude to prevent bacterial growth against both bacteria.

RESULTS AND DISCUSSION

Alkaloids, carbohydrates, tannins, steroids, and flavonoids were detected in the phytochemical screening of leaves. These chemical group compounds have the ability to be an as reducing agent to convert the metal from its salt by changing its charge from +1, +2 to zero. In the present study, the leaves *Meliadubia* has been shown to present the major chemical group compounds in which they are able to work as a reducing agent (Table 1).

According to Allen et al (2016), the compounds may be volatile or semi-volatile organic compounds. *Melia dubia* leaves underwent the GC-MS analysis to identify the exact composition of the leaves and the content of the compound, which can be determined based on the area of the peak, the retention time, and the molecular formula. Based on the analysis, 22 compounds were discovered in the leaves' composition. Three of these compounds exhibited high percentage in the leaves, which are 1-azabicyclo (3.1.0) hexane with the composition of 12.16%, Pyridine, 2, 3, 4, 5-tetrahydro-3-methyl with 17.03%, and 2-Undecanol with 7.63%. The molecular formulas of these compounds are; $C_{18}H_{29}F_7O_2$ and $C_6H_{10}O$ respectively. Generally, the overall composition of these compounds was 0.79% (Table 2). The EDX technique detects the X-ray beams emitted from the sample during the process of the bombardment to characterize the composition of the element from the sample. The outcome of the technique highlighted the elements with the atomic number, which ranges from beryllium to uranium, to indicate that the relative x-ray counts at a certain energy level of the sample's constituent can assist in obtaining the quantitative result (Zhao et al 2019). Besides that, EDX also helps to identify the percentage of the elements that exist in the crude of leaves. The two elements that had been discovered in the crude were oxygen (O) and carbon (C). These elements were associated with the organic

Table 1. Phytochemical screening of M*eliadubia* leaves

Phytochemical test Alkaloid test	Indicator	Result
Mayer's	Appearance of white or creamy precipitate	Positive
Wagner's,	Appearance of reddish- brown precipitate	Positive
Dragendroff's	Aprominent yellow precipitate	Positive
Saponins Test	Not foam on the surface of the mixture	Negative
Carbohydrates	formation of violet ring	Positive
Cardiac glycosides	No appearance of greenish blue colour	Negative
Tannins test	Appearance of blue black colouration	Positive
Steroids test	Appearance of green fluorescent	Positive
Terpenoids test	No appearance of reddish brown colouration	Negative
Flavonoids test	Appearance of yellow colour	Positive
Coumarins test	No appearance of yellow coloration	Negative

compounds that exist naturally in the plant (Fig. 2).

Besides EDX, the Fourier Transform Infrared Spectroscopy (FTIR) was also used as it was known to be a substantial analytical method as it can detect a few functional groups in the compounds. It was discovered that there were significant impacts to the chemical bond in a liquid once it interacted with the infrared light. The chemical bond will elongate, contract, and absorb the radiation when other molecules were present at a particular wavelength. Therefore, the main functional groups in the compound were recorded. Based on the record, the FTIR spectra noted the *M.*

Fig. 1. Fresh and dried leaves of *Meliadubia*

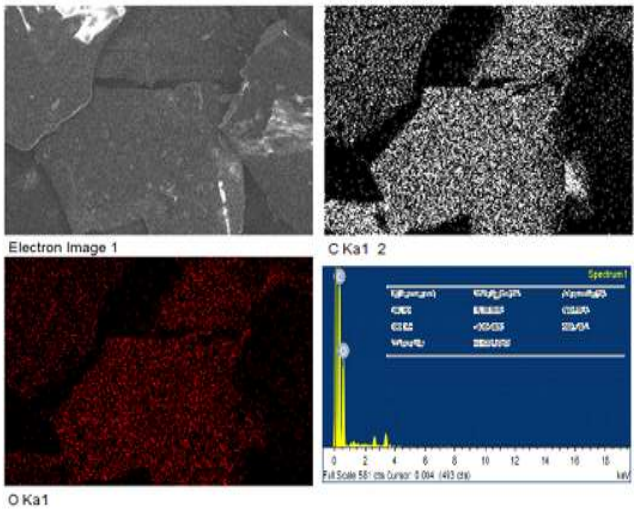

Fig. 2. EDX mapping of leaves *Meliadubia*

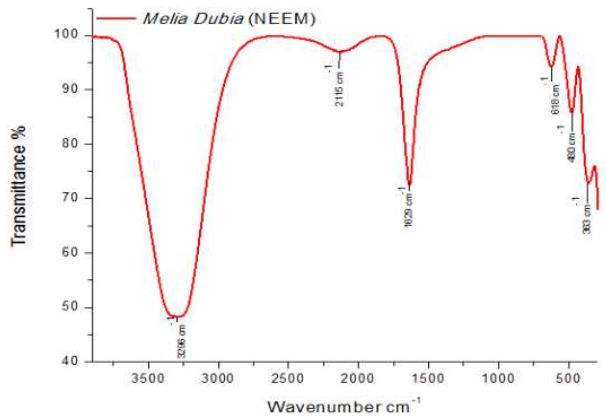

Fig. 3. FTIR spectroscopy of *Meliadubia* leaves

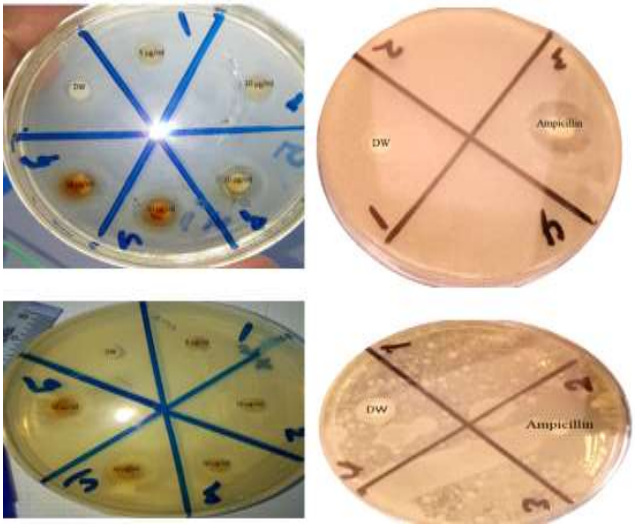

Fig. 4. Antibacterial activities of extract of plant and control samples against *E. coli* and B) AgNPs against *S. aureus*

Table 2. Main Components (%) of *M. dubia* leaves

R. Time	Composition (%)	Compound	Molecular formula
9.887	3.29	Acetyl cyanide	C_3H_3NO
9.887	3.29	2-Propynenitrile, 3-fluoro-	C_3FN
9.887	3.29	Ethyl isocyanide	C_3H_5N
10.154	12.16	1-azabicyclo(3.1.0)hexane	C_5H_9N
10.154	17.03	Pyridine, 2,3,4,5-tetrahydro-3-methyl-	$C_6H_{11}N$
10.264	1.45	1,2,3,6-Tetrahydropyridine	C_5H_9N
10.319	1.96	1-Methoxy-2-propyl acetate	$C_6H_{12}O_3$
12.079	1.02	Tans-1-Propenylcyclopropane	C_6H_{10}
12.361	3.88	1,6-Heptadiene	C_7H_{12}
13.037	7.62	2-Undecanol	$C_{11}H_{24}O$
13.131	1.49	2-Furanmethanamine	C_5H_7NO
13.171	1.35	Acetamide, N-2-propynyl-	C_5H_7NO
13.288	2.59	Acetonitrile, 2,2'-iminobis-	$C_4H_5N_3$
13.343	2.42	Cyclobutanone, 2-methyl-2-oxiranyl-	$C_7H_{10}O_2$
13.587	2.73	2-Hexenal	$C_6H_{10}O$
13.343	2.42	1-Butene, 2-ethyl-3-methyl-	C_7H_{14}
13.367	1.32	2-Pentanone, 3-methylene-	$C_6H_{10}O$
13.516	1.78	2(5H)-Furanone, 5-methyl-	$C_5H_6O_2$
13.642	0.79	Heptafluorobutyric acid, n-tetradecyl ester	$C_{18}H_{29}F_7O_2$
13.642	0.79	4-Methyl-3-pentenal	$C_6H_{10}O$
13.673	1.95	1,1,2,3-Tetramethylcyclopropane	C_7H_{14}
13.775	1.32	1,1,3-Trimethylcyclopentane	C_8H_{16}

dubia band at 1668 cm-1 that referred to amide I C=O stretching (Liu et al., 2018). The peak of 2140 cm^{-1} was associated with the alkaline group that exists in the phyto constituents of *M. dubia* (Kumar et al 2018). At 3301 cm^{-1}, the peak was assigned to the amide A (N-H). Generally, the detected peaks are known to be as flavanoids and terpenoids that exist significantly in the extract of plants (Mu et al 2016).

It was reported that the distilled water inhibition was zero in both bacteria. Meanwhile, the inhibition zones of ampicillin were 28.6 against *E. coli* and 22.1 against *S. aureus.* Overall, the inhibition bacteria growth of the *M. dubia* was 10, 11, 13, 15 and 16 mm against *E. coli* and 8, 10, 12, 13, 15 mm against *S. aureus*.

CONCLUSION

The aqueous solutions of *M. dubia* have been shown to have 22 compounds in the GC-mass spectroscopy. Three of these compounds have been shown high availability. FT-IR was identified to factional groups that belonged to phyto-constituents of extract. Antibacterial activity of extract has been investigated, which can use it as an antibacterial agent in the medical and biological filed.

ACKNOWLEDGMENTS

This research was funded to grant no. GPU 2017-0189-104-01, UPSI. Prof Dr. Ismail Zainol. Lab of polymer, department of chemistry, faculty of science and mathematics, UPSI, Perak, Malaysia.

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Received 27 February, 2020; Accepted 08 May, 2020

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