# PHYTOCHEMICAL ANALYSIS AND BIOACTIVITY STUDIES OF *ZIZIPHUS MAURITIANA* (TWIGS AND LEAVES)

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## Abstract

*Malaysia lack and inadequate research about Ziziphus mauritiana. Two part of Z. mauritiana plant obtained which are leaves and twigs for this study. Z. mauritiana extraction processes conducted using three difference polarity of solvents which are hexane, chloroform and methanol by cool extraction method. The highest yield percentage is chloroform leaves extract which is 3.71%. The phytochemical analysis study revealed that many secondary metabolites presence inside Z. mauritiana such as alkaloid, flavonoid, glycoside, phenol, saponin, steroid, sterol, tannin and terpenoid but for leave part only absence of saponin detected. In thin layer chromatography (TLC) method, the mixture of solvent system used are hexane and chloroform with ratio 3:7 or 1:9 to obtain good separation of compound under short (254 nm) and long (366 nm) wavelength UV lamp. Antibacterial activity examined using disc diffusion method toward Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Salmonella sp. conducted. The highest maximum zone inhibition diameter recorded is hexane leaves crude extract on S. aureus bacteria which is 15 mm. In addition, antioxidant study discovered the highest percentage found on crude extract of methanol.*

Keywords: *Ziziphus mauritiana, Phytochemical analysis, Thin layer chromatography, Antibacterial, Antioxidant*

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## 1. Introduction

Plants can easily obtain in large group and economically serve as basic raw materials for industry. The cost of plants as source in healthy field with their function to cure and prevent diseases is cheap (Adeyemo, 2011). In Malaysia, Z. *mauritiana* is also known as Bidara, Jujub and Epal Siam. The native species Z. *mauritiana* can be found in Afghanistan, Algeria, Australia, Bangladesh, China, Egypt, India, Indonesia, Iran, Kenya, Libyan Arab Jamahiriya. Malaysia, Nepal, Pakistan, Thailand, Tunisia, Uganda, Vietnam (Orwa *et al.*, 2009). The background scientific classification about this plant, as stated in Table 1.

Table 1. Scientific classification of *Ziziphus mauritiana*

| Kingdom | Plant |
|---|---|
| Division | Magnoliophyta |
| Subdivision | Agiosperm |
| Class | Magnoliopsida |
| Order | Rosales |
| Family | Rhamnaceae |
| Tribe | Paliureae |
| Genus | *Ziziphus* |
| Species | *Mauritiana* |

Source: (Palejkar*et al.,* 2012)

On the leaves part, it is helpful function as poultices and liver in problems, asthma, fever (Jain *et al.,* 2011), high blood pressure and diabetes mellitus (Ray *et al*., 2014). Furthermore, use to cure and prevent skin diseases (Abalaka *et al*., 2010). It have antioxidant activity, reducing power, and scavenging effect on free radicals. Each medical plant has the most valuable phytochemical which only it plant contains. Phytochemicals are the non-nutritive secondary metabolites that have defensive or disease preventive properties. Almost all medicinal plants have different phytochemical secondary metabolites that exhibit medical properties including antisplasmodic, anti-inflammatory, antiviral, anticancer, antifungal, antibacterial, antioxidant, antimalarial, antiulcer, antihypertensive, antidepressive, hypochlolestrerolemic, immunomodulatory clot dissolving, detoxifying and many others potential (Talmale *et al.*, 2014).

The objectives of this study including to extract the leaves and twigs part of *Ziziphus mauritiana* using difference polarity of solvents, to perform phytochemical analysis on extracted samples, to determine Thin Layer Chromatography profile of extracted samples by using (TLC) method with variety of solvent systems, and to screen antibacterial and antioxidant activities of extracted samples.

Despite some researches have been conducted from species of *Z. mauritiana* in Malaysia, but the raw materials were imported from other countries such as Pakistan and India. This is sign that research about *Z. mauritiana* species planted from Malaysia country need to be conduct. Hence, to validate, confirm and encourage*Z.mauritiana* development and scientific method study as medicinal plant for future. A proper documentation and scientific validation about *Z. mauritiana* need to perform and establish.

## 2. Materials and methods

The twigs and leaves of *Ziziphus mauritiana* were collected from Selangor. The samples were cleaned, air-dried at room temperature for three weeks and ground into fine powder.

### 2.1 Extraction of samples

Individually, powder from each part of *Z. mauritiana* of twigs and leaves was soaked in three difference polarity of solvents which are hexane, chloroform and methanolfor at least 48 hour in order to accomplished cool extraction method. The crude extracts of

*Z. mauritiana* were obtained by using vacuum rotary evaporator. The yield percentage of the crude sample was determined by using Equation 1 according to Ramadevi (2005).

$$Yield = \frac{weight\ of\ the\ extract\ crude\ (g)}{weight\ of\ ground\ sample\ (g)} x\ 100 \qquad \text{Equation 1}$$

## 2.2 Phytochemical analysis on the extract samples

### 2.2.1 Test for alkaloid (Wagner's reagent)

The extract solution was mixed with little amount of Wagners's reagent. The formation of reddish or brown precipitate were formed indicate the presence of alkaloid.

### 2.2.2 Test for flavonoid

The crude extract was mixed with sodium hydroxide. The appearance of yellow intense coloration become colorless when added dilute hydrochloric acid indicate the presence of flavonoid.

### 2.2.3 Test for glycoside

The extract was mixed with sodium hydroxide solution. The appreance of greenish yellow color solution was confirmed the presence of glycoside.

### 2.2.4 Test of phenol

The 2 mL of extract solution was added with 0.5 mL iron (III) chloride solution. The formation of intense color indicate the presence of phenol (Parmar *et al*., 2012).

### 2.2.5 Test for saponin

The extract was dissolved with distilled water. The 2 mL of extract vigorously was shaked in test tube for 2 minutes. The absence of frothing indicate the presence of saponin (Adesegun *et al*., 2008).

### 2.2.6 Test for steroid

The crude extract was dissolved in acetic acid. A drop of concentrate sulphuric acid was added along side of the test tube. Appearance of greenish yellow color indicate the presence of steroid (Talmale *et al.,* 2014).

### 2.2.7 Test for sterol

The crude extract was dissolved in chloroform. The 2 mL of acetic anhydride mixed with 2 drops of sulphuric acid was added. The apperance of red, blue, or green color indicate the presence of sterol.

### 2.2.8 Test for tannin

The crude extract was mixed with 1 % ferric chloride solution. The formation of green black color indicate the presence of tannin (Parmar *et al*., 2012).

#### 2.2.9 Test for terpenoid (Salkowski test)

The crude extract was dissolved with chloroform. A few drops of sulphuric acid and acetic anhydride were take placed. The formation of red or violet color indicate the presence of terpenoid (Adesegun *et al.*, 2008).

### 2.3 Thin Layer Chromatography (TLC)

Each extract from three polarity of difference solvent was obtained. The separation of components are running on thin layer chromatography plates. The detection position of the spot was obtained by using ultraviolet lamp with short (254 nm) and long (366 nm) wavelength.

### 2.4 Antibacterial activity

Antibacterial assay was tested using disc diffusion method. The pathogenic bacteria was spreaded on the agar plate with the help of sterile cotton swab. Each petri plate was inoculated and fresh growth of baterial culture was collected for each bacterium by sterile streaked loop method. The well grown bacterial colony was picked from Nutrient agar (NA) media and sub-cultured in Nutrient broth (NB) media. The bacterium was incubated for 24 hour and maintained at 37°C. The test was performed on disc diffusion method for all bacterial strains. The inhibition zone was measured after incubation period that express as value in mm unit.

### 2.5 Antioxidant activity

The difference extracts of *Ziziphus mauritiana* was evaluated for 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Ascorbic acid was used as positive control. Cude extracts of *Z. mauritiana* sample solution were dissolved with methanol. Each different solvent has different concentration between 500 until 100 µg/mL was added with DPPH. Then, the absorbance was measured at 517 nm by using a Ultraviolet-Visible (UV-Vis) spectrophotometer. The percentage of DPPH scavenging effect was calculated using Equation 2 (Gupta *et al.*, 2009).

$$Inhibition\ (\%) = \frac{A_0 - A_1}{A_0}\ x\ 100 \qquad \text{Equation 2}$$

Notes:
$A_0$ : Absorbance of the control reaction
$A_1$ : Absorbance of the standard sample or plants extract

## 3. Results and discussions

### 3.1 Extraction of samples

The difference polarity of solvents chosen to extract the samples. The process of extraction started from non-polar solvent to semi-polar solvent and finally polar solvent. This order made, to ensure the compound of extraction will be retain according to their polarity.

Table 2. Result of leaves extraction

| Solvent | Dry powder leaves (g) | Mass of samples (g) | Extraction of samples (%) | Yields (%) |
|---|---|---|---|---|
| Hexane | 1125.45 | 5.22 | 99.54 | 0.46 |
| Chloroform | 900.54 | 33.47 | 96.28 | 3.71 |
| Methanol | 700.32 | 11.81 | 98.31 | 1.69 |

Table 3. Result of twigs extraction

| Solvent | Dried powder twigs (g) | Mass of crude samples (g) | Extraction of samples (%) | Yields (%) |
|---|---|---|---|---|
| Hexane | 711.18 | 3.34 | 99.53 | 0.47 |
| Chloroform | 700.62 | 5.08 | 99.27 | 0.73 |
| Methanol | 700.32 | 10.81 | 98.46 | 1.55 |

Both leaves and twigs extraction shown in Table 2 and Table 3 give better percentage extraction when hexane was used as solvent. On leaves part, the highest yield percentage is chloroform extract which 3.71 % and for twigs part the highest 1.55 % yield percentage is methanol extract. Hexane give the better extraction samples because solvent used for extraction non-polar compounds usually take the shortest time compare semi-polar and non-polar solvents. Meanwhile, yields percentage justified that on leaves part contain more semi-polar compounds. Moreover, twigs part contain more polar compounds.

### 3.2 Phytochemical screening of crude extracts

The results of phytochemical screening of *Z. mauritiana* as shown in Table 4.

Table 4. Phytochemical analysis of *Z. mauritiana*

| Phytochemical tests | Leaves | Twigs |
|---|---|---|
| Alkaloid | + | + |
| Flavanoid | + | + |
| Glycoside | + | + |
| Phenol | + | + |
| Saponin | - | + |
| Steroid | + | + |
| Sterol | + | + |
| Tannin | + | + |
| Terpenoid | + | + |

Key: + presence, - absence

Many saponin available on twigs, branches or barks of the plant compare to leaves part of the plant. This explain the absence of saponin in the twigs part of *Z. maurtiana*. The same result absence of saponin was obtained by Parmar *et al.* (2012). Investigation shows phytochemical analysis activity of *Z. mauritiana* in

twigs part significantly better compare to leaves part. Presence of secondary metabolites in the plant give indication that plant have many bioactivity activities.

### 3.3 Thin layer chromatography (TLC) of crude extracts

By using ultraviolet lamp, the position spot of the compound was detected under short (254 nm) and long (366 nm) wavelength. Frequently, UV short wavelength at 254 nm specified for natural compound which contain aromatic rings and conjugated double bonds which have unsaturated compound. In addition, plant pigment color dye stuffs on the layer in sunlight and local fluorescence compound such as riboflavin and quinines was easily detected in UV long wavelength at 366 nm (Fried and Sherma, 1999).

The combination hexane and $CHCl_3$ solvents shown that many non-polar compounds were obtained from hexane extract that make high distance compound migrate on TLC compare to methanol extract. The most solvent systems suitable to use are 3:7 and 9:1 of (hexane:$CHCl_3$). The more polar ratio of compound applied the better separation results produced.

### 3.4 Antibacterial activity

The study witness *Z. mauritiana* extracts influence competent inhibitory effects against test isolate were established broad spectrum of antibacterial activities (Goyal *et al*., 2012; Najafi, 2013; Sameera and Mandakini, 2015). Result of antibacterial activities performed in this study shown in Table 5.

Table 5. Antibacterial activity of difference *Z. mauritiana* extracts

| Diameter of inhibition zone (mm) | | | | |
|---|---|---|---|---|
| **Test organisms** | ***Bacillus subtilis*** | ***Staphylococcus aureus*** | ***Escherichia coli*** | ***Salmonella sp*** |
| Streptomycin | 15.0 | 23.0 | 21.0 | 14.0 |
| Methanol | 5.0 | 5.0 | 5.0 | 5.0 |
| LHZM | 13.0 | 15.0 | 9.0 | 8.0 |
| LCZM | 8.0 | 5.0 | 7.0 | 7.0 |
| LMZM | 9.0 | 9.0 | 8.0 | 8.0 |
| THZM | 7.0 | 8.0 | 9.0 | 7.0 |
| TCZM | 9.0 | 10.0 | 8.0 | 8.0 |
| TMZM | 11.0 | 8.0 | 10.0 | 8.0 |

Notes:
Diameter of inhibition zone including the diameter of disc (5 mm)
Diameter of disc is 5 mm.
LHZM- Leaves hexane extract of *Z. mauritiana*
LCZM- Leaves chloroform extract of *Z. mauritiana*
LMZM- Leaves methanol extract of *Z. mauritiana*
THZM- Twigs hexane extract of *Z. mauritiana*
TCMZ- Twigs chloroform extract of *Z. mauritiana*
TMZM- Twigs methanol extract of *Z. mauritiana*.

Streptomycin was used as positive control meanwhile methanol as negative control. The leaves and twigs crude extracts have antibacterial activities but only crude

extract of chloroform leaves do not has any antibacterial activity against *S. aureus*. Similar result found by Sameera and Mandakini (2015) on *Ziziphus xylopyra* there do not have bacterial inhibition activity on aqueous-dioxane crude extract on *E. coli* and *Proteus vulgaris*. The leaves crude hexane extraction shows the highest antibacterial activity when against gram positive bacteria *B. subtillis* and *S. aureus* which each diameter inhibition zone is 15 mm and 13 mm.

For twigs part of plant, the highest antibacterial activity found on methanol extract. Basically, for gram negative bacterium the highest inhibition 10 mm was obtained from twigs methanol crude extract on *E. coli.* The better antibacterial activity on gram positive bacterium compare to gram negative bacterium because presence of barrier against enzymes and antibiotic membrane in gram positive bacterium.

## 3.5 Antioxidant activity

Indicator of the radical nature reaction is based on rate reduction of a chemical when DPPH was added at 517 nm which at strong absorption band center wavelength of UV-Vis for antioxidant (Abalaka, 2011). DPPH radical scavenging activity obtained from all crude extracts of *Z. mauritiana* stated in Table 6 and Figure 1.

Table 6. DPPH radical scavenging activity (%) of different extracts of *Z. mauritiana*

| **Extract** | **Concentration of samples (µg/mL)** | | | | |
|---|---|---|---|---|---|
| | **100** | **200** | **300** | **400** | **500** |
| AA | 67.23 | 87.09 | 87.54 | 96.06 | 96.63 |
| LHZM | 43.14 | 44.40 | 45.67 | 46.38 | 49.19 |
| LCZM | 42.05 | 43.50 | 44.58 | 46.38 | 46.93 |
| LMZM | 43.32 | 46.21 | 51.35 | 51.99 | 91.43 |
| THZM | 43.23 | 45.31 | 45.49 | 47.20 | 63.72 |
| TCZM | 40.79 | 44.85 | 46. 75 | 47.92 | 48.47 |
| TMZM | 43.95 | 44.04 | 44.54 | 46.30 | 82.85 |

AA- Ascorbic acid
LHZM- Leaves hexane extract of *Z. mauritiana*
LCZM- Leaves chroloform extract of *Z. mauritiana*
LMZM- Leaves methanol extract of *Z. mauritiana*
THZM- Twigs hexane extract of *Z. mauritiana*
TCMZ- Twigs chloroform extract of *Z. mauritiana*
TMZM- Twigs methanol extract of *Z. mauritiana*.

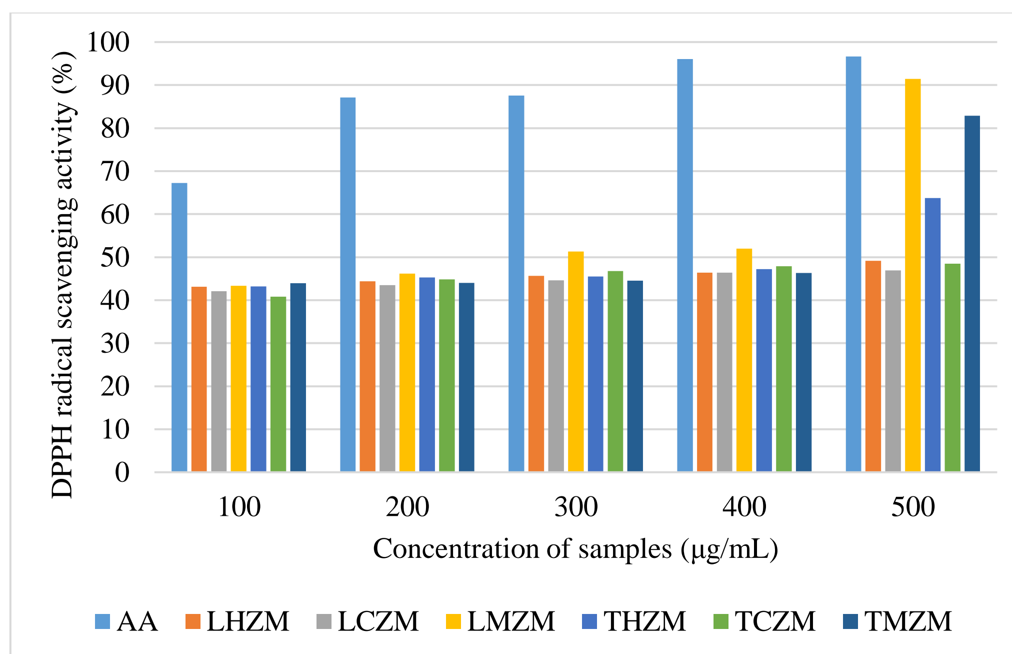


Figure 1. DPPH radical scavenging activity versus concentration of extract samples of *Z. mauritiana*

In the presence analysis, the result of hexane leaves extract of *Z. mauritiana* has 43.14 % same as discovered according to Abalaka*et al*. (2011) which is 43.09±1.05 %. Basically, as shown in Figure 1 both leaves and twigs extracts shown the increase quantity in (µg/mL) effect the increase DPPH radical scavenging activity % obtained. The increasing order of leaves and twigs antioxidant percentage as followed: methanol > hexane > chloroform. Result from twigs extract shown: methanol > hexane > chloroform was not similar in agreement with previous study by Ashraf *et al*. (2015) as the result in previous study found: methanol > chloroform > hexane. However, the result from leaves extraction similar with previous study (Heo *et al*., 2007; Bhuiyan *et al*., 2009; Ashraf *et al*., 2011) which revealed methanol extract significantly have the highest antioxidant compare to hexane and chloroform in DPPH radical scavenging activity.

## 4. Conclusions

The extraction results revealed the most efficient solvent extraction for both parts is hexane. In leaves, the highest 3.71 % yield is chloroform extract and for twigs the highest 1.55 % yield is methanol extract. Almost all secondary metabolites are found inside *Z. mauritiana* except the absence of saponin on leaves part. Fractionation of compound have a better result when ratio 7:3 and 9:1 of hexane: chloroform solvent system were used. The methanol crude extract of twigs part has the highest inhibition zone diameter which is 10 mm on gram negative *E.coli* species. The highest maximum inhibition zone diameter for gram positive was recorded from leaves methanol crude extract on *S. aureus* which is 15 mm. In addition, only leaves crude extract of chloroform do not has any antibacterial activity against *S. aureus*.The results established antioxidant activity of *Z. mauritiana* contain the highest percentage of DPPH radical

scavenging activity when methanol crude extract was used contra to when hexane and chloroform crude extracts were used.

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