

EVALUATION OF PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF WHITE MULBERRY (*Morus alba*)

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ABSTRACT

Traditional Chinese Medicine is mainly derived from medicinal herbal plant sources, which are easily obtained and cheaper than modern medicines. One such plant used as a remedy to treat various illnesses is *Morus alba*, known as white mulberry. This study aimed to screen the phytochemicals of *M. alba* and examine the potential antibacterial activity against several pathogenic bacteria. Leaves, fruits, and stems of *M. alba* were extracted using three solvents of different polarities (hexane, ethyl acetate, methanol) to screen the presence of phytochemical constituents, followed by an evaluation of their antimicrobial potential. The qualitative phytochemical tests revealed that carbohydrates, flavonoids, tannins, saponins, steroids, coumarins, alkaloids, and terpenoids were detected in the crude extracts of *M. alba*. Extracts of ethyl acetate and methanol preparations were subjected to antibacterial susceptibility test using disk diffusion method against *Bacillus* sp., *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella* sp. Interestingly, *M. alba* methanolic leaf extract showed noticeable antibacterial activity in a dose-dependent manner (concentration range 6.25–100%) against all tested Gram-positive bacteria. Data of this study provide preliminary findings on the potential use of *M. alba* leaf for the treatment of infections caused by the Gram-positive bacteria.

Key words: *Morus alba*; Moraceae, phytochemicals, antibacterial, qualitative assessment

INTRODUCTION

Traditional Chinese Medicine (TCM) utilizes natural sources and various herbs in Chinese medical practice. The white mulberry or *Morus alba* is one such TCM herbal plant, which belongs to the major flowering group of the family *Moraceae*, along with other members; *Morus nigra* (black mulberry) and *Morus rubra* (red mulberry). Species of *Morus* can be found in subtropical and temperate regions, widely cultivated in Korea and Japan (Chan *et al.*, 2016).

Most TCM herbal plants are produced in the form of crude extract containing a complex mixture of different phytochemicals. These include flavonoids, alkaloids, and phenolic acids commonly present in the *Morus* genus (Natić *et al.*, 2015; Chan

et al., 2016). Phytochemical constituents derived from *M. alba* have been extensively investigated for biological properties. For instance, flavonoids from the branches of *M. alba* have been shown to contain a high antioxidant that scavenges superoxide radicals (Hussain *et al.*, 2017), whereas polysaccharides of *M. alba* leaf improve flavonoid antioxidant activity (He *et al.*, 2018). Polyphenols, such as (E)-resveratrol and moracin M extracted from *M. alba* stem have anti-inflammatory activity (Rivière *et al.*, 2014). Moracins have also been reported to possess antifungal and antibacterial properties (Naik *et al.*, 2015). Besides that, isolation of steroid compounds of *M. alba* stem bark; albosteroid, demonstrated effective antiulcer ability by diminishing gastric ulcer lesion of experimental rats (Ahmad *et al.*, 2013). Research has also shown that the treatment of convulsion models rats with Morusin, a novel flavonoid glycoside isolated from

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M. alba was able to decrease seizures of epilepsy (Gupta *et al.*, 2014).

To date, interest in developing therapeutic agents derived from TCM plant sources has increased due to the development of microbial resistance to the current antibiotics. Considerable efforts have been made to discover potential phytochemicals and secondary metabolites of TCM plants that may have antimicrobial properties. Considering *M. alba* is one of the TCM plants easily grown in Malaysia, the potential use of this plant as an antimicrobial source is economical. Thus, this study was carried out in order to determine the phytochemical constituents of *M. alba* and evaluate its potential antibacterial properties.

MATERIALS AND METHODS

Plant materials

Fresh *M. alba* leaves, stems, and fruits were collected from Subang Jaya, Selangor. The samples were washed and shaded dry at room temperature for five days before pulverized into a powder. The plant powder was then stored in sealed containers at 4°C.

Plant extraction

Morus alba powder samples were dissolved in three different solvents with increasing polarity (hexane, ethyl acetate, and methanol) at room temperature using ultrasonic sonication technique (Elma D-78224 Singen Htw, Germany). The extracts were then concentrated using a rotary evaporator (Büchi Rotavap R-200 CH-9230, Switzerland) at 35–40°C under reduced pressure. Each extract was then weighed and stored at 4°C.

Phytochemical screening

Detection of carbohydrate (Fehling's Test)

Plant extracts (0.5 g) were dissolved in distilled water and filtered. The filtrates were hydrolyzed with hydrochloric acid (HCl) and subsequently neutralized with sodium hydroxide (NaOH). Fehling's A and B solutions were added to the mixture. The formation of brick red precipitates indicated the presence of carbohydrates.

Detection of flavonoid (Alkaline Reagent Test)

Plant extracts (0.5 g) were treated with drops of NaOH. The addition of a few drops of HCl caused decolorization of intense yellow which indicated the presence of flavonoids.

Detection of polyphenol and tannin (Ferric Chloride Test)

Ferric chloride (2–3 drops of 5% solution) were added into 0.5 g plant extracts. The presence of

phenols was shown by the formation of greenish-black or blue-green color.

Detection of saponin (Foam Test)

Plant extracts (0.5 g) were vigorously shaken with 2 mL distilled water. The formation of foam that lasted for 10 min indicated the presence of saponins.

Detection of steroid/triterpenoid (Liebermann-Burchard Test)

Chloroform was added to the plant extract along with a few drops of sulphuric acid. The mixture was shaken and left at room temperature for a few minutes. The formation of red and yellow color indicated the presence of steroids and triterpenoids, respectively.

Detection of coumarin

Plant extracts (0.5 g) were treated with 3 mL of 10% NaOH. The formation of a yellow color indicated the presence of coumarins.

Detection of alkaloid (Wagner's Test)

Wagner's reagent (2–3 drops) were added to the plant extracts. The formation of brownish or reddish precipitate was an indication of the presence of alkaloids.

Detection of protein (Biuret Test)

Plant extracts (0.5 g) were treated with 4% NaOH, followed by two drops of 1% cuprum sulphate. The presence of proteins was indicated by the formation of violet color.

Detection of anthocyanin

Plant extracts (0.5 g) were mixed with 2 mL of 2N HCl. Anthocyanins were present when the pinkish-red solution turned purplish-blue after the addition of ammonia.

Preparation of bacteria inoculum

Bacteria isolates were obtained from the Microbiology Laboratory, Faculty of Science and Marine Environment, Universiti Malaysia Terengganu. These include three Gram-Positive bacteria; *Bacillus* sp., *Staphylococcus aureus*, and *Enterococcus faecalis*, and three Gram-negatives; *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella* sp. Bacteria inoculum were prepared fresh by mixing bacteria colonies into 0.9% NaCl. Turbidity of each suspension was adjusted equivalent to 0.5 McFarland before inoculated onto Mueller-Hinton agar (MHA).

Determination of antibacterial activity

Crude extracts of *M. alba* leaf, fruit, and stem prepared in ethyl acetate and methanol were

subjected to antibacterial assay using the Kirby-Bauer disk diffusion method (Clinical and Laboratory Standards Institute *et al.*, 2019). Each extract was serially diluted (100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56% and 0.78%) and pipetted (20 μ L) onto 6 mm sterile discs. Each disc was firmly placed on MHA plates inoculated with bacteria. Experiments were carried out in triplicates for each plant extract. Standard antibiotic disc gentamicin (10 mg/mL) was used as a positive control, whereas, ethyl acetate and methanol were used as negative controls. The MHA plates were then incubated at 37°C overnight. The antibacterial activity demonstrated by *M. alba* extracts was recorded based on the diameter of clear inhibition zones on MHA plates.

Statistical analysis

Data of the bacterial growth inhibition zones were analyzed using Microsoft Excel and reported as means \pm standard deviation of the three replicates (n=3) of each plant sample.

RESULTS AND DISCUSSION

Phytochemical analysis

A series of qualitative phytochemical screening was conducted on *M. alba* extracts (leaf, fruit, and stem) to determine the presence of metabolites. In our study, overall data showed that carbohydrates, flavonoids, tannins, saponins, steroids, coumarins, alkaloids, and terpenoids were present in *M. alba* crude extracts (Table 1). Most compounds are considered as semi-polar and polar due to the presence of more phytochemicals in ethyl acetate and methanol extracts. Regardless of the type of solvent used, carbohydrates were present in all *M. alba* extracts. Carbohydrates are sugar biomolecules that act as a vital source of energy for all living

organisms. Our data are consistent with a previous report on the presence of carbohydrates in fruits and leaves of *M. alba* (He *et al.*, 2018). This study also found that flavonoids are present in both methanolic and ethyl acetate of *M. alba* extracts. Flavonoids have been suggested as the primary bioactive molecule in *M. alba* (Ramesh *et al.*, 2014; Chan *et al.*, 2016). The compound is semi or high polar due to the presence of hydroxyl (-OH) and carbonyl (C=O) groups in the structural backbone.

Polyphenols, tannins, and saponins were also detected in all parts; leaf, fruit, and stem of *M. alba*, which correlates well with previous findings (Devi *et al.*, 2013; Grajek *et al.*, 2015). Polyphenols and tannins are general antioxidants that can serve as potential anticancer (Rivière *et al.*, 2014). The presence of these compounds in *M. alba* suggests the plant as a great source of natural antioxidants. Besides that, *M. alba* was found to contain steroids, which correlates with previous data (Ahmad *et al.*, 2013; Hussain *et al.*, 2017). Furthermore, coumarins were detected in *M. alba* ethyl acetate and methanol extracts. Of note, this is the first report of detecting coumarin in *M. alba* fruits, rather than in the leaves and stems (de Oliveira *et al.*, 2015; Chan *et al.*, 2016).

Alkaloid has been reported as the main phytochemical in *M. alba* fruits (Chan *et al.*, 2016). Findings from the current study, however, detected alkaloids only in the leaves and stem of *M. alba*. It is known that several factors such as light intensity, heat, high moisture, and oxygen reduce the stability of alkaloids (El-Sakka *et al.*, 2010). We speculate improper storage of the plant samples leading to excessive moisture may have caused degradation of alkaloids in our fruit samples. Our study also detected terpenoids in methanol extract of both fruits and stems of *M. alba*, which correlates with previous reports (Grajek *et al.*, 2015; Chan *et al.*, 2016). Unfortunately, proteins and anthocyanins

Table 1. Phytochemical compounds of *M. alba* crude extracts

Phytochemical	Leaf			Fruit			Stem		
	H	EA	M	H	EA	M	H	EA	M
Carbohydrate	+	+	+	+	+	+	+	+	+
Flavonoid	-	+	+	-	+	+	-	+	+
Polyphenol and Tannin	-	+	+	-	+	+	-	+	+
Saponin	-	+	+	-	-	+	+	-	+
Steroid	+	+	+	+	+	-	+	-	-
Coumarin	-	+	+	-	+	+	-	+	+
Alkaloid	-	+	-	-	-	-	-	+	-
Terpenoid	-	-	-	-	-	+	-	+	+
Protein	-	-	-	-	-	-	-	-	-
Anthocyanin	-	-	-	-	-	-	-	-	-

+ : Detected. - : Non-detected. H: Hexane. EA: Ethyl acetate. M: Methanol.

Table 2. Antibacterial activity exhibited by the leaf, fruit, and stem of *M. alba* extracts

Bacteria	Diameter of inhibition zones (mm)									
	Positive control		Negative control		Leaf		Fruit		Stem	
	G	M	EA	M	EA	M	EA	M	EA	
<i>Bacillus</i> sp.	19.3 ± 0.16	–	–	10 ± 0.12	–	–	–	–	–	8 ± 0.14
<i>Staphylococcus aureus</i>	21.8 ± 0.08	–	–	11 ± 0.04	–	8 ± 0.07	–	8 ± 0.16	–	7 ± 0.23
<i>Enterococcus faecalis</i>	15.4 ± 0.05	–	–	12 ± 0.09	–	–	–	–	–	–
<i>Klebsiella pneumoniae</i>	18.2 ± 0.13	–	–	–	–	–	–	–	–	–
<i>Salmonella</i> sp.	16.9 ± 0.22	–	–	–	–	–	–	–	–	–
<i>Escherichia coli</i>	17.1 ± 0.30	–	–	–	–	–	–	–	–	–

G: gentamicin. M: methanol. EA: ethyl acetate. – : No antibacterial activity. Values are the means of diameter ± standard deviation.

were not detected in any of *M. alba* extracts. This could be due to the limited amount produced or they could have been degraded by physical factors such as pH and high temperatures. These factors have been shown to affect their stability (Aramwit *et al.*, 2010), thus appropriate storage of the plant extracts is vital to avoid degradation.

Antibacterial activity

The leaf, fruit, and stem of *M. alba* extract prepared in methanol and ethyl acetate, which were found high in phytochemical constituents were subsequently screened for antibacterial activity using the Kirby-Bauer disk diffusion method. Based on the results, *M. alba* leaf extracted in methanol inhibited the growth of all three Gram-positive bacteria; *Bacillus* sp., *S. aureus*, and *E. faecalis* at 100% concentration (Table 2). The largest inhibition zone was seen in *E. faecalis* (12±0.09 mm), followed by *S. aureus* (11±0.04 mm) and *Bacillus* sp. (10±0.12 mm). Further dilution of the extract down to 6.25% concentration showed an inhibition towards the bacterial growth in a concentration-dependent manner.

Findings of this study correlate well with others, demonstrating *M. alba* methanolic leaf extract can inhibit the growth of *S. aureus* and *E. faecalis* (Sheikhlar *et al.*, 2013; Cui *et al.*, 2019). Ethanolic extract of *M. alba* leaf has been previously shown to be more effective against fungi, and capable of inhibiting the growth of Gram-negative bacteria (Omidiran *et al.*, 2012; de Oliveira *et al.*, 2015). This highlights the importance of *M. alba* leaves due to the potential presence of antimicrobial components against a wide range of microorganisms. On the contrary, ethyl acetate extracts of *M. alba* leaf did not show any inhibition activity. This could be due to the organic solvent used, which produced different bioactive phytochemical constituents.

Apart from the leaf, the fruit and stem of *M. alba* have been previously investigated for antimicrobial properties with various findings. In this study, we

found that the fruit and stem methanolic extracts of *M. alba* demonstrated milder antibacterial activity than the methanolic leaf. They inhibited the growth of *S. aureus* (at 100% concentration) with a reduced diameter of inhibition zones compared to the leaf extract (Table 2). Fruit extract of *M. alba* has been reported to contain high antioxidants and possess antibacterial activity against *B. subtilis*, *S. aureus*, *E. coli*, and *Salmonella typhimurium* (Dimitrijevic *et al.*, 2014). This is also true for *M. alba* stem extract which was able to inhibit the growth of oral pathogens (Zafar *et al.*, 2013). Similar observations were obtained in this study with *M. alba* stem prepared in ethyl acetate, which was shown to be effective towards inhibiting the growth of *Bacillus* sp. and *S. aureus* at 100% concentration.

Environmental factors such as temperature, humidity, and the location of *M. alba* cultivations may contribute to the quality of *M. alba* leaves, hence the presence of bioactive constituents (Hao *et al.*, 2018). Besides, the extraction method and polarity of solvents used are equally important. The different polarity of solvents results in the extraction of different types of phytochemical constituents with varying amount and level of stability. Of note, the test bacteria used in this study were those commonly associated with various forms of human infections. Thus, the demonstration of antibacterial activity of *M. alba* particularly towards the Gram-positive bacteria suggests that the plant serves as a potential source of bioactive substances, useful for the development of therapeutic agents against pathogenic bacteria.

CONCLUSION

Several phytochemical constituents including carbohydrates, flavonoids, tannins, saponins, steroids, coumarins, alkaloids, and terpenoids were detected in methanol and ethyl acetate extracts of *M. alba*. The methanolic extract of *M. alba*,

particularly in the leaf has demonstrated a noticeable antibacterial activity towards the Gram-positive bacteria in a dose-dependent manner. This finding suggests that *M. alba* leaf possesses a substantial level of bioactive components that play a role in inhibiting the growth of Gram-positive bacteria. Further investigation involving isolation and identification of the extract components is essential for the development of a potential therapeutic agent against Gram-positive bacterial infection.

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