



Characterization of Antimicrobial, Antioxidant, Anticancer Property and Chemical Composition of *Michelia champaca* Seed and Flower Extracts

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ABSTRACT

This study was carried out to characterize antimicrobial, antioxidant and anticancer activities of *Michelia champaca* seed and flower extracts. The main objective of the present study was to reveal the medicinal values of *M. champaca* seed and flower for human uses. Antimicrobial property of *M. champaca* seed and flower extracts were revealed by using two fold microdilution method whereas antioxidant activity of the extract was determined with DPPH radical scavenging method. The anticancer property of the plant extract was revealed through Colorimetric MTT (tetrazolium) assay. The minimum inhibitory concentration values of *M. champaca* seed and flower extracts ranged from 15.6 to 125mg/l and 7.8 to 62.5mg/l, respectively in which both of the plant extracts were found can inhibit the growth of all the tested bacterial isolates namely *A. hydrophila*, *E. tarda*, *E. coli*, *Flavobacterium* sp., *Klebsiella* sp., *P. aeruginosa*, *Salmonella* sp., *V. alginolyticus*, *V. cholerae* and *V. parahaemolyticus*. *M. champaca* flower extract was able to control the growth of *E. tarda*, *E. coli*, *Flavobacterium* sp., *P. aeruginosa* and *V. cholerae* at the concentration of 7.8mg/l whereas *A. hydrophila*, *Klebsiella* sp. and *V. alginolyticus* were failed to grow at the concentration 15.6mg/l. The *M. champaca* flower extract was also able to control the growth of *Salmonella* sp. and *V. parahaemolyticus* at the concentration of 62.5mg/l. At the maximum concentration of *M. champaca* seed and flower extracts were found can inhibit only 40% of DPPH whereas the IC₅₀ value of *M. champaca* seed and flower extract against MCF-7 cells was 1.98 ± 0.31µg/ml and 1.86 ± 0.21µg/ml, respectively. A total of 9 chemical compounds were successfully identified in *M. champaca*'s flower extract whereas 37 chemical compounds were found in the leaf extract. The findings of the present study indicated that medicinal values of *M. champaca* seed & flower extracts in terms of antimicrobial and anticancer are promising.

Key words: Antioxidant, Anticancer, Antimicrobial, Chemical compound, *Michelia champaca*

INTRODUCTION

Michelia champaca is a member of family Magnoliaceae. It is well known and widely used in traditional medicine such as fever, colic, leprosy, post partum protection (Perry, 1980), eye disorder and many more. This plant was

claimed possesses various pharmacological properties such as antipyretic, anti-inflammatory (Vimala *et al.*, 1997), insecticidal, antimicrobial and etc. Furthermore, Atjanasuppat *et al.* (2009) reported that this plant can be as remedy of antiuretic, carminative and antidiabetic. Several compounds of this plant were also characterized and identified such as alkaloids, saponins, tannins, sterols, flavonoids and triterpenoids in the study of Khan *et al.* (2002). The increasing of incidence of antibiotic resistance case among pathogenic bacteria lead to the most of commercial antibiotics were no longer effectively in controlling bacteria

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diseases. Subsequently, it is a must to find the alternative antimicrobial agent especially from plants. As oxidative stress may lead to many human diseases, the use of antioxidants in pharmacology is intensively studied, particular as treatment for stroke and neurodegenerative diseases. Apart of oxidative stress, cancer is recognized as the new emerged human disease that had killed a lot of people. Thus, this study was carried out to reveal the potential of *Michelia champaca* to be used as a natural health food supplement. Till present, the information of medicinal values of *M. champaca* is still lacking in the literature. Therefore, this study was carried out to enrich the information of the medicinal property of *M. champaca* in terms of antimicrobial, antioxidant and anticancer as well as its chemical composition in the literature.

MATERIALS AND METHODS

Plant material

The plant sample was purchased from herbal nursery located at Pasir Puteh, Kelantan, Malaysia. The fresh plant sample was oven dried at 37 °C for 4 days. Next, the plant sample was freeze dried prior to extraction using 70% methanol and concentrated at 1 g/ml. Finally, the plant extraction was kept in -20°C until further use.

Bacteria isolates

All bacterial isolates were provided by Universiti Malaysia Kelantan namely *Aeromonas hydrophila*, *Escherichia coli*, *Edwardsiella tarda*, *Flavobacterium* spp., *Klebsiella pneumonia*, *Salmonella typhi*, *Vibrio alginolyticus*, *V. parahaemolyticus*, *V. cholerae* and *Pseudomonas aeruginosa*. These bacteria were isolated from various aquatic animals and kept in tryptic soy agar (TSA) for further uses.

Minimum inhibitory concentration (MIC) determination

The values of minimum inhibitory concentration (MIC) of *M. champaca* seed and flower extracts against bacterial isolates were determined through a two-fold broth micro dilution method

(Lee and Najiah, 2008; Lee *et al.*, 2009). The bacterial isolates were cultured in tryptic soy broth for 24 h at room temperature and the concentration of these cultures were adjusted to 10⁹ CFU mL⁻¹ by using physiological saline. The concentration was cross check with a Biophotometer (Eppendorf, Germany). The bacterial suspensions were then inoculated into a microtiter plate that contained a serial dilution of *M. champaca* seed and flower extracts. The microplate was then incubated at room temperature for 24 h. The MIC values were defined as the lowest concentration of the *M. champaca* seed and flower extracts in the wells of the microtiter plate that showed no visible turbidity after 24 h incubation.

Determination of antioxidant activity with α , α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging method

DPPH radical scavenging method was conducted as described by Blois (1958), Yen and Duh (1994), Brand-William *et al.* (1995) and Gadow *et al.* (1997) with some modifications. The assay was carried in a 96 wells elisa plate with three replicates. 5 μ l of the sample (0.5 mg/ml) solution was added into the well followed by 200 μ l DPPH. The absorbance of the sample was recorded by using ELISA reader for ever interval 6 s. The percentage inhibition of DPPH radical was calculated based on the absorbance.

Cancer cell lines

The human breast adenocarcinoma (MCF-7) cell line was derived from Institute of Marine Biotechnology, Universiti Malaysia Terengganu. All the cells were grown in standard cell medium (RPMI 1640) supplemented with 5% fetal bovine serum in a 5% CO₂ atmosphere. The cells was then transferred into microplate at the concentration of 1 X 10² cells per well for cytotoxicity test of the plant extract. At 48 h, proliferation was measured by the MTT colorimetric assay. The IC₅₀ value was calculated from the following formula as described Adebayo *et al.* (2010).

Table 1. The sensitivity of bacterial isolates against *Michelia champaca* seed and flower extracts.

Bacterial isolates	Minimum Inhibitory Concentration (MIC) (mg/l)	
	<i>Michelia champaca</i> seed	<i>Michelia champaca</i> flower
<i>Aeromonas hydrophila</i>	31.3	15.6
<i>Edwardsiella tarda</i>	15.6	7.8
<i>Escherichia coli</i>	15.6	7.8
<i>Flavobacterium</i> sp.	15.6	7.8
<i>Klebsiella</i> sp.	31.3	15.6
<i>Pseudomonas aeruginosa</i>	15.6	7.8
<i>Salmonella</i> sp.	125.0	62.5
<i>Vibrio alginolyticus</i>	31.3	15.6
<i>Vibrio cholerae</i>	15.6	7.8
<i>Vibrio parahaemolyticus</i>	125.0	62.5

$$\log_{10}(IC_{50}) = \frac{\log_{10} C_L (I_H - 50) + \log_{10} C_H (50 - I_L)}{I_H - I_L}$$

$$IC_{50} = 10^{\log_{10}(IC_{50})}$$

Where:

I_H: I% above 50%

I_L: I% below 50%

C_H: High drug concentration

C_L: Low drug concentration

Colorimetric MTT (tetrazolium) assay

Colorimetric MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma, USA) assay was carried out as described by Mosmann (1983). 10 µl of MTT solution (5mg/ml) was added to all wells of 96 wells micro plate followed by 4h incubation at 37°C. Acid isopropanol was added to all wells for dissolving the dark blue crystals. The microplate was then read on an ELISA reader at wavelength 570nm within 1h after adding isopropanol.

Bioactive compound characterization

The chromatographic procedure was carried out using a Shimadzu QP2010-GC-MS with auto-sampler. The sample was diluted 25 times with

Table 2. IC₅₀ value of *M. champaca* seed and flower extract against MCF-7 cells.

<i>Michelia champaca</i>	IC ₅₀ (µg/ml)
Seed	1.98 ± 0.31
Flower	1.86 ± 0.21

acetone and 1 µl (1 µg/ml) of sample was injected into a column. A fused silica capillary column HP5-MS (30m x 0.32mm, film thickness 0.25 µm) was used. Helium was the carrier gas, and a split ratio of 1/100 was used. The oven temperature used was maintained at 60°C for 8 min. The temperature was then gradually raised at a rate of 3°C per min to 180°C and maintained at 180°C for 5 min. The temperature at the injection port was 250°C. The components of the test solution were identified by comparing the spectra with those of known compounds stored in internal library as described by Lee *et al.* (2009). The identification was accomplished using computer searches on a internal data library. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the basis of its mass-spectral fragmentation. If available, references compound were co-chromatographed to confirm GC retention times (Bankova *et al.*, 2002).

RESULT AND DISCUSSION

The MIC values of *M. champaca* seed and flower extracts ranged from 15.6 to 125 mg/l and 7.8 to 62.5 mg/l, respectively (Table 1). *E. tarda*, *E. coli*, *Flavobacterium* sp., *P. aeruginosa* and *V. cholerae* were failed to grow at the concentration of 15.6mg/l of *M. champaca* extract. At the concentration of 31.3mg/l of *M. champaca* seed extract was found can inhibit the growth of *A. hydrophila*, *Klebsiella* sp. and *V. alginolyticus* whereas at the concentration of 125mg/l of *M.*

Table 3. Compound composition of *Michelia champaca*'s flower.

Compound	Compound Composition (%)
9,12-Octadecadienoic acid, methyl ester, (E,E)-	39.55
2-Propanone, 1-phenoxy	25.50
Benzofuran, 2,3-dihydro-	9.89
5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	7.05
Butanoic acid, 2-methyl-3-oxo-, ethyl ester	4.10
7-Oxabicyclo[4.1.0]heptanes, 1-methyl-4-(2-methyloxiranyl)-	3.69
7-Oxabicyclo[4.1.0] heptanes, 1-methyl-4-(2-methyloxiranyl)-	2.95
Oleic acid	2.79
Camphorsulfonic acid	1.98
3 unidentified compounds	2.50
Total	100

champaca seed extract was able to control the growth of *V. parahaemolyticus*. *M. champaca* flower extract was able to control the growth of *E. tarda*, *E. coli*, *Flavobacterium* sp., *P. aeruginosa* and *V. cholerae* at the concentration of 7.8mg/l whereas *A. hydrophila*, *Klebsiella* sp. and *V. alginolyticus* were failed to grow at the concentration 15.6mg/l. The *M. champaca* flower extract was also able to control the growth of *Salmonella* sp. and *V. parahaemolyticus* at the concentration of 62.5mg/l. At the maximum concentration of *M. champaca* seed and flower extracts were found can inhibit only 40% of DPPH whereas the IC₅₀ value of *M. champaca* seed and flower extract against MCF-7 cells was 1.98 ± 0.31µg/ml and 1.86 ± 0.21µg/ml, respectively (Table 2). A total of 9 chemical compounds were successfully identified in *M. champaca*'s flower extract in which 9,12-Octadecadienoic acid, methyl ester, (E,E)- 39.55% and 2-Propanone, 1-phenoxy 25.50% were the major compounds (Table 3). This was followed by Benzofuran, 2,3-dihydro- 9.89%, 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)- 7.05%, Butanoic acid, 2-methyl-3-oxo-, ethyl ester 4.10%, 7-Oxabicyclo[4.1.0]heptanes, 1-methyl-4-(2-methyloxiranyl)- 2.95%, Oleic acid 2.79%, Camphorsulfonic acid 1.98% and another 3 unidentified compounds 2.50%. A total of 37 chemical compounds were successfully identified in the *M. champaca*'s leaf extract (Table 4). They were Naphthalenemethanol 7.70%, Acetic acid 6.68%, 2, 3-Butanediol 6.46%, Phenol 5.34%, Andrographolide 4.56%, 5-Dodecyne 3.61%, Pimaric acid 3.08%, Ortho-Formylphenoxyacetic acid 2.80%, Succinamic

acid 2.78%, Benzoic acid 2.66%, Methyl β-d-galactopyranoside 2.45%, 2-Propanone, 1-hydroxy- 1.93%, Trimethoxyvinylsilane 1.81%, Androstane-3, 17-diol, 17-methyl- 1.59%, Cyclooctane, (methoxymethoxy) 1.52%, 1, 2-Benzenediol 1.44%, Propane, 1-bromo-2-methyl- 1.34%, Octadecatrienoic acid 1.19%, 1-Pentyl-hexobarbital 1.13%, Hydroquinone 1.12%, 1-Alanine, N-isobutoxycarbonyl-, butyl ester 1.01%, Benzofuran, 2, 3-dihydro 0.96%, Phenol 0.91%, (E)-3(10)-Caren-4-ol 0.91%, 1-Alanine, N-allyloxycarbonyl-, undec-10-enyl ester 0.83%, Heptenoic acid 0.73%, Imidazole-5-pentanoic acid 0.70%, Bisnor-7-desoxycholic acid 0.68%, 2, 5-Dimethoxy-4-(methylsulfonyl) amphetamine 0.67%, Valeric acid 0.65%, 2-Furanmethanol 0.64%, 5-Eicosyne 0.63%, Tromethamine 0.55%, 2-Cyclopenten-1-one, 2-hydroxy- 0.54%, Glycerin 0.50%, 1-Pentyl-hexobarbital 0.50%, Pholedrine 0.47% and 11 unidentified compounds 22.97%.

In the present study, both of the plant extracts were found successfully inhibited the growth of all the tested bacterial isolates. This was supported by the study of Khan *et al.* (2002) in which they found that leaves and seeds extracts of *M. champaca* showed inhibitory activity against Gram positive, negative bacteria and fungi as well as a protozoan, *Trichomonas vaginalis*. Furthermore, Octadecadienoic acid, Butanoic acid, Oleic acid, Camphorsulfonic acid, Acetic acid and Pimaric acid in the plant extracts which were responsible to the antimicrobial activity of the plant extracts. Thus, we may conclude that the antimicrobial property of *M.*

Table 4. Compound composition of *Michelia champaca*'s leaf.

Compound	Compound Composition (%)
Naphthalenemethanol	7.70
Acetic acid	6.68
2, 3-Butanediol	6.46
Phenol	6.25
Andrographolide	4.56
5-Dodecyne	3.61
Pimaric acid	3.08
Ortho-Formylphenoxyacetic acid	2.80
Succinamic acid	2.78
Benzoic acid	2.66
Methyl β -d-galactopyranoside	2.45
2-Propanone, 1-hydroxy-	1.93
Trimethoxyvinylsilane	1.81
Androstane-3, 17-diol, 17-methyl-	1.59
Cyclooctane, (methoxymethoxy)	1.52
1, 2-Benzenediol	1.44
Propane, 1-bromo-2-methyl-	1.34
Octadecatrienoic acid	1.19
1-Pentyl-hexobarbital	1.13
Hydroquinone	1.12
1-Alanine, N-isobutoxycarbonyl-, butyl ester	1.01
Benzofuran, 2, 3-dihydro	0.96
(E)-3(10)-Caren-4-ol	0.91
1-Alanine, N-allyloxycarbonyl-, undec-10-enyl ester	0.83
Heptenoic acid	0.73
Imidazole-5-pentanoic acid	0.70
Bisnor-7-desoxycholic acid	0.68
2, 5-Dimethoxy-4-(methylsulfonyl)amphetamine	0.67
Valeric acid	0.65
2-Furanmethanol	0.64
5-Eicosyne	0.63
Tromethamine	0.55
2-Cyclopenten-1-one, 2-hydroxy-	0.54
Glycerin	0.50
1-Pentyl-hexobarbital	0.50
Pholedrine	0.47
11 unidentified compounds	22.97
Total	100.00

champaca was undoubtedly. Although, there are studies revealed the antimicrobial property of *M. champaca* but they do not further the study on the MIC values of the plant extracts. Therefore, the present study provided the MIC values of the plant extracts against the tested bacterial isolates that may be useful for the *in vivo* test in the near future.

In so far, there is only one study involved in the work of antioxidant activity of *M. champaca* in the literature where Hossain *et al.* (2009) claimed that *M. champaca* leaf extract possesses antioxidant activity against DPPH. In addition, in the present study found that *M. champaca* seed and flower extracts have antioxidant activity against DPPH as well. Hence, we may conclude that *M. champaca* leaf, seed and flower can be used as an antioxidant agent for medicinal uses. Furthermore, this was supported by the finding of the present study in which Octadecadienoic acid, Butanoic acid, Oleic acid, Camphorsulfonic acid, Acetic acid, Pimaric acid, Phenol and Benzoic acid were found in the plant extracts that responsible to the antioxidant activity of the plant extracts.

From the literature survey, no much works were done on the anticancer activity of *M. champaca*. For instance, only the study of Atjanasuppata *et al.* (2009) reported *M. champaca* leaf extract showed the inhibitory activity against C32 cells but failed to response to Hela cells. Therefore, this is the first report on the positive response of *M. champaca* flower and seed extracts to MCF-7 cells. Furthermore, several compounds such as Octadecadienoic acid, Butanoic acid, Oleic acid, Camphorsulfonic acid, Acetic acid, Pimaric acid, Phenol and Benzoic acid that responsible to the anticancer activity were found in the plant extracts. Therefore, the findings of the present study convince us that *M. champaca* possesses anticancer property.

In conclusion, the findings of the present study and the information from the literature survey, we may conclude that *M. champaca* possesses high medicinal values. However, further study still need to be carried out in terms of *in vivo* test before it was commercialized for public uses.

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