

**CHARACTERIZATION OF ANTIMICROBIAL, ANTIOXIDANT, ANTICANCER  
PROPERTIES AND CHEMICAL COMPOSITION OF MALAYSIAN  
ANDROGRAPHIS PANICULATA LEAF EXTRACT**

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**Summary**

This study was carried out to characterize antimicrobial and antioxidant activities of Malaysian *Andrographis paniculata* leaf extract. The main objective of the present study is to reveal the potential of *A. paniculata* leaf to be used as antimicrobial and antioxidant agent for aquaculture feed use. Antimicrobial property of *A. paniculata* leaf extract against *Aeromonas hydrophila*, *Escherichia coli*, *Edwardsiella tarda*, *Flavobacterium* sp., *Klebsiella* sp., *Salmonella* sp., *Vibrio alginolyticus*, *V. parahaemolyticus*, *V. cholerae* and *Pseudomonas aeruginosa*. was revealed by using broth micro-dilution method whereas anticancer activity of the extract was determined with Colorimetric MTT (tetrazolium) assay against human breast adenocarcinoma (MCF-7). Compounds of the plant extract were screening and identified by using gas chromatography-mass spectrometry (GC-MS). Antioxidant activity of the plant extract was also characterized by using  $\alpha$ ,  $\alpha$ -diphenyl- $\beta$ -picrylhydrazyl (DPPH) radical scavenging method. The result of the present study showed that *A. paniculata* leaf extract possesses antimicrobial, antioxidant and anticancer activities. The minimum inhibitory concentration (MIC) values were ranged from 31.25 to 125 mg/l in which the plant extract was found can inhibit the growth of *Edwardsiella tarda* *Escherichia coli*, *Flavobacterium* sp., *Pseudomonas aeruginosa* and *Vibrio cholerae* at 31.25 mg/l, *Aeromonas hydrophila*, *Klebsiella* sp, *Salmonella* sp. and *Vibrio alginolyticus* at 62.5 mg/l and it was able to control the growth of and *Vibrio parahaemolyticus* at 125 mg/l. The plant extract's antioxidant activity was recorded almost same like Quercetin at the concentration of 10 ppt whereas the value of IC<sub>50</sub> of the plant extract against MCF-7 cell was 20.72  $\pm$  1.10  $\mu$ g/ml. A total of 16 chemical compound were identified (n-hexadecanoic acid, 7.65 %, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, 6.58 %,  $\beta$ -sitosterol, 6.35 %,  $\alpha$ -D-Glucopyranoside, methyl, 5.12 %, 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- 5.05 %, 2H-1-Benzopyran-6-ol,3,4-dihydro-2,8-dimehtyl-2-(4,8,12-trimethyltridecyl)- 4.20 %, Phytol, 4.17 %, Pentadecanoic acid, 14-methyl-, methyl ester, 3.47 %, Methyl 2-O-benzyl- $\beta$ -D-xylopyranoside, 2.68 %, 9, 12-Octadecadienoic acid (Z, Z)-, 2.61 %, 3-phenylpropanoic acid, 4-hexadecyl ester, 2.48 %, Heneicosanoic acid, 9,12-Octadecadienoic acid, methyl ester, (E,E)-, 1.72 %, Oleic acid, 1.72 %, Cholest-5-en-7-one, 3-fluoro-, (3  $\beta$ )-, 1.61 %, Glycerin, 1.27 % and 4H-Chromene-3-carbonitrile, 2-amino-7-benzyloxy-4-cyclohex-3-enyl-, 1.10 %). The findings of this study indicating that methanol extract of *A. paniculata* leaf extract possess high antimicrobial and antioxidant properties.

**Keywords:** antioxidant, anticancer, antimicrobial, chemical compound, *Andrographis paniculata*

## Introduction

*Andrographis paniculata* is a popular herb and commonly known as king of bitter. As a member of family *Acanthaceae* this plant can be found abundant in many Asia countries including Malaysia (1). It is well known and traditionally used in anti-cold, anti-hepatotoxic, anti-urothelial, fever, herpes and sore throat. It was reported can be used in the treatment of various chronic and infectious diseases (1). There are several studies were conducted to reveal the medicinal property of *A. paniculata* extract. For instance, *A. paniculata* was found can be as insect repellent (2), possess antioxidant activity for diabetes treatment (3), in relief of rheumatoid arthritis symptoms (4) and in controlling *Streptococcus agalactiae* infection in Nile tilapia (5). It was also reported possess anti-malarial (6), antiviral (7), antihepatotoxic (8), anti-inflammatory (9), antivenom (10), antileishmanial (11), Anti HIV (12), antidiarrhoeal (13), antifertility (14), antifilaricidal, and antimalarial (15). Therefore, in the present study, antimicrobial, antioxidant, anticancer activities of Malaysian *A. paniculata* leaf extract were studied to reveal the potential of this plant to be used in medicinal. The chemical composition in the plant extract was also screened to characterize bioactive compound in the plant extract.

## 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.

### Bacterial isolates

All bacterial isolates were provided by Universiti Malaysia Kelantan namely *Aeromonas hydrophila*, *Escherichia coli*, *Edwardsiella tarda*, *Flavobacterium* sp., *Klebsiella* sp., *Salmonella* sp., *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 *Andrographis paniculata* leaf extract against bacterial isolates were determined through a two-fold broth micro dilution method (16; 17). The bacterial isolates were cultured in tryptic soy broth for 24 h at room temperature and the concentration of these cultures were adjusted to  $10^9$  CFU mL<sup>-1</sup> by using physiological saline. The concentration was cross checked with a Biophotometer (Eppendorf, Germany). The bacterial suspensions were then inoculated into a microtiter plate that contained a serial dilution of *A. paniculata* leaf extract and positive control. The microplate was then incubated at room temperature for 24 h. The MIC values were defined as the lowest concentration of the *A. paniculata* leaf extract and positive control in the wells of the microtiter plate that showed no visible turbidity after 24 h incubation.

### Determination of antioxidant activity with $\alpha$ , $\alpha$ -diphenyl- $\beta$ -picrylhydrazyl (DPPH) radical scavenging method

DPPH radical scavenging method was conducted as described by Blois (1958) (18), Yen and Duh (1994) (19), Brand-William et al. (1995) (20) and Gadov et al. (1997) (21) with some modifications. The assay was carried in a 96 wells elisa plate with three replicates. 5  $\mu$ l of the sample (0.5 mg/ml) solution was added into the well followed by 200  $\mu$ 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 % CO<sub>2</sub> atmosphere. The cells was then transferred into microplate at the concentration of 1 X 10<sup>2</sup> cells per well for cytotoxicity test of the plant extract. At 48 h, proliferation was measured by the MTT colorimetric assay. The IC<sub>50</sub> value was calculated from the following formula as described Adebayo et al. (2010) (22):

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

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

Where:

I<sub>H</sub> : I% above 50%

I<sub>L</sub> : I% below 50%

C<sub>H</sub> : High drug concentration

C<sub>L</sub> : 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) (23). 10  $\mu$ l of MTT solution (5 mg/ml) was added to all wells of 96 wells micro plate followed by 4 h incubation at 37 °C. Acid isopropanol was added to all wells for dissolving the dark blue crystals. The microplate plate was then read on an ELISA reader at wavelength 570 nm within 1 h after adding isopropanol.

### Bioactive compound characterization

The chromatographic procedure was carried out using a Shimadzu QP2010-GC-MS with autosampler. The sample was diluted 25 times with acetone and 1  $\mu$ l of sample was injected into a column. A fused silica capillary column HP5-MS (30 m x 0.32 mm, film thickness 0.25  $\mu$ m) 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.

## Results

The result of the present study showed that *A. paniculata* leaf extract possesses antimicrobial, antioxidant and anticancer activities. The MIC values were ranged from 31.25 to 125 mg/l in which the plant extract was found can inhibit the growth of *Edwardsiella tarda*, *Escherichia coli*, *Flavobacterium* sp., *Pseudomonas aeruginosa* and *Vibrio cholerae* at 31.25 mg/l, *Aeromonas hydrophila*, *Klebsiella* sp., *Salmonella* sp. and *Vibrio alginolyticus* at 62.5 mg/l and it was able to control the growth of and *Vibrio parahaemolyticus* at 125 mg/l (Table 1). The value of IC<sub>50</sub> of the plant extract against DPPH and MCF-7 cell was 1.12 ± 0.93 ppt and 20.72± 1.10 µg/ml, respectively. The major compounds of the plant extract was n-hexadecanoic acid (7.65 %), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (6.58 %), β-sitosterol (6.35 %) (Table 2). This was followed by α-D-Glucopyranoside, methyl (5.12 %), 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (5.05 %), 2H-1-Benzopyran-6-ol,3,4-dihydro-2,8-dimehtyl-2-(4,8,12-trimethyltridecyl)- (4.20 %), Phytol (4.17 %), Pentadecanoic acid, 14-methyl-, methyl ester (3.47 %), Methyl 2-O-benzyl-β-D-xylopyranoside (2.68 %), 9, 12-Octadecadienoic acid (Z, Z)- (2.61 %), 3-phenylpropanoic acid, 4-hexadecyl ester (2.48 %), Heneicosanoic acid, 9,12-Octadecadienoic acid, methyl ester, (E,E)- (1.72 %), Oleic acid (1.72 %), Cholest-5-en-7-one, 3-fluoro-, (3 β)- (1.61 %), Glycerin (1.27 %), 4H-Chromene-3-carbonitrile, 2-amino-7-benzyloxy-4-cyclohex-3-enyl- (1.10 %). However, a total of 7 chemical compounds in which consists of 39.79 % of the total chemical compounds in the plant extract cannot be identified.

**Table 1. Minimum inhibition concentration (MIC) of *Andrographis paniculata* leaf extract against bacterial isolates**

Bacterial isolates	MIC (mg/l)
<i>Aeromonas hydrophila</i>	62.5
<i>Edwardsiella tarda</i>	31.25
<i>Escherichia coli</i>	31.25
<i>Flavobacterium</i> sp.	31.25
<i>Klebsiella</i> sp.	62.5
<i>Pseudomonas aeruginosa</i>	31.25
<i>Salmonella</i> sp.	62.5
<i>Vibrio alginolyticus</i>	62.5
<i>Vibrio cholerae</i>	31.25
<i>Vibrio parahaemolyticus</i>	125

**Table 2. Compound composition of *Andrographis paniculata* leaf extract**

Compound	Compound Composition (%)
n-hexadecanoic acid	7.65
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	6.58
β-sitosterol	6.35
α-D-Glucopyranoside, methyl	5.12
9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	5.05
2H-1-Benzopyran-6-ol,3,4-dihydro-2,8-dimehtyl-2-(4,8,12-trimethyltridecyl)-	4.20
Phytol	4.17
Pentadecanoic acid, 14-methyl-, methyl ester	3.47
Methyl 2-O-benzyl-β-D-xylopyranoside	2.68
9, 12-Octadecadienoic acid (Z, Z)-	2.61
3-phenylpropanoic acid, 4-hexadecyl ester	2.48
Heneicosanoic acid	2.18
9,12-Octadecadienoic acid, methyl ester, (E,E)-	1.97

Oleic acid	1.72
Cholest-5-en-7-one, 3-fluoro-, (3 $\beta$ )-	1.61
Glycerin	1.27
4H-Chromene-3-carbonitrile, 2-amino-7-benzyloxy-4-cyclohex-3-enyl-	1.10
Unidentified compounds	39.79
Total	100

### Discussion

This study was carried out to document the antimicrobial, antioxidant and anticancer activities of Malaysian *A. paniculata* leaf extract and its chemical composition. Malaysian *A. paniculata* leaf extract showed positive results to all tests in the present study as described in the literature reviews. In the present study, the plant extract was able to show inhibitory activity against all the tested bacterial isolates. Similar finding was also reported in the study of Leelarasamee et al. (1990) (24) in which they found *A. paniculata* extract can inhibit the growth of *Salmonella*, *Shigella*, *E. coli*, *Streptococci* and *Staphylococcus aureus*. Mishra et al. (2009) (25) also revealed that *A. paniculata* extract exhibit inhibitory activity against both Gram positive and negative bacterial isolates. Furthermore, the chemical compound that found in the plant extract of the present study such as hexadecanoic acid, Octadecatrienoic acid, Pentadecanoic acid, Heneicosanoic acid and Oleic acid may responsible to the antimicrobial property of *A. paniculata* extract. Therefore, the antimicrobial property of *A. paniculata* is undoubtedly.

The antioxidant activity of *A. paniculata* was well documented in the literature. For instance, Lin et al. (2009) (26) claimed that *A. paniculata* extract can inhibit xanthine oxidase, a free radical scavenging. Furthermore, Zhang and Tan (2000) (27) proved the antioxidant property of *A. paniculata* by using normal and diabetic rats. In addition, the finding of the present study showed *A. paniculata* extract possesses several compounds such as hexadecanoic acid, Octadecatrienoic acid, Pentadecanoic acid, Heneicosanoic acid, Oleic acid,  $\beta$ -sitosterol and Phytol that may responsible to the antioxidant activity of the plant extract. Thus, the antioxidant property of *A. paniculata* cannot be question.

From the literature survey, the studies of anticancer property of *A. paniculata* have been carried out extensively. This plant was found can inhibit the growth of various cancer cells such as HCT-116, colon cancer cell (Jada et al., 2007) (28) and leukemia cell (29). Similar finding was reported by Matsuda et al. (1994) (29) in which they also found that *A. paniculata* exhibited inhibitory activity against MCF – 7, breast cancer cell as described in the present study. Furthermore,  $\beta$ -sitosterol and Phytol were found in the present plant extract that may responsible to the anticancer activity. Further study on clinical test should be carried out in the near future, before this plant can come to a commercial sense in cancer treatment.

There is a different in chemical composition between Malaysian *A. paniculata* leaf extract and other studies. In the other study found that Andrographolide is the major diterpenoid compound in *A. paniculata* (30; 31; 32) and the other compounds are deoxyandrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographide, isoandrographolide, 5-hydroxy-7,8-dimethoxyflavone, 5-hydroxy-7,8,2',5'-tetramethoxyflavone, 5-hydroxy-7,8,2',3'-tetramethoxyflavone, 5-hydroxy-7,8,2'-trimethoxyflavone, 7-*O*-methylwogonin and 2'-methyl ether. However, all the compounds that can be found in the plant extract of the present study were not mentioned in the other studies and vice versa. This may due to different approach that applied in this study compared to the others in which the chemical compounds of the plant extract in the present study were

screening using GC-MS whereas the other studies applied high-performance liquid chromatography (HPLC) and proton nuclear magnetic resonance (HNMR) to characterize compound in the plant extract.

In conclusion, although different of chemical compounds were reported in the present study compared to the other studies, however, Malaysian *A. paniculata* possesses similar biological property such as antimicrobial, antioxidant and anticancer compared to the other studies.

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