Evaluation of total phenolic compound and cytotoxic activity of  
Murraya paniculata

1Bovornvattanangkul, T. and 2Jiraungkoorskul, W.

1Mahidol University International College, Mahidol University, Salaya Campus, Nakhon Pathom 73170, Thailand
2Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

Abstract

_Murraya paniculata_ leaf aqueous extractions in 0.5, 1, 3, 5 and 24 hours were determined the highest amount of total phenolic compound and used for evaluating the cytotoxicity test against _Artemia salina_ at varied concentrations as 0, 5, 50, 100, 500, 2,500 and 5,000 ppm, by determining the median and 90% lethal concentration, LC$_{50}$ and LC$_{90}$, respectively, within 24 hours. The result revealed that the total phenolic compound measurements in each time extraction were 134.71±3.46, 136.08±7.47, 124.86±10.61, 146.66±9.01 and 129.65±3.53 mg of gallic acid equivalent per g of extract, respectively. Due to the highest amount of total phenolic compound, the 5-hour aqueous extract of _M. paniculata_ leaf expressed the 24-h LC$_{50}$ and LC$_{90}$ values in _A. salina_ were 2,572.03 and 4,565.79 ppm, respectively.

Introduction

The widespread of traditional medicines to relieve symptoms of diseases have increased significantly in the past decades (Bent, 2008). Medicinal plants are widely studied around the world for their contribution to health care because of their pharmacological activities and antioxidant properties (Gurib-Fakim, 2006). Natural antioxidants in medicinal plants have many advantages over synthetic antioxidants in chemical medicines due to the fact that they are safer, natural, and more affordable (Fawzi Mahomoodally, 2013). Antioxidants are important because it can prevent the destructive processes caused by oxidative stress (Lobo et al., 2010). Many diseases related to oxidative stress are as an outcome of free radicals in the body (Scheibmeir et al., 2005). The uses of herbal extracts have anti-inflammatory properties, which are used for the treatment of inflammatory diseases (Muluye et al., 2014). For centuries, Murraya paniculata in synonyms with _M. alata_; _M. crenulata_; _M. euchrestifolia_; _M. koenigii_; _M. kwangsiensis_; _M. microphylla_; _M. ovatifoliolata_; _M. stenocarpa_; _M. tetrameria_; and _M. paniculata_. The vernacular name of _M. paniculata_ is also known as Kamini (Bengali), Thanaka (Burmese), Chiu li xiang, Kau lei heung (Chinese), Kamuning (Filipino/Tagalog), Buis de Chine (French), Gacharisha, Marchula (Hindi), Gekkitsu, Inutsuge (Japanese), Falscher jasmin, Orangenraute (German), Angara gida, Konji (India), Kemuning (Indonesian), Sarika keo (Kheimer), Keo (Lao), Kemuning (Malay), Kunti (Marathi), Kemoening (Nederland), Etteriya (Singhalese), Naranjo jazmín (Spanish), Keo (Thai), Satinwood, Simaikkonji (Tamil), Nguyen quat (Vietnamese), and Banati (Visayan) (Seidemann, 2005).

_M. paniculata_ is a tropical, evergreen plant that grows in small shrubs, usually 2 to 3 m in height but reaching 7.5 m (Fig. 1A) and 9-13 cm in stem diameter (Fig. 1B) with small, scented, white flowers (Fig. 1C). The fruit is elliptic shape about 0.8-1.2 cm long, with one or two light green seeds in tear-drop shaped (Fig. 1D). The leaves, 6-11 cm long, are pinnately compound with three to nine leaflets alternating on the rachis. The leaflets are dark-green, stiff, ovate, and smell of citrus when crushed (Fig. 1E). This plant is native to southern China, Taiwan, and the sub-continent, southeastern Asia including Thailand.
and Malaysia, and northern Australia (Ng et al., 2012). However, now it is cultivated and can be easily found in many countries in the tropics. Phytochemical analysis has shown that *M. paniculata* contains several kinds of coumarins and derivative (Aziz et al., 2010; Ito et al., 2005; Saeed et al., 2011), alkaloids (Gill et al., 2014; Rehman et al., 2014), flavonoids (Zhang et al., 2013; 2012), phenolic compounds (Gautam et al., 2012) and essential oil (Rajendran et al., 2014; Shah et al., 2014). *M. paniculata* has been used in ethnomedicine (Olawore et al., 2005). The benefits of *M. paniculata* are from its anti-inflammatory properties that can heal dermatological (Mehmood and Khan, 2012) and gastrointestinal diseases (Rahman et al., 2010). In Thailand, the leaves are crushed and mixed with alcohol to soothe sprains, joint pain, bone pain, contusions, and swollen, painful insect and snake bites (Rodanant et al., 2012). In India, *M. paniculata* is used for the treatment of toothache by boiling the leaves with a little bit of salt (Gautam and Goel, 2012). Medicinal plants have gained huge interests from researchers around the world because of their positive bioactivity effects (Gurib-Fakim, 2006). However, there is still not much data available about the toxicity of medical plants. For this reason, this experiment is set out to observe the cytotoxic effect of *Murraya paniculata* extract against *Artemia salina*. The brine shrimp lethality assay is a top method used to indicate general toxicity because of its simplicity (Meyer et al., 1982). The findings from this study would give basic contributions for the development of new treatments for health providers.

**Methods and Methods**

**Plant collection and extraction**

Fresh, mature, green leaves of *M. paniculata* were randomly collected in Mahidol University, Faculty of Science, Bangkok, Thailand (13° 45’ 51” N, 100° 31’ 32” E) in January 2015. The voucher specimen was numbered and kept in our research laboratory for the further reference. The leaves were washed with tap water and air dried in shade for 24 hours and dried in a hot air oven at 70°C for 6 hours, and crushed with a blender. The extraction procedure was determined by the method of Kjanijou et al. (2012) with modifications. Five grams of leaf powder was extracted with 100 ml of distilled water on a shaker at 180 rpm for 0.5, 1, 3, 5, and 24 hours at room temperature. The whole mixture was then filtered through a fresh gauge plug, and centrifuged at 4,000 rpm for 10 minutes. Finally supernatant was filtered with a Whatman number 1 filter paper, the clear filtrate used as a stock solution for total phenolic compound measurement and bioassay experiment.

**Total phenolic compound measurement**

Total phenolic compound was determined using Folin–Ciocalteu reagent according to methods of Jiraungkoorskul (2016) and Mcdonald et al. (2001) with modifications. Briefly, the 50 µl of the extraction in each time (0.5, 1, 3, 5 and 24 hours) was mixed with 250 µl of 10% Folin–Ciocalteus and 200 µl of 0.7 M sodium carbonate then add distilled water until 5 ml and incubated at room temperature for 2 hours in the dark room. The mixture was measured at 724 nm by using a spectrophotometer. Quantification was based on the standard curve of the gallic acid and expressed as gallic acid equivalent (GAE) using the following linear equation based on the calibration curve as shown in this equation (OD=9649.4C² - 3697C + 132.38), where OD was the absorbance and C was concentration as GAE.

**Brine shrimp lethality bioassay**

The brine shrimp lethality assay was assigned to determine the cytotoxic effect of plant extract. It followed the method by Meyer et al. (1982). Due to the highest amount of total phenolic compound, the required concentrations (0, 5, 50, 100, 500, 2500 and 5000 ppm) were prepared through mixing up of the 5 hour-extraction with variable amounts of 2.5% NaCl. Ten Artemia salina were added into five replicates of each concentration of the leaf extract. The bioassay was maintained at 26±1°C throughout the test. The mortality was recorded for a maximum of 24 hours of exposure. They were considered dead or moribund if they stopped moving for a prolonged period even after gentle probing with a small spatula. The LC₅₀ was analyzed by the probit method of Finney (1971) using the SPSS 18.0 (Statistical Package of

![Figure 1](M. paniculatais small shrub (A), stem (B) flowers (C), fruits (D) and leaves (E).)
Social Sciences) software. It estimated the lethal concentration and the slope of the regression line with its confidence interval (p<0.05).

**Results**

The total phenolic compound from leaves of *M. paniculata* measurement in each time extraction 0.5, 1, 3, 5 and 24 hours were 27.29±3.29, 28.82±7.35, 18.94±9.05, 39.90±9.78 and 22.61±3.18 mg/g GAE, respectively. The properties of the aqueous leaf extract of *M. paniculata* against *A. salina* were presented in Figure 2. The result of brine shrimp assay was expressed in percentage of mortality. The dose dependent mortality was observed, as the rate of mortality (Y) was positively correlated with the concentration (X) of the leaf extract as evident from established regression equations (y=49.844x+79.833). The percentage mortality increased as the concentration of aqueous extract of *M. paniculata* increased. The 5-hour aqueous extract of *M. paniculata* leaf expressed the 24-h LC$_{50}$ and LC$_{90}$ values in *A. salina* were 2,572.03 and 4,565.79 ppm, respectively. *M. paniculata* showed a significant effect against brine shrimp. The correlation (R$^2$) between concentration and mortality was 0.9977.

**Discussion**

The levels of antioxidants defense mechanism in normal state are not sufficient for the prevention of the free radical induced injury (Paramaguru et al., 2012). Therefore, there is an increasing interest in the supplementation of antioxidants from a natural plant. Due to avoiding any solvent effect, the aqueous solvent was used to extract *M. paniculata* in the present study. Extracts of medicinal herbs are the most studied natural antioxidants (Yanishlieva et al., 2006). Literature survey has revealed a direct relationship between antioxidant activity and total phenolic content (Conforti et al., 2009; Kumar et al., 2010). The present result revealed that the total phenolic compound measurement in 0.5, 1, 3, 5 and 24 hours extraction were 134.71±3.46, 136.08±7.47, 124.86±10.61, 146.66±9.01 and 129.65±3.53 mg/g GAE, respectively. These results were in agreement with earlier reports. Naresh et al. (2014) extracted *M. paniculata* seed with 50% ethanol for 72 hours and reported the total phenolic compound measurement was 55 mg GAE/g of extract. Paramaguru et al. (2012) extracted *M. paniculata* leave with 50% ethanol for 48 hours and reported the total phenolic compound measurement was 172.61 mg GAE/g of extract. Kumar et al. (2010) extracted *M. exotica* leave with 80% methanol for 24 hours and reported the total phenolic compound measurement was 510 mg GAE/g of extract. Moreover, Gautam et al. (2012) and Menezes et al. (2014), both extracted *M. paniculata* leaves with 50% ethanol for 72 hours and reported the total phenolic compound measurements were 66.5 and 24.8 mg GAE/g of extract, respectively. Using brine shrimp lethality bioassay tested the cytotoxic activity of the aqueous extract of leaves of *M. paniculata* were found to show a little toxicity as expressed the 24-h LC$_{50}$ and LC$_{90}$ values in *A. salina* were 2,572.03 and 4,565.79 ppm, respectively. Each of the different concentrations samples showed different mortality rates. When graphed, the

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Weight</th>
<th>Extraction</th>
<th>LC$_{50}$</th>
<th>LC$_{90}$</th>
<th>Researchers</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. paniculata</em></td>
<td>50 g/L</td>
<td>5 hours in water</td>
<td>2.572.03 ppm</td>
<td>4,565.79 ppm</td>
<td>the present study</td>
</tr>
<tr>
<td><em>M. paniculata</em></td>
<td>412 g/L</td>
<td>15 days in 80% ethanol</td>
<td>32 µg/ml</td>
<td>158 µg/ml</td>
<td>Sharker et al., 2009</td>
</tr>
<tr>
<td><em>M. paniculata</em></td>
<td>125 g/L</td>
<td>7 days in methanol</td>
<td>0.773 µg/ml</td>
<td>No data</td>
<td>Mita et al., 2013</td>
</tr>
<tr>
<td><em>M. exotica</em></td>
<td>258 g/L</td>
<td>10 days in methanol</td>
<td>1.27 µg/ml</td>
<td>5.09 µg/ml</td>
<td>Khatun et al., 2014</td>
</tr>
</tbody>
</table>
concentrations versus mortality percentage showed an approximate linear correlation. These results were not in agreement with earlier reports because most of the studies on *M. paniculata* cytotoxicity have been done using crude extracts. Various researchers have reported the LC$_{50}$ and LC$_{90}$ of *M. paniculata* in different doses, time and solvent extraction, as shown in Table 1. This activity could be explained by the phenols, flavonoids and coumarins present in the extract. There are reports in the literature describing the antimicrobial activity correlated high content of phenolics and flavonoids in *M. paniculata* leaf extract (Gautam et al., 2012; Sundaran, 2011; Aziz et al., 2010).

**Conclusions**

In conclusion, the aqueous extract of *M. paniculata* can be the alternative used as the natural product. However, further studies are necessary to find out what the active substances are and how they do or the mechanism of them in the target species.

**Acknowledgements**

The authors are thankful to the members of the Fish Research Unit, Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok, for the technical support in their laboratory. This study was funded by the Thailand Research Fund and the Commission on Higher Education: Research Grant for Mid-Career University Faculty. Many thanks to the anonymous referees and editors for their perceptive comments and positive criticism of this manuscript.

**References**


