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Evaluation of total phenolic compound and cytotoxic activity of *Murraya paniculata*

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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. exotica, Chalcas exotica,* and *C. paniculata*, has been widely used as "Traditional Medicinal Plant" in Asia to relief symptoms of various diseases (Sayar *et al*., 2014). *M. paniculatais* is commonly known as satinwood, orange jasmine or China box tree, and belongs to the family Rutaceae. The Rutaceae is a large family, comprised of about 150 genera and 1,200 species distributed in the world (Bhatterjee, 2000; Shah *et al*., 2014). There are around 10 global species belonging to the genus Murraya i.e., *M. alata; M. crenulata; M. euchrestifolia; M. koenigii; M. kwangsiensis; M. microphylla; M. ovatifoliolata; M. stenocarpa; M. tetramera;* and *M. paniculata*. The vernacular name of *M. paniculatais* 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 (Khmer), Keo (Lao), Kemuning (Malay), Kunti (Marathi), Kemoening (Nederland), Etteriya (Singhalese), Naranjo jazmín (Spanish), Keo (Thai), Satinwood, Simaikkonji (Tamil), Nguyet quat (Vietnamese), and Banati (Visayan) (Seidemann, 2005).

M. paniculatais 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

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Figure 1 *M. paniculatais* small shrub (A), stem (B) flowers (C), fruits (D) and leaves (E).

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 sooth 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^{o} 45’ 51’’ N, 100^{o} 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^{o}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 LC_{50} was analyzed by the probit method of Finney (1971) using the SPSS 18.0 (Statistical Package of

Table 1 Compare the brine shrimp lethality bioassay of *Murraya paniculata* of the present study with past researches

Plant Part	Weight	Extraction	Brine shrimp lethality bioassay		Researchers
			LC_{50}	LC_{90}	
M. paniculata Leave	50 g/L	5 hours in water	2,572.03 ppm	4,565.79 ppm	the present study
M. paniculata Leave	412 g/L	15 days in 80% ethanol	32 µg/ml	158 µg/ml	Sharker *et al.*, 2009
M. paniculata Leave	125 g/L	7 days in methanol	0.773 µg/ml	No data	Mita *et al.*, 2013
M. exotica Leave	258 g/L	10 days in methanol	1.27 µg/ml	5.09 µg/ml	Khatun *et al.*, 2014

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

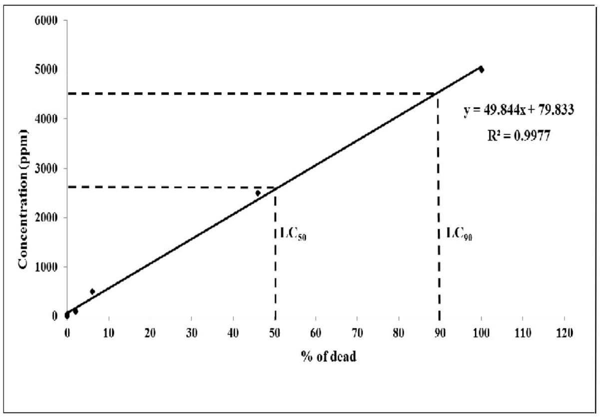

Figure 2 The relationship between the percentage of *Artemia salina* dead and *Murraya paniculata* leaf extract concentration.

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 show 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* leave 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.

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References

Aziz, S.S.S.A., Sukari, M.A., Rahmani, M., Kitajima, M., Aimi, N. and Ahpandi, N.J. 2010. Coumarins from *Murraya paniculata* (Rutaceae). Malaysian Journal of Analytical Sciences 14: 1-5.

Bent, S. 2008. Herbal medicine in the United States: Review of efficacy, safety, and regulation. Journal of General Internal Medicine 23: 854-859.

Bhatterjee, S.K. 2000. Hand Book of Aromatic Plants. Popular Offset Services Pvt. Ltd., Jaipur, pp. 299-448.

Conforti, F., Sosa, S., Marrelli, M., Menichini, F., Statti, G.A., Uzunov, D., Tubaro, A. and Menichini, F. 2009. The protective ability of Mediterranean dietary plants against the oxidative damage: The role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents. Food Chemistry 112: 587-594.

Fawzi Mahomoodally, M. 2013. Traditional medicines in Africa: An appraisal of ten potent African medicinal plants. Evidence-Based Complementary and Alternative Medicine Article ID 617459, 14 pages, doi:10.1155/2013/617459

Finney, D.J. 1971. Probit analysis, Cambridge University Press, Cambridge, UK.

Gautam, M.K. and Goel, R.K. 2012. Exploration of preliminary phytochemical studies of leaves of *Murraya paniculata* (L.). International Journal of Pharmacy and Life Sciences 3: 1871-1874.

Gautam, M.K., Gangwar, M., Nath, G., Rao, C.V. and Goel, R.K. 2012. In-vitro antibacterial activity on human pathogens and total phenolic, flavonoid contents of *Murraya paniculata* Linn. leaves. Asian Pacific Journal of Tropical Biomedicine 2: S1660-S1663.

Gill, N.S., Kaur, N. and Arora, R. 2014. An overview on *Murraya paniculata* Linn. International Journal of Institutional Pharmacy and Life Sciences 4: 1-11.

Gurib-Fakim, A. 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine 27: 1-93.

Ito, C., Itoigawa, M., Onoda, S., Hosokawa, A., Ruangrungsi, N., Okuda, T., Tokuda, H., Nishino, H. and Furukawa, H. 2005. Chemical constituents of *Murraya siamensis*: Three coumarins and their anti-tumor promoting effect. Phytochemistry 66: 567-572.

Jiraungkoorskul, W. 2015. Larvicidal and histopathological effects of *Andrographis paniculata* leaf extract against *Culex quinquefasciatus* larva. Walailak Journal of Science and Technology (WJST), North America, Available at: *http://wjst.wu.ac.th/index.php/wjst/article/view/1455*.Date accessed: 27 Jan. 2015.

Khatun, A., Rahman, M. and Jahan, S. 2014. Preliminary phytochemical, cytotoxic, thrombolytic and antioxidant activities of the methanol extract of *Murraya exotica* Linn. Leaves. Oriental Pharmacy and Experimental Medicine 14: 223-229

Kjanijou, M., Jiraungkoorskul, K., Kosai, P. and Jiraungkoorskul, W. 2012. Effect of *Murraya paniculata* leaf extract against *Culex quinquefasciatus* larva. Asian Journal of Biological Sciences 5: 201-208.

Kumar, A., Kaur, R. and Arora, S. 2010. Free radical scavenging potential of some Indian medicinal plants. Journal of Medicinal Plants Research 4: 2034-2042.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. 2010. Free radicals, antioxidants and unctional foods: Impact on human health. Pharmacognosy Review 4: 118-126.

Mcdonald, S., Prenzler, P.D., Antolovich, M. and Robards, K. 2001. Phenolic content and antioxidant activity of olive oil extracts. Food Chemistry 73: 73-84.

Mehmood, F. and Khan, Z.U.D. 2012. Determination of skin irritancy by essential oils from some members of Family Rutzceae of Pakistan. Biologia 58: 161-166.

Menezes, I.R.A., Santana, T.I., Varela, V.J.C., Saraiva, R.A., Matias, E.F.F., Boligon, A.A., Athayde, M.L., Coutinho, H.D.M., Costa, J.G.M. and Rocha, J.B.T. 2014. Chemical composition and evaluation of acute toxicological, antimicrobial and modulatory resistance of the extract of *Murraya paniculata.* Pharmaceutical Biology 26: 1-7.

Meyer, B.N., Ferrigni, N.R., Putnam, J.B., Jacobsen, L.B., Nichols, D.E. and McLaughlin, J.L. 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Medicine 45: 31-34.

Mita, T.A., Shihan, M.H., Rahman, M., Sharmin, T., Maleque, M., Alvi, M.R.H. and Chowdhury, S.R. 2013. In vitro antioxidant, cytotoxic, thrombolytic, antimicrobial and membrane stabilizing activities of *Murraya paniculata.* American Journal of Research Communication 1: 226-237.

Muluye, R.A., Bian, Y. and Alemu, P.N. 2014. Anti-inflammatory and antimicrobial effects of heat-clearing Chinese herbs: A current review. Journal of Traditional and Complementary Medicine 4: 93-98.

Naresh, S., Narinder, K. and Rashmi, A. 2014. Isolation and characterization of *Murraya paniculata* ethanol seed extract for their antioxidant components. International Research Journal of Pharmacy 5: 690-694.

Ng, M.K., Abdulhadi-Noaman, Y., Cheah, Y.K., Yeap, S.K. and Alitheen, N.B. 2012. Bioactivity studies and chemical constituents of *Murraya paniculata* (Linn) Jack. International Food Research Journal 19: 1307-1312.

Olawore, N.O., Ogunwande, I.A., Ekundayo, O. and Adeleke, K.A. 2005. Chemical composition of the leaf and fruit essential oils of *Murraya paniculata* (L.) Jack. (Syn. *Muraya exotica* Linn.). Flavour and Fragrance Journal 20: 54-56.

Paaramaguru, R., Silambu Janaki, P., Bavani Eswaran, M., Rao, C.V., Rawat, A.K.S. and Vijayakumar, M. 2012. Free radical scavenging and lipid peroxidation inhibition potential of various fractions of *Murraya paniculata.* Pharmacologia 3: 138-143.

Rajendran, M.P., Pallaiyan, B.B. and Selvaraj, N. 2014. Chemical composition, antibacterial and antioxidant profile of essential oil from *Murraya koenigii* (L.) leaves. Avicenna Journal of Phytomedicine 4: 200–214.

Rahman, M.A., Hasanuzzaman, M. and Uddin, N. 2010. Antidiarrhoeal and anti-inflammatory activities of *Murraya paniculata* (L.) Jack. Pharmacology 3: 768-776.

Rehman, F., Khan, M.F., Khan, I., Shareef, H. and Marwat, S.K. 2014. Analgesic activity of carbazole alkaloid from *Murraya paniculata* Linn. (Rutaceae). American-Eurasian Journal of Agricultural and Environmental Science 14: 240-245.

Rodanant, P., Surarit, R., Srichan, R. and Korsuwanwong, S. 2012. Cytotoxic and anti-inflammatory activity of some Thai medicinal plants. Journal of Medicinal Plants Research 6: 4063-4068.

Saeed, S., Shah, S., Mehmood, R. and Malik, A. 2011. Paniculacin, a new coumarin derivative from *Murraya paniculata.* Journal of Asian Natural Products Research 13: 724-727.

Sayar, K., Paydar, M. and Pingguan-Murphy, B. 2014. Pharmacological properties and chemical constituents of *Murraya paniculata* (L.) Jack. Medicinal and Aromatic Plants 3: 173-178.

Scheibmeir, H.D., Christensen, K., Whitaker, S.H., Jegaethesan, J., Clancy, R. and Pierce, J.D. 2005. A review of free radicals and antioxidants for critical care nurses. Intensive and Critical Care Nursing 21: 24-28.

Seidemann, J. 2005. World Spice Plants: Economic Usage, Botany, Taxonomy. Springer Science & Business Media, 592p.

Shah, S., Saied, S., Mahmood, A. and Malik, A. 2014. Phytochemical screening of volatile constituents from aerial parts of *Murraya paniculata*. Pakistan Journal of Botany 46: 2051-2056.

Sharker, S.M., Shahid, I.J. and Hasanuzzaman, M. 2009. Antinociceptive and bioactivity of leaves of *Murraya paniculata* (L.) Jack, Rutaceae. Revista Brasileira de Farmacognosia 19: 746-748.

Sundaram, M. 2011. Studies on in vitro antibacterial, antifungal property and antioxidant potency of *Murraya paniculata.* Pakistan Journal of Nutrition 10: 925-929.

Yanishlieva, N.V., Marinova, E. and Pokorny, J. 2006. Natural antioxidans from herbs and spices. European Journal of Lipid Science and Technology 108: 776-793.

Zhang, J.Y., Lu, J.Q. and Gao, X.Y. 2013. Characterization of thirty-nine polymethoxylated flavonoids (PMFs) in the branches of *Murraya paniculata* by HPLC-DAD-ESI-MS/MS. Chinese Journal of Natural Medicines 11: 63-70.

Zhang, J.Y., Li, N., Zhou, Y., Jiang, Y. and Tu, P.F. 2012. Simultaneous qualitative and quantitative determination of major polymethoxylated flavonoids in the leaves of *Murraya paniculata* by RRLC-DAD-ESI-MSn. Analytical Methods 4: 3399–3406.