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Antioxidant and Cytotoxic Activities of Methanolic Extracts from *Mimusops elengi* Flowers

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Abstract The total phenolic content, antioxidant and cytotoxic activities of methanolic extracts from the petals and sepals of *Mimusops elengi* flowers were examined. The total phenolic content of methanolic extracts from petals and sepals using Folin-Ciocalteu method showed 49.32±3.04 and 93.36±3.58 mgGAE /g extract, respectively. The methanolic extract from sepals showed higher antioxidant activity than petals with IC_{50} (50% inhibitory concentration) values in DPPH and ABTS radical scavenging assay of 98.20 and 236.13 µg/ml, respectively. In addition, Fe^{3+}-TPTZ reduction in FRAP method was 63.11 mgAAE /g extract. Furthermore, MTT assay was applied to evaluate cytotoxic activity against eight cell lines. The sepals extract exhibited high cytotoxic activity against six cancerous cell lines: TK6, HeLa, HepG2, HT-29, MCF-7 and KB with CC_{50} (50% cytotoxic concentration) values of 179.38, 379.06, 442.61, 500.66, 517.98 and 541.06 µg/ml, respectively. Nevertheless, the sepals extract exhibited cytotoxic activity against normal cell lines (L929 and Vero) with CC_{50} of 493.96 and 413.69 µg/ml. The results suggested that methanolic extract from sepals of *M. elengi* flowers revealed high antioxidant and cytotoxic activities. Further studies are needed to evaluate the constituent of bioactive compounds that beneficial for medicinal and cosmetic applications.

Keywords: Antioxidant activity, Cytotoxic activity, *Mimusops elengi*, Total phenolic content

Introduction

In the present, natural extracts from the medicinal plants are interesting because of their biological activities including antioxidant and cytotoxic activities that are beneficial to prevent any diseases. Free radical such as reactive oxygen species (ROS) that is produced by living organism and environment is unpaired electron molecule that can react with biomolecule and can damage cell structure such as nucleic acids, carbohydrates, proteins, and lipids (Birben *et al*., 2012). In addition, ROS have been involved in the aging process and in different diseases including cancer (Datta *et al*., 2000).

Normally our body processes can produce antioxidant compounds such

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as enzymes and vitamins to neutralize free radicals. If our body processes can't control free radicals it is known as oxidative stress condition and related to any disease (Lobo *et al*., 2010). Synthetic antioxidant compounds have been considered unsafe (Brewer *et al*., 2011). Therefore, the natural antioxidant compounds need to be used to reduce oxidative stress and related damage. Vegetables and fruits are important natural antioxidant source that contain bioactive compounds and are useful to health (Serrano *et al*., 2011).

Mimusops elengi Linn belongs to Sapotaceae that is a medicinal plant which commonly known as Pikul in Thailand. In traditional used plant, *M. elengi* have been used as astringent, tonic and febrifuge properties (Shivatare *et al*., 2013). In the previous report, most part of *M. elengi* extracts revealed various pharmacological properties such as leaves have presented antioxidant (Saha *et al*., 2008) and antibacterial (Nair and Chanda, 2008) activities. Bark showed anti-hyperglycemic (Ganu *et al*., 2010) and cytotoxic effects (Ganesh *et al*., 2013) as well as the fruits showed antioxidant activity (Boonyuen *et al*., 2009). However studying about bioactivities of flowers has a few reports and we are interested in part of flowers extracts and their bioactivities. Therefore, the purpose of this study was to evaluate total phenolic content, antioxidant and cytotoxic activities of flowers extract from *M. elengi*.

Materials and methods

Preparation and extraction plant material

M. elengi flowers were collected from Ratchaburi province, Thailand. Dried the flowers in hot air oven at 40 °C. Then, separate the petals and sepals of the flowers. Each part was grinded with electric grinder to obtain a fine powder and macerated in methanol at room temperature for a week. After that solvent extracts were filtered with Whatman No.1 and evaporated by the rotary evaporator to obtain methanolic extracts of petals and sepals from *M. elengi* flowers.

Evaluation of total phenolic content

The total phenolic content of *M. elengi* flowers extracts were determined by Folin-Ciocalteu method with few modifications according to Magalhaes *et al.* (2010). Then, 50 µl of sample at 1,000 µg/ml and Folin-Ciocalteu reagent were mixed in 96-well plate. The mixture was kept in the dark for 6 min, after that 100 µl of 7.5% Na_2CO_3 was added, shaken and incubated for 30 min. Absorbance was measured at 765 nm using microplate reader. The total

phenolic content was determined using gallic acid as a standard curve and expressed as mgGAE /g extract.

Quantification of antioxidant activities

DPPH free radical scavenging assay

Sample and standard trolox were diluted to different concentrations according to Shahwar and Raza (2012) with few modifications. Then, 100 µl of sample or standard were mixed with 200 µl of 0.2 mM DPPH solution, the reaction mixture was incubated in the dark for 30 min. After that the absorbance was measured at 517 nm against blank, calculated percentage of DPPH radical scavenging activity following this formula. The IC_{50} value of sample and standard were determined using GraphPad Prism 6.0 and trolox equivalent antioxidant capacity was expressed as mgTE /g extract.

$$\% \text{ DPPH radical scavenging activity} = [(A_{control}-A_{sample})/A_{control}] \times 100$$

ABTS free radical scavenging assay

$ABTS^{+\bullet}$ was activated by oxidation of ABTS solution with potassium persulfate according to Re *et al* (1999) with a few modifications. The mixture consisted of the different concentration of sample (100-500 µg/ml) or trolox as standard (20-80 µg/ml) with ratio 1: 10. Then, it was incubated for 6 min and measured at 734 nm against blank. After that the percentage of ABTS radical scavenging activity was calculated following below equation. The IC_{50} values were determined using GraphPad Prism 6.0 and trolox equivalent antioxidant capacity was expressed as mgTE /g extract.

$$\% \text{ ABTS radical scavenging activity} = [(A_{control}-A_{sample})/A_{control}] \times 100$$

Ferric reducing antioxidant power assay *(*FRAP*)*

The absorptions of sample in this method are related to reducing power of antioxidants in test compound. Firstly the FRAP solution was prepared according to Benzie and Strain (1996) by mixing 300 mM acetate buffer, 10 mM TPTZ solution and 20 mM $FeCl_3$ in ratio 10: 1: 1. Then 500 µg/ml of extracts were mixed with FRAP solution, left for 6 min and mixture was measured at 593 nm compared with standard ascorbic acid and expressed as mgAAE /g extract.

Cytotoxic activity

Cells culture

Eight cell lines were used to test the cytotoxicity consisted of six cancerous cell lines including TK6 (Human B-lymphoblastoid), HeLa (Human cervical carcinoma), KB (Human oral cavity carcinoma), HepG2 (Human hepatoma), HT-29 (Human colon adenocarcinoma) and MCF-7 (Human breast adenocarcinoma). In addition, two normal cell lines that were also used consist of Vero (African green monkey kidney) and L929 (Mouse fibroblast). All cell lines were cultured in RPMI-1640 medium supplemented with 8% fetal bovine serum and gentamicin in the 5% CO_2 incubator at 37 °C.

MTT assay

The stock solution of methanolic extracts from petals and sepals of *M. elengi* flowers were prepared at 20 mg/ml and diluted to 1,000 µg/ml at the final concentration by RPMI-1640 supplemented with 5% FBS for preliminary screening of cytotoxicity. For evaluated 50% cytotoxic concentration (CC_{50}) values of various cell lines the stock solution of extract was diluted to different concentration (125-1,000 µg/ml).

MTT assay was applied to test cytotoxicity according to Mahavorasirikul *et al* (2010) with slight modification. For the experiment, 100 ul of various cell lines were seeded in 96-well plate at cells density of $0.8\text{-}1.8x10^5$ cells/ml, then incubated in the CO_2 incubator with 5% CO_2 at 37 ^{0}C for 48 hours. After that various cell lines were treated with 100 µl of extracts and incubated for 20 hours using DMSO and mitomycin C as negative and positive control, respectively. Afterwards, 50 µl of 2 mg/ml MTT solution was added and incubated for 4 hours. Then, supernatant was removed and 100 µl of DMSO: ethanol was added to dissolve formazan crystal. The absorbance was measured at 570 nm and calculated % cytotoxicity following below equation. The CC_{50} values of extracts against various cell lines were determined using GraphPad prism 6 software.

$$\% \text{ Cytotoxicity} = \frac{[(\text{ODcontrol}-\text{ODblank})-(\text{ODsample}-\text{ODblank})]\ x100}{(\text{ODcontrol}-\text{ODblank})}$$

Where as Control = cells and medium

Sample = cells and extracts

Statistical analysis

Student's t-test of SPSS Statistics 23 software was used to analyze intergroup differences. The IC_{50} and CC_{50} values were determined by GraphPad

Prism 6 software. Data are represented as the mean ± SD and all experiments in this study were repeated at least three times, p-value of less than 0.05 was considered to be statistically significant differed.

Results

Extraction yield

The extraction yield of petals and sepals extracts from *M. elengi* flowers were 5.80 and 4.53 %, respectively. Furthermore, the methanolic extracts of each part from *M. elengi* flowers by maceration method showed different appearance. The methanolic extract from petals showed red-brown color and is viscous nevertheless the methanolic extract from sepals was dark brown color and be a powdered solid.

Total phenolic content

The total phenolic content of *M. elengi* flowers extracts was estimated using gallic acid (20-100 µg/ml) as a standard compound. A linear calibration curve of gallic acid showed $y = 0.0125x$ with r^2 value of 0.9991. The results showed methanolic extract from petals and sepals of *M. elengi* flowers have the total phenolic content of 49.32±3.04 and 93.36±3.58 mgGAE /g extract, respectively. The values of total phenolic content from petals and sepals extracts were significantly different ($p < 0.05$).

Antioxidant activities

The free radical scavenging activity of *M. elengi* flowers extracts in various assays are presented in table 1. Both petals and sepals methanolic extract from *M. elengi* flowers demonstrated antioxidant activities. The antioxidant capacity of methanolic extract from sepals revealed high antioxidant activities than petals, significantly different for all assays ($p < 0.05$). The methanolic extract from sepals showed the antioxidant capacity of 206.72±10.38 and 187.67±8.15 mgTE /g extract in DPPH and ABTS assay, respectively. However, methanolic extract from petals showed 135.03±5.64 and 161.03±5.63 mgTE /g extract, respectively. In addition, in FRAP method the sepals extract also showed higher Fe^{3+}-TPTZ reducing power than petals extract with the values of 63.43±2.95 and 34.50±9.55 mgAAE /g extract, respectively when compared with ascorbic acid as a standard antioxidant compound.

Table 1. Antioxidant activities of the methanolic extracts from *M. elengi* flowers in various assays.

Methanolic extracts	Antioxidant activity			IC_{50} values (µg/ml)	
	DPPH (mgTE/g extract)	ABTS (mgTE/g extract)	FRAP (mgAAE/g extract)	DPPH	ABTS
Petals	135.03±5.64	161.03±5.63	34.50±2.95	151.79	280.96
Sepals	206.72±10.38	187.67±8.15	63.43±9.55	98.20	236.13

Cytotoxic activity

The preliminary screening for antiproliferative effect of extracts at concentration 1,000 ug/ml against eight cell lines were estimated by MTT assay. The methanolic extract from petals showed low cytotoxicity. On the other hand, the methanolic extract from sepals of *M. elengi* exhibited high cytotoxicity against eight cell lines. In addition, the morphology of treated cell lines had an irregular shape. Some cells are unattached to the bottom of well-plate when compared with control that the morphology of some cell lines is presented in figure 1.

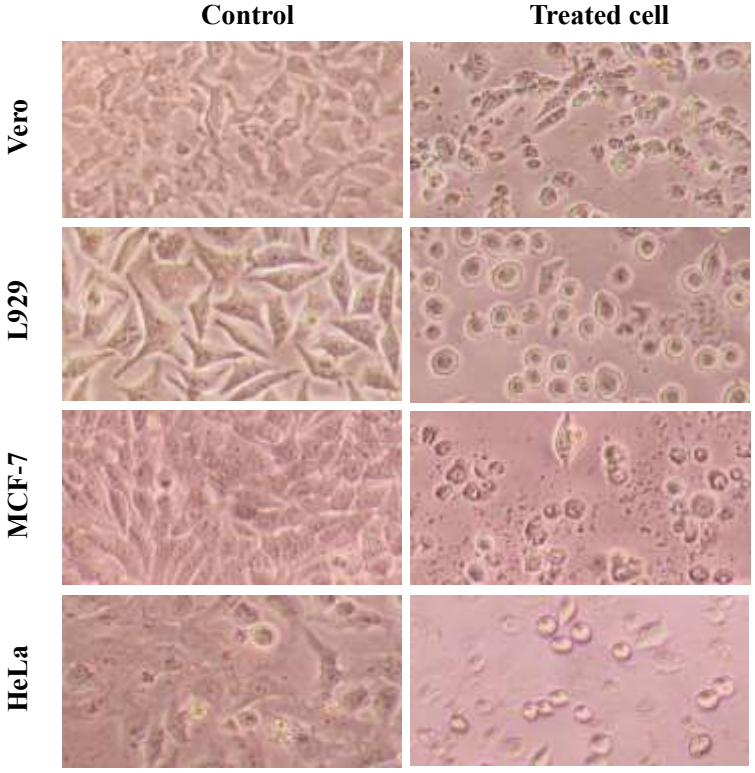

Figure 1. The morphology change of normal cell line (Vero and L929)nand cancerous cell lines (MCF-7 and HeLa) after treated with methanolic extract from *M. elengi* flowers at 1,000 ug/ml for 20 hours under phase contrast microscope (100X)

Therefore, the methanolic extract from sepals of *M. elengi* flowers was used to estimate the CC_{50} (50% cytotoxic concentration) values with different concentration against cancerous and normal cell lines in figure 2. The percentage of cytotoxicity of extract was revealed in a dose-dependent manner. The result of this study showed the methanolic extract from sepals exhibited high cytotoxicity against six cancerous cell lines including TK6, HeLa, HepG2, HT-29, MCF-7 and KB with CC_{50} values of 179.38, 379.06, 442.61, 500.66, 517.98 and 541.06 µg/ml, respectively. Nevertheless, the sepals extract exhibited cytotoxic activity against normal cell lines (L929 and Vero) with CC_{50} of 493.96 and 413.69 µg/ml as presented in table 2.

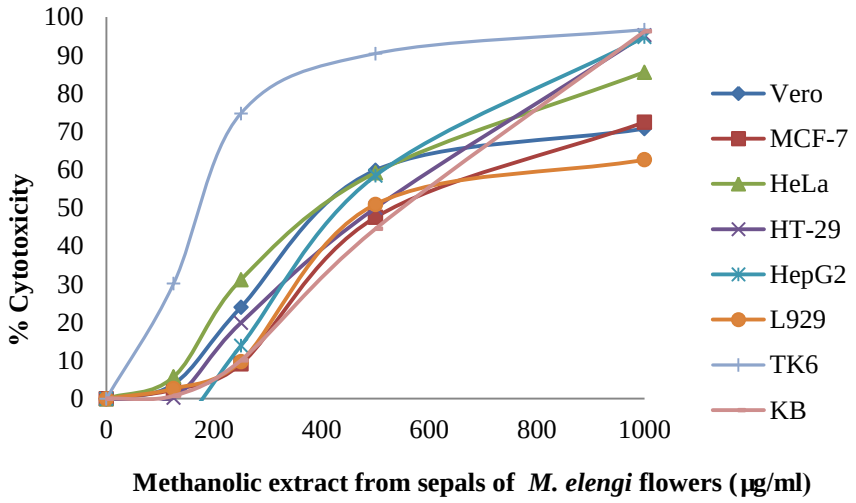

Figure 2. Cytotoxic activity of sepals extract from *M. elengi* flowers at different concentration against eight cell lines

Table 2. The CC_{50} values of the methanolic extract from sepals of *M. elengi* in various cell lines.

The methanolic extract	CC_{50} values (µg/ml)							
	Cancerous cell lines						**Normal cell lines**	
	TK6	**HeLa**	**HepG2**	**HT-29**	**MCF-7**	**KB**	**L929**	**Vero**
Sepals	179.38	379.06	442.61	500.66	517.98	541.06	493.96	413.69

Discussion

In traditional medicine, each part of *M. elengi* has been used for many diseases such as astringent, tonic and febrifuge properties (Shivatare *et al*.,

2013). In the previous report, we found leaves, bark, seed and fruit extracts of *M. elengi* were used to test various biological activities but in flowers extract have a few reports. Therefore in this study, we focus on the phenolic content, antioxidant and cytotoxic activities of petal and sepal extracts from *M. elengi* flowers. According to results of this study, the methanolic extracts from petal and sepal of *M. elengi* are compose of the phenolic compound that sepal extract revealed the higher total phenolic content (93.36±3.58 mgGAE /g extract) than petal extract (49.32±3.04 mgGAE /g extract). Our results are consistent with previous study reported that the methanolic extract from bark of *M. elengi* have total phenolic content of 160.3±3.20 mgGAE /g extract (Patil *et al*., 2016).

Moreover, the result of antioxidant activity in this study is related to the total phenolic content of extracts. The methanolic extract of sepal demonstrated a higher phenolic content and also showed higher antioxidant activity than the petal extract for DPPH, ABTS and FRAP method. In previous studies, the methanolic extract from *M. elengi* leaves revealed antioxidant activity with IC_{50} value of 43.26 µg/ml in DPPH assay (Saha *et al*., 2008) that are higher than flowers extracts from this study. However, the IC_{50} value of *M. elengi* bark extract in ABTS assay was 206.58±7.63 µg/ml (Patil *et al*., 2016) that is similar to this study.

Cytotoxic activity in this study showed the methanolic extract from sepal of *M. elengi* flowers also presented higher cytotoxic activity than petal extract. The previous report showed the ethanolic extract from *M. elengi* flowers presented cytotoxic activity against CL-6 (human cholangiocarcinoma), HepG2 (human hepatocarcinoma) and Hep-2 (human laryngeal) cell lines with CC_{50} values of 48.84, 54.44 and 109.99 µg/ml, respectively (Mahavorasirikul *et al*., 2010). Furthermore, the methanolic extract from leaf and bark of *M. elengi* exhibited cytotoxicity against SiHa (human cervical cancer cell line) by MTT assay with CC_{50} values of 67.46±4.21 and 35.08±2.92 µg/ml, respectively (Ganesh *et al*., 2013).

Conclusion

This is the first study that carried out for measuring antioxidant and cytotoxic activities of petals and sepals extracts from *M. elengi* flowers. The results of this study revealed both parts of *M. elengi* flowers contain the phenolic compound and presented antioxidant activity for DPPH, ABTS and FRAP assays. In addition, the antioxidant activity is related to the total phenolic content of both extracts. Moreover, the methanolic extract from sepals showed high cytotoxic activity against six cancerous cell lines. Nevertheless, the sepals extract also exhibited cytotoxicity with two normal cell lines. Further studies

are needed to evaluate the constituent of bioactive compounds that are beneficial for the medicinal and cosmetic applications.

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