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Antioxidant activities and anticancer screening of ethanolic extracts from *Baccaurea macrophylla* Muell and *Elateriospermum tapos* Blume

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Abstract

Two local plants in Euphorbiaceae family, *Baccaurea macrophylla* Muell.(Langkha) and *Elateriospermum tapos* Blume. (Pra), are traditionally used in local cuisine and herbal medicines in Thailand.In this study, ethanolic extracts prepared from fruits, leaves, seeds and stem barks of these plants were tested for antioxidant properties using DPPH assay and also screened for anticancer activity using MTT assay against four human cancer cell lines: breast (MCF-7), colon (HT29, HCT116) and cervical (HeLa). The ethanolic crude extracts from the leaves of Pra and Langkha showed the highest total phenolic contents (TPC) (198.77±6.26 and 161.17± 0.32 mg gallic acid equivalent (GAE)/1 g of sample, respectively) compared to the other parts of the plants. Whereas, the ethanolic extracts of Pra's seed and Langkha's dried pulp had the lowest amounts of TPC (0.76± 0.42 and 1.06± 0.12 mg GAE/1 g of sample, respectively). In addition, *E. tapos* fresh leaf and *B. macrophylla* dried leaf ethanolic extracts exhibited the highest antioxidant activities with IC_{50} values of 0.10 and 1.70 μg/mL, respectively. The dried leaf ethanolic extract of *E. tapos* had the highest cytotoxicity against HeLa, HCT116, and MCF-7 cells (97.44, 98.58, 98.57% inhibition, respectively, at 100 μg/mL), whereas its seed skin ethanolic extract had the highest cytotoxic activity against HT29 cells (98.76% inhibition at 100 μg/mL) for 72 h-exposure. Among all ethanolic extractsof *B. macrophylla*, its dried leaf ethanolic extract had the highest cytotoxicity against all cancer cell lines tested. The leaf ethanolic extracts of *E. tapos* had greater anticancer activity than those of *B. macrophylla*.

Keywords: *Baccaurea macrophylla* Muell, *Elateriospermum tapos* Blume, anticancer, antioxidant

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1. Introduction

Recently, the research on local herbal plants has had increasingly attention due to the Royal Thai Government Policy. Various types of herbal research have been focused and developed, especially in pharmaceutical and cosmetic applications. The studies of anticancer properties and biological activities from various plants have been widely reported. For example, a large number of research indicated that different parts of some edible plants' extracts contained high amounts of total phenolic content and had significant anticancer activities against prostate cancer cell lines, colon cancer cell lines and breast cancer cell lines [1, 2, 3, 4]. The extract of *Casearia capitellata* leaves showed the best anticancer activity on breast cancer cell line (MCF-7), with IC_{50} value of 2.0 μg/mL and its methanolic extract also exhibited an outstanding anticancer activity on lung cancer cell line (H460). The highest yields of phenolics were obtained for methanolic extracts. The methanolic extracts of *Strobilanthus crispus* leaves showed the highest amount of total phenolic compound [1].

In addition, a number of studies indicated that the various parts of the plants such as the branch, leaves, fruits and the peels of fruits could be potentially rich sources of natural antioxidant activity and total phenolic contents [5, 6, 7, 8, 9]. Wetwitayaklung *et al*. studied twenty-one methanolic extracts of fruit meats, barks and peels of native and exotic fruits in Thailand using Folin-Ciocalteu reagent and Tolox equivalent antioxidant capacity (TEAC) assays. They found these fruit extracts contained the amount of total phenolic and antioxidant activities. The *Terminaliachebula* fruit meat and *Diospyros peregrine* fruit extracts showed the highest amount of total phenolic contents and antioxidant activities [10]. A research found most Thai herbal plants have a lot of bioactivity sources such as vitamin C, vitamin E, carotenoids, phenolic

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and antioxidant compounds with high antioxidant activities. The plant chemical compounds are contributors to the antioxidant activity [11]. Moreover, some studies reported that the antioxidant activities of the fruits were in direct proportion to their amount of total phenolic compound [5, 10, 12, 13]. It can be concluded that variety parts of fruits and vegetables are the main sources of antioxidant capacity, which act as free radical scavengers, making these plants essential to human health.

Baccaurea macrophylla Muell. and *Elateriospermum tapos* Blume. Are in Euphorbiaceae family. They are normally found in the highland forests wild, especially in the Southern Region of the Kingdom of Thailand. *B. macrophylla* is a species of fruit tree locally known as Langkha. It is a medium to large deciduous tree. The fruits are approximately 5 to 8 centimeters wide. Langkha fruits are eaten fresh and are rich in nutrients and minerals containing a large amount of vitamin C, Zn, P and K [6]. The pulp is sweet to acidic in taste. Various parts of Langkha are used in traditional medicine in Thailand for treatment of stomachache and sore eyes as well as used as antiulcer and anti-flammatory [1]. *E. tapos*is a large deciduous tree about 20 to 40 meters in height which grows in the highland rain forest, locally known as Pra. Pra fruits are oval, approximately 5 to 6 centimeters long and grow in strands. Each fruit has brown skin, which wrinkles at ripening. It is filled with pale brown pulp containing 3-5 seeds, which separate readily with each segment containing brown oval seeds. The seeds are used as flavoring agent for curries, sweet and are used in the preparation of snacks. The eatable seeds are cooked. The raw seeds are hazardous because they contain cyanide. The ground seeds are rich in nutrients containing considerable amounts of protein, carbohydrate and fat high in unsaturated fatty acids [14], therefore it has been used in the nutrition area.

Phenolic compounds and polyphenol-rich extracts from plants are extensively using in clinical trials for chemoprevention and therapy [15]. The present study deals with the screening of the two local plants in the Euphorbiaceae family (Langkha and Pra) for their antioxidant, total phenolic content and anticancer potential. These plants were chosen because of their use in local traditional cuisine and local medicines. In addition, the results from this research will be useful for the local plant promotion and value added as food for dietary prevention of cancer. The aims of this preliminary study areto investigate antioxidant activity, total phenolic content and anticancer activity of ethanolic extracts of fruits, seed, leaves and stem bark of these plants against four human cancer cell lines: breast cancer cell line (MCF-7), colon cancer cell lines (HT29 and HCT116), and cervical adenocarcinoma cell line (HeLa).

2. Materials and methods

2.1 Plant materials

Fresh and dried leaves, barks, fruits and seeds of Langkha*(B. macrophylla*) and Pra (*E. tapos*) were collected from Yala and Nakornsrithamaraj Provinces, Thailand, during August-October, 2015. Taxonomic identification was approved by the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand. The plant samples were divided into different parts as shown in Figure 1 and Figure 2.

2.2 Preparation of plant extracts

The leaves, fruit peel and fruit pulps of Langkha *(B. macrophylla*) and the leaves, bark, fruit pulps, seed skin and seeds of Pra (*E. tapos*) were cleaned with running tap water to remove any external material. The leaves and fruits of Langkha were divided into fresh and dried samples. For Pra, only the leaves were divided into fresh and dried samples. To prepare the dried samples, the plant parts were dried in a hot air oven at 40 to 50 ºC for 24 h. The fresh plant parts were cut into small pieces, whereas the dried plant samples were ground. Both fresh and dried samples of the plant parts (500 grams) were soaked in 95 % ethanol (2,000 mL) for seven days at room temperature. The supernatant was collected and filtered through a filter paper. The solid residue was repeatedly extracted 3 times with ethanol. The filtrates from each extraction were combined and the solvent was evaporated under reduced pressure using a rotary evaporator to yield the crude ethanolic extract of each plant part. All the crude extracts were weighed and kept in a refrigerator.

2.3 Determination of antioxidant activity

The antioxidant activities of ethanol crude extracts were analyzed by the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay with some modification. The solution of DPPH 0.1 mM in ethanol was prepared using absolute ethanol and stored in the dark before use. The stock solutions of the extracts were prepared at concentration of 100 μg mL^{-1}. Various concentrations of Trolox standard solutions were prepared using absolute ethanol as a solvent. This experiment was carried out with all samples in the concentrations of 20, 40, 60, 80 and 100 μg mL^{-1}. The sample solution (2,000 μL) was added immediately with 0.1 mM DPPH solution (2,000 μL). The mixture was incubated at room temperature for 30 minutes in the dark to check for the colorimetric change (from deep violet to light yellow) when DPPH was reduced. The absorbance of all samples was measured at 517 nm. Trolox was used as the reference standard. Radical scavenging capacity was calculated by using the formula (Equation 1):

$$\% \text{ Inhibition} = [(Ac - As) \times 100] / Ac \quad (1)$$

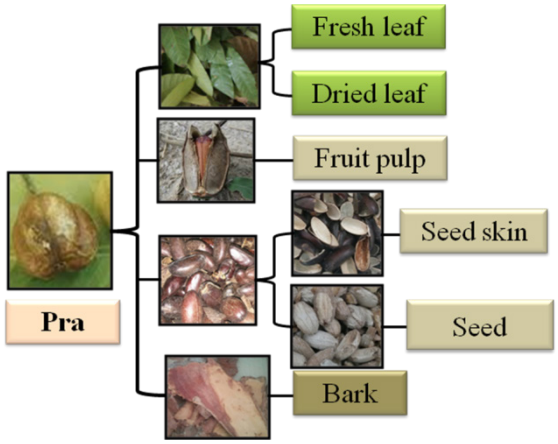

Figure 1 Parts used of Pra

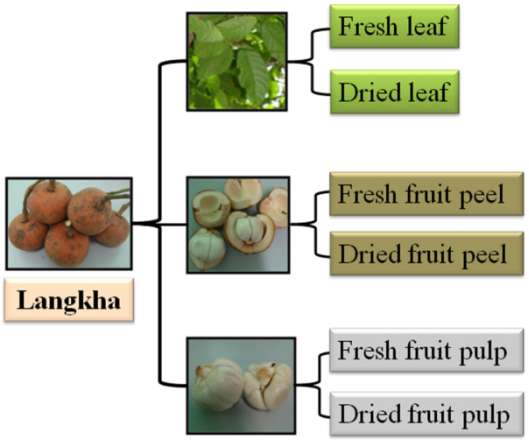

Figure 2 Parts used of Langkha

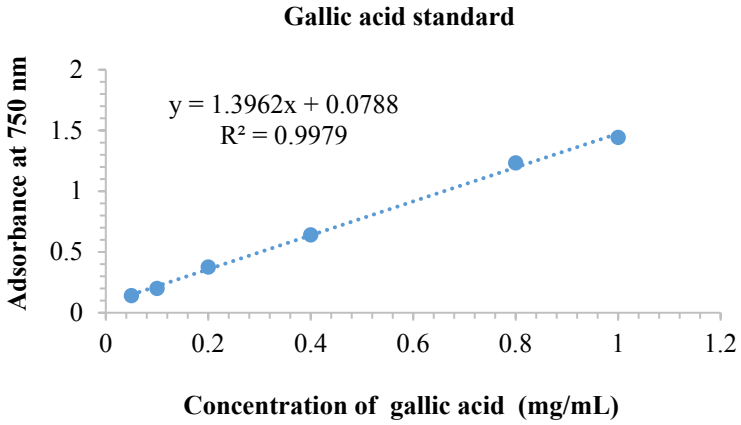

Figure 3 Calibration plot for phenolic determination

where Ac is absorbance of the control and As is absorbance of the test sample after incubation for 30 min. The values of % inhibitions were obtained from Equation 1. For the 50% Inhibitory Concentration (IC_{50}) evaluation of the extract, graphs showing the concentration of the test samples versus % inhibition (% DPPH reduction) were plotted. All measurements were carried out in triplicate and mean values were calculated.

2.4 Determination of total phenolic contents

The total phenolic contents of all ethanolic extracts were determined using Folin-Ciocalteu reagent according to the method described by Singleton and Rossi with some modifications. Standard solutions of gallic acid was prepared using absolute ethanol as solvent at various concentrations (1.0, 0.8, 0.4, 0.2, 0.1 and 0.05 μg mL^{-1}). A reagent blank was prepared using deionized water. The 100 μL of ethanolic extracts were mixed with 500 μL of Folin & Ciocalteu's phenol reagent and 1,000 μL of 20% Na_2CO_3. The mixture was shaken and placed in the dark at room temperature for 1 hour before measuring the absorbance at 760 nm. The calibration curve was prepared using gallic acid with a concentration range between 0.05 to 1.0 μg mL^{-1}. Total phenolic content was calculated and reported as mg gallic acid equivalent (GAE)/1 g of crude extracts by comparison with the gallic acid standard curve (Figure 3). All samples were analyzed in three replications.

2.5 Screening for anticancer activity

The MTT cytotoxicity assay was performed following protocol describe by Senawong *et al.* with some modifications [16]. Cells were seeded at 8,000 cells in each well of a 96-well plate in 100 μL of fresh culture medium and were allowed to attach overnight. Cells were tested with ethanolic extracts at 100 μg $mL^{-1}$for 24, 48 and 72 h, respectively. After each period of exposure, 10 μL of MTT (3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl-2H-tetrazoliumbromide) (5 μg mL^{-1}) was added per well and incubated for 2 h at 37 °C. The formazan dye was then dissolved with 100 μL of DMSO (dimethyl sulphoxide). The absorbance

Table 1 Antioxidant activity of the extracts from Langkha *(B. macrophylla*) and Pra (*E.tapos*) by DPPH assays

Plant	Part used	IC_{50} (μg mL^{-1})	Plant	Part used	IC_{50} (μg mL^{-1})
Pra	Fresh leaf	0.10±0.00	Langkha	Fresh leaf	>1,000
	Dried leaf	20.00±0.01		Dried leaf	1.70 ±0.03
	Dried fruit pulp	84.00±0.03		Fresh fruit peel	14.00±0.05
	Seed skin	21.00±0.01		Dried fruit peel	8.00±0.01
	Seed	>1,000		Fresh fruit pulp	66.00±0.01
	Bark	9.00±0.01		Dried fruit pulp	612.00±0.05
	Trolox	3.10±0.01			

Trolox was used as the reference standard.
* Values are given as mean ± S.D. of triplicate experiments.

was measured at 550 and 655 nm using a microplate reader (Bio-Rad Laboratories, Hercules, CA). The absorbance at 655 nm was used as a reference wavelength. Each assay was replicated three times. The survival of cancer cell lines was reflected by the percentage of cell viability.

3. Results and discussion

3.1 Antioxidant activity

Table 1 shows the antioxidant activities of all parts of Pra (*E. tapos*) and Langkha *(B. macrophylla*) evaluated in this study. Antioxidant activities of the ethanol extracts (leaves, barks, fruits and seeds) of Langkha and Pra were determined by using DPPH assay. The tested samples can reduce the purple-colored radical DPPH into yellow colored DPPH-H. The IC_{50}values of the plant extracts were evaluated referencing to the calibration curve. The results were compared with the antioxidant standard, Trolox as shown in Table 1. For Pra, the antioxidant activities (IC_{50}) of thee thanolic extracts were found varying from 0.10±0.00 to 84.00±0.03 μg mL^{-1}. The ethanolic extracts prepared from the fresh leaves of Pra presented the most potent antioxidant activities with the lowest IC_{50} values of 0.10±0.00 μg mL^{-1}, followed by the extracts from barks and dried leaves with the IC_{50} values of 9.00±0.01 and 20.00±0.01 μg mL^{-1},respectively. Whereas the ethanol extract prepared from the dried fruit pulp of Pra presented poor antioxidant activity, with the IC_{50}value of 84.00±0.03 μg mL^{-1}.

In addition, the antioxidant activities (IC_{50}) of the ethanol extracts from various parts of Langkha were found varying from 1.70±0.03 to >1,000 μg mL^{-1}. The extracts prepared from the dried leaves of Langkha had the highest antioxidant capacities with the lowest IC_{50} values of 1.70±0.03 μg mL^{-1}, followed by the extracts from dried fruit peel and fresh fruit peel of Langkha fruits, with the IC_{50} values of 8.00±0.01 and 14.00±0.05 μg mL^{-1}, respectively. Whereas the ethanol extract prepared from the fresh leaves of Langkha exhibited poor antioxidant activities, with the IC_{50}>1,000 μg mL^{-1}.

According to the results, it can be concluded that the extracts from the leaves (either fresh or dried leaves) of Pra and Langkha possessed higher antioxidant capacities than that of the extracts from the other parts of these fruits. These results were consistent with a number of studies reported that the extracts prepared from the leaves or bark of plants presented higher antioxidant capacities than the extracts prepared from the flowers, fruits, roots, stems and barks of the plants [10, 11, 13]. In addition, Jakkrit also found that the water extracts prepared from the fruit peel of Lankha and Champuling had higher antioxidant capacities than those prepared from their fruit pulpsagainst DPPH [17].

3.2 Total Phenolic Content (TPC)

Quantitative determination of the concentration of phenolic compound in the ethanol extracts of leaf, bark, fruit and seed from Langkha (*B. macrophylla*) and Pra (*E. tapos*) was carried out using spectrophotometric methods. The total phenolic contents of the extracts were determined using the Folin Ciocalteu reagent. The results were reported in Table 2. These results are an estimation of total phenolic content in chemical equivalents (gallic acid). The amounts of TPC of these ethanol extracts were found varying from 1.06 to 198.77 mg GAE/1 g of sample. Among all parts of the Langkha extracts, the extracts of the fresh leaves had the highest amount of TPC (161.17 mg GAE/1 g of sample), followed by the extract of its fresh fruit peel (151.33 mg GAE/1 g of sample), whereas the lowest total phenolic content was found in its dried pulp (1.06 mg GAE/1 g of sample).

For Pra, the amount of TPC of all parts of tested extract vary from minimum to maximum (0.76-198.77 mg GAE/1 g of sample). The highest amount of TPC was found in its dried leaf extract (198.77 mg GAE/1 g of sample), whereas the lowest level was found in

Table 2 Total phenol contents of the extracts of Langkha (*B. macrophylla*) and Pra (*E. tapos*).

Plants	Parts used	Total phenolic content (mg GAE/1 g of sample)	Plants	Parts used	Total phenolic content (mg GAE/1 g of sample)
Pra	Fresh leaf	161.16±6.81	Langkha	Fresh leaf	161.17±0.32
	Dried leaf	198.77±6.26		Dried leaf	95.45±2.60
	Dried fruit pulp	195.64±9.42		Fresh fruit peel	151.33±17.27
	Seed skin	69.02±1.66		Dried fruit peel	137.78±7.21
	Seed	0.76±0.42		Fresh fruit pulp	5.81±1.78
	Bark	121.86±4.36		Dried fruit pulp	1.06± 0.12

All analyses are the mean of triplicate measurements ± standard deviation (SD).

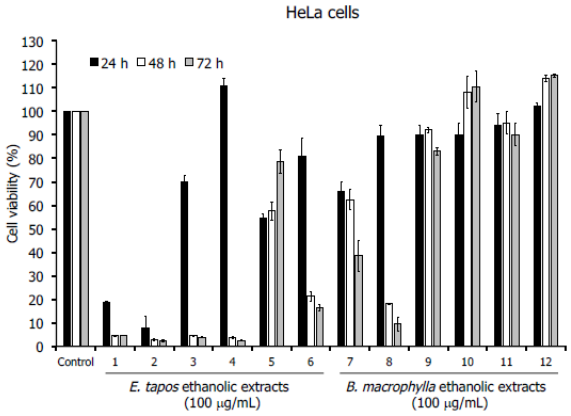

Figure 4 Graphical representation of antiproliferative activity of 12 ethanolic extracts (*E. tapos* parts; 1 = fresh leaf, 2 = dried leaf, 3 = dried fruit pulp, 4 = seed skin, 5 = seed, 6 = bark, and *B. macrophylla* parts; 7 = fresh leaf, 8 = dried leaf, 9 = fresh fruit peel, 10 = dried fruit peel, 11 = fresh fruit pulp, 12 = dried fruit pulp**)** against human cervical cancer cell line (HeLa cells). The bar graphs are expressed as mean ± S.D. (n = 6)

the seeds (0.76 mg GAE/1 g of sample). These results indicated that the ethanolic extracts from the leaves of both Pra and Langkha had the highest amount of phenolic compound comparing to the other parts of these plants.

The variation among TPC may be assumed due to the presence of different types of phenol and other constituents in different parts of plants. It was found that the leaves of plants contained high amounts of TPC [1, 12, 18]. The results from this study were consistent with previous studies, which the leaves of Langkha and Pra contained high amounts of TPC. In addition, the antioxidant capacities of the fruits of Langkha and Praare in direct proportion to their amount of total phenolic. These findings are according to some studies exhibited that the antioxidant activities of the fruits are in direct proportion to their amount of total phenolic [5, 10, 12, 13, 19].

3.3 Anticancer activity

The anticancer activities of 12 ethanolic extracts (*E. tapos* parts; 1 = fresh leaf, 2 = dried leaf, 3 = dried fruit pulp, 4 = seed skin, 5 = seed, 6 = bark, and *B. macrophylla* parts; 7 = fresh leaf, 8 = dried leaf, 9 = fresh fruit peel, 10 = dried fruit peel, 11 = fresh fruit pulp,12 = dried fruit pulp) against human cervical cancer cell line (HeLa cells), human colon cancer cell lines (HT29 and HCT116 cells), human breast cancer cell line (MCF-7 cells), and a non-cancer cell line (African green monkey kidney epithelial cells; Vero cells) were determined using the MTT assay. The ethanolic extracts of all plant parts at 100 μg/mL were used for this anticancer screening.

The results showed that all plant parts except seed of *E. tapos* exhibited significant anticancer activity against HeLa cells at exposure times of 48 and 72 h (Figure 4). Specifically, dried leaf ethanolic extract showed the most toxicity to HeLa cells with cell viabilities of 7.99 ± 4.92, 2.97 ± 0.49, 2.56 ± 0.44 % at exposure times of 24, 48 and 72 h, respectively. In contrast, only one plant part (dried leaf) of *B. macrophylla* inhibited HeLa cell growth greater than 70% at exposure time of 48 and 72 h (Figure 4). Fresh leaf, dried leaf, dried fruit pulp and seed skin ethanolic extracts of *E. tapos* significantly inhibited

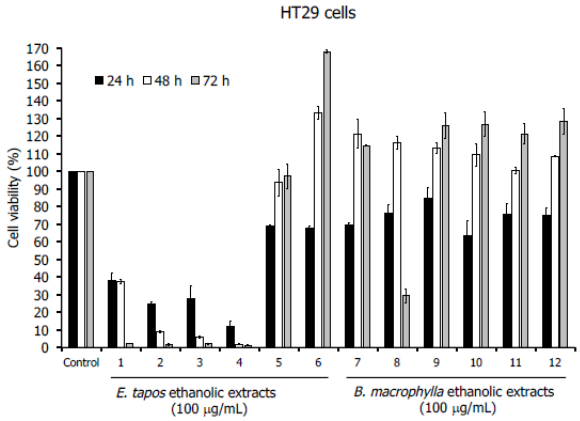

Figure 5 Graphical representation of antiproliferative activity of 12 ethanolic extracts (*E. tapos* parts; 1 = fresh leaf, 2 = dried leaf, 3 = dried fruit pulp, 4 = seed skin, 5 = seed, 6 = bark, and *B. macrophylla* parts; 7 = fresh leaf, 8 = dried leaf, 9 = fresh fruit peel, 10 = dried fruit peel, 11 = fresh fruit pulp, 12 = dried fruit pulp**)** against human colon adenocarcinoma cell line (HT29 cells). The bar graphs are expressed as mean ± S.D. (n = 6)

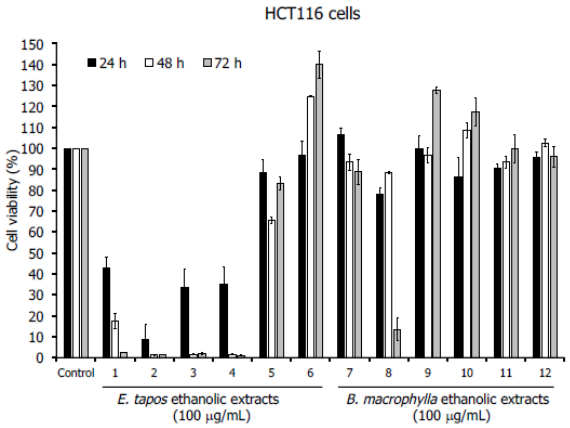

Figure 6 Graphical representation of antiproliferative activity of 12 ethanolic extracts (*E. tapos* parts; 1 = fresh leaf, 2 = dried leaf, 3 = dried fruit pulp, 4 = seed skin, 5 = seed, 6 = bark, and *B. macrophylla* parts; 7 = fresh leaf, 8 = dried leaf, 9 = fresh fruit peel, 10 = dried fruit peel, 11 = fresh fruit pulp, 12 = dried fruit pulp**)** against human colorectal carcinoma cell line (HCT116 cells). The bar graphs are expressed as mean ± S.D. (n = 6)

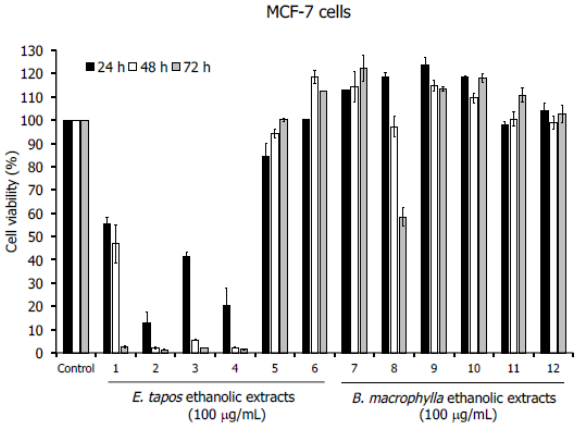

Figure 7 Graphical representation of antiproliferative activity of 12 ethanolic extracts (*E. tapos* parts; 1 = fresh leaf, 2 = dried leaf, 3 = dried fruit pulp, 4 = seed skin, 5 = seed, 6 = bark, and *B. macrophylla* parts; 7 = fresh leaf, 8 = dried leaf, 9 = fresh fruit peel, 10 = dried fruit peel, 11 = fresh fruit pulp, 12 = dried fruit pulp**)** against human breast adenocarcinoma cell line (MCF-7 cells). The bar graphs are expressed as mean ± S.D. (n = 6)

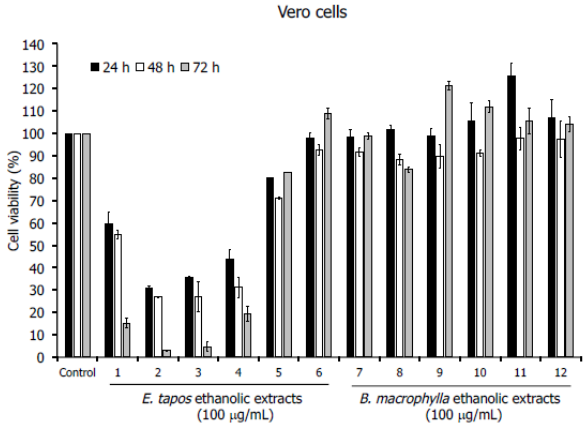

Figure 8 Graphical representation of antiproliferative activity of 12 ethanolic extracts (*E. tapos* parts; 1 = fresh leaf, 2 = dried leaf, 3 = dried fruit pulp, 4 = seed skin, 5 = seed, 6 = bark, and *B. macrophylla* parts; 7 = fresh leaf, 8 = dried leaf, 9 = fresh fruit peel, 10 = dried fruit peel, 11 = fresh fruit pulp, 12 = dried fruit pulp**)** against non-cancer cell line (Vero cells). The bar graphs are expressed as mean ± S.D. (n = 6)

the growth of HT29 (Figure 5), HCT116 (Figure 6), and MCF-7 (Figure 7) cells, especially at exposure time of 72 h. The dried leaf ethanolic extract of *B. macrophylla* exhibited significant toxicity to HeLa (Figure 4), HT29 (Figure 5) and HCT116 (Figure 6) cells only at exposure time of 72 h. None of the *B. macrophylla* ethanolic extracts showed toxicity to MCF-7 cells (Figure 7). The less cytotoxicity against a non-cancer cell line (Vero cells) of *E. tapos* ethanolic extracts was observed (Figure 8). While all *B. macrophylla* ethanolic extracts showed no toxicity to Vero cells (Figure 8). To the best of our knowledge, the significant cytotoxicity against human carcinoma cell lines of *E. tapos* and *B. macrophylla* is reported for the first time in this work. However, further study on their half maximal inhibitory concentration (IC_{50}) values, apoptosis and cell cycle arrest induction activities is required in order to understand more on their anticancer activity. Mechanistically, bioactive components of these plant parts may prevent cancer through various mode of action such as anti-oxidant activity, anti-proliferative activity, apoptosis induction activity, anti-invasive activity and subcellular signaling pathways [20, 21]. The individual bioactive components are also required to be identified, although the actions of individual pure compound alone may lose its bioactivity or may not act the same way as it is in crude extracts.

4. Conclusions

The dried leaf ethanolic extract of Pra and the fresh leaf ethanolic extract of Langkha exhibited the highest amount of total phenolic content (198.77±6.26 and 161.17 ± 0.32 mg GAE/1 g of sample), respectively compared to the other parts of the plants. Whereas, the pulp ethanolic extract of Langkha and the seed extract of Pra contained the lowest amounts of TPC (0.76±0.42 and 1.06±0.12mg GAE/1 g of sample, respectively). In addition, the ethanolic extracts from the leaves of both plants exhibited the highest antioxidant activities determined by DPPH assay. The dried leaf ethanolic extract of *E. tapos* had the highest cytotoxicity against HeLa, HCT116, and MCF-7 cells, whereas its seed skin ethanolic extract had the highest cytotoxic activity against HT29 cells. Among all ethanolic extracts of *B. macrophylla*, its dried leaf ethanolic extract had the highest cytotoxicity against all cancer cell lines tested. The leaf ethanolic extracts of *E. tapos* had greater anticancer activity than those of *B. macrophylla*. Accordingly, parts of these plants could be useful for application in areas such as alternative medicine for cancer treatment and functional food for dietary prevention of cancer.

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