INVESTIGATION OF SOME BIOACTIVITIES OF *Peperomia pellucida* L. (THIT-YAY-GYI) AND *Enhydra fluctuans* L. (KANA-PHAW)

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Abstract

In the present work, *Peperomia pellucida* L. (Thit–yay–gyi) and *Enhydra fluctuans* L. (Kana-phaw) were chosen to investigate some bioactivities such as antimicrobial, antioxidant, antitumor, anticancer or antiproliferative activities. The antimicrobial activity of different crude extracts from the Thit-yay-gyi and Kana-phaw was determined by agar well diffusion method. Ethyl acetate extracts of both plants samples showed the antimicrobial activity on all tested microorganisms with inhibition zone diameters in the range between 18 mm-35 mm for Thit-yay-gyi and 21 mm-35 mm for Kana-phaw. The antioxidant activity of ethanol and watery crude extracts of Thit-yay-gyi and Kana-phaw were investigated by using DPPH free radical scavenging assay. The antioxidant activity of Kana-phaw ethanol extract (IC_{50} = 22.53 µg/mL) was found to be highest followed by Thit-yay-gyi ethanol extract (IC_{50} = 37.75 µg/mL), then by Kana-phaw watery extract (IC_{50} = 40.63 µg/mL) and Thit-yay-gyi watery extract (IC_{50} = 44.81 µg/mL). In addition, the cytotoxicity of EtOH and H_2O crude extracts from Thit-yay-gyi and Kana-phaw were evaluated by brine shrimp cytotoxicity bioassay. The cytotoxicity of Thit-yay-gyi EtOH extract (LD_{50}=68.2µg/mL) showed more cytotoxic effect than other crude extracts. The antitumor activity of crude extracts was pre-screened by PCG test. It was found that they can inhibit the growth of tumor in the rangs of concentration 1 g/mL, 0.5 g/mL and 0.25 g/mL, 0.12 g/mL. Furthermore, antiproliferative activity of EtOH and H_2O extracts of Kana-phaw and Thit-yay-gyi was investigated by using seven human cancer cell lines. Antiproliferative activity of two extracts were found to be in order of EtOH extract of Thit-yay-gyi>EtOH extract of Kana-phaw> H_2O extract of Thit-yay-gyi> H_2O extract of Kana-phaw. Among the tested crude extracts, EtOH extract of Thit-yay-gyi were found to be more potent than other crude extracts. Therefore, it could be inferred that EtOH extract of Thit-yay-gyi possessed the higher antiproliferative activity than Kana-phaw. By silica gel column chromatographic separation technique, compound A (kaurenoic acid) (white crystal, 0.015 %, m.pt. 172 °C) from PE extract of Kana-phaw was isolated. The isolated compound was identified by physicochemical

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properties and modem spectroscopic technique such as FT IR, NMR, HMBC and ESI MS spectrometry as well as by comparing with their reported data. Isolated compound A (kaurenoic acid) was evaluated by brine shrimp cytotoxicity bioassay. Compound A (kaurenoic acid) showed 50 % death of brike shrimp at concentration of 0.00001 μg/mL.

Keywords: *Peperomida pellucida, Enhydra flucuans*, antimicrobial activity, antioxidant activity, DPPH, brine shrimp cytotoxicity, antitumor activity, antiproliferative activity

Introduction

Peperomida pellucida L. and *Enhydra flucuans* L. belongs to the families Piperaceae and Asteraceae respectively and are called Thit-yay-gyi and Kana-phaw in Myanmar. These plants have been used for treatments of antifungal, antioxidant, anticancer, antiflammatory and antidiarrheal. It is a remarkable medicinal plants that grow wild in India, China and throughout South East Asia (Sarma, 2014).

An antimicrobial activity is an agent that kills microorganisms or inhibits their growth. Medicinal plants represent a rich source of antimicrobial agents. Antioxidants means “against oxidation”. Antioxidants, also known as “free radical scavengers” are compounds that either reduce formation of free radicals or react with an neutralize them. Cytotoxicity is the quality of being toxic to cell. Example of toxic agents are an immure cell or some types of venom.

The extra cells can form a mass called a tumor. Tumors can be benign or malignant. Benign tumors are not cancer while malignant ones are. Cells from malignant tumors can invade nearby tissues. They can also break away and spread to other parts of body. Cancer is not just one disease but many diseases. Most cancers are named for where they start.

Antiproliferative activity is the activity relating to a substance used to prevent or retard the spread of cells, especially malignant cells, into surrounding tissues. Antiproliferative activity were studied *in vitro* using human cancer cell lines.

Materials And Methods

Sample collection

Thit-yay-gyi has been collected from Yangon University Campus and Kana-phaw has been collected from Hlaingtharyar Township, Yangon Region. Its scientific name has been identified at Department of Botany, University of Yangon.

Extraction and Preparation of Crude Extracts from Thit-yay-gyi and Kana-phaw

The dried powdered sample (500 g) was defatted with (2 L) for 95 % EtOH for one week at room temperature by percolation method and followed by filtration. This procedure was repeated for three times. The total combined filtrate was evaporated under reduced pressure by means of a rotary evaporator. Consequently, the defatted EtOH extract was obtained. The defatted sample was then partitioned with PE: H_2O (1 : 2) (300 mL). The combined petroleum ether layers were concentrated under reduced pressure by means of a rotary evaporator. Consequently, pet-ether soluble extract was obtained. The defatted residue was further partitioned between ethyl acetate with water. The combined ethyl acetate layers were concentrated by means of a rotary evaporator. After that ethyl acetate soluble extract was obtained. In this way, 95 % ethanol, pet-ether and ethyl acetate soluble extracts of both plants samples were prepared.

Screening of Antimicrobial Activity by Agar Well Difussion Method

Antimicrobial activities of different crude extracts were tested on six microorganism such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Escherichia coli* and *Candida albicans* species at the Pharmaceutical Research Department (PRD), Ministry of Industry, Yangon.

To a mixture of 1 g of meat extract, 1 g of peptone, 0.5 g of NaCl and 1.5 g of agar powdered were placed in a sterilized 250 mL conical flask, 100 mL of sterile distilled water were added to obtain nutrient agar medium. The resulting mixture was heated to dissolve the contents. Then the pH of the

resulting solution was adjusted to 7.2 with 0.1 M NaOH solution. It was sterilized in an autoclave at 121 °C for 15 min. About 20-25 mL of agar medium contained test organisms were poured into the sterile petri-dishes under aseptic condition near the flame of the spirit burner and left the agar solid, the cork borer about 10 mm in diameter was sterilized and made a well in the agar plate previously described. Then the extract samples were introduced into the well (about 0.2 mL). They were then incubated at 36 °C for 24 h. The formation of inhibition zone around the well was observed. This observation indicates the presence of antimicrobial active compounds in the extract.

Screening of Antioxidant Activity by DPPH Free Radical Scavenging Assay

In this experiment, the antioxidant activity of ethanol and watery extracts of two selected plant samples was studied by DPPH free radical scavenging assay.

DPPH free radical scavenging activity was determined by UV-visible spectrophotometric method according to the procedure described by Marinova and Batchvarov (2011). The control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol in the brown bottle. The sample solution was also prepared by mixing 1.5 mL of 0.002 % DPPH solutions and 1.5 mL of test sample solution. These bottles were incubated at room temperature and were shaken on shaker for 30 min. After 30 min, the absorbance values of these solutions were measured at 517 nm by using UV-visible spectrophotometer. The percent radical scavenging activity was calculated by the following equation.

$$\% \text{ RSA} = [(A_{DPPH} - A_{Sample}) - A_{Blank} / A_{DPPH}] \times 100$$

where,

% RSA = % radical scavenging activity
A_{DPPH} = absorbance of DPPH in EtOH solution
A_{sample} = absorbance of sample + DPPH solution
A_{Blank} = absorbance of sample + EtOH solution

The antioxidant power (IC_{50}) is expressed as the test substances concentration (μg/mL) that result in a 50% reduction of initial absorbance of DPPH solution and that allow to determine the concentration. IC_{50} (50% inhibitory concentration) value were calculated by linear regressive excel program. The standard deviation was also calculated by the following equation.

$$\text{Standard Deviation (SD)} = \sqrt{\frac{(\bar{x} - x_1)^2 + (\bar{x} - x_2)^2 + ...(\bar{x} - x_n)^2}{(n-1)}}$$

Investigation of Cytotoxicity by Brine Shrimp Lethality Bioassay

Cytotoxicity of crude extracts from Kana-phaw and Thit-yay-gyi was investigated by brine shrimp lethality bioassay according to the procedure described by Dockery and Tomkins, 2000.

Artificial sea water (9 mL) and (1 mL) of different concentrations of samples and standard solutions were added to each chamber. Alive brine shrimps (10 nauplii) were then taken with pasteur pipette and placed into each chamber. They were incubated at room temperature for about 24 h. After 24h, the number of dead or survival brine shrimps was counted and 50 % lethality dose (LD_{50}) was calculated (Dockery and Tomkins, 2000). The control solution was prepared as the above procedure by using distilled water instead of sample solution.

Screening of Antitumor activity by Potato Crown Gall (PCG) Test or Potato Disc Assay Method

Fresh potato tubers were obtained from Hledan market, Kamayut Township in Yangon Region and were used within 48 hours before transfer to the laboratory.

Tubers of moderate size were surface-sterilized by immersion in 50% sodium hypochlorite (Clorox) for 20 min. The ends were removed and soaked for 10 min more in Clorox. A core of the tissue was extract from each tuber by using surface-sterlized (ethanol and flame) 1.0 cm wide cork borer and 2 cm pieces were removed from each end and discarded and the remainder of

the cylinder is cut into 0.5 cm thick dics with a surface-sterilized cutter. The discs were then transferred to agar plates (1.5 g of agar was dissolved in 100 mL distilled water, autoclaved for 20 min at 121 °C, 20 mL poured into each petri dish). Each plate contained four potato discs and 4 plates were used for each sample dilution.

Sample (0.125, 0.25 g, 0.5 g and 1 g) of crude extracts were respectively dissolved in dimethyl sulphoxide (DMSO) (2mL) and filtered through Millipore filter (0.22 μm) into sterile tube. This solution (0.5 ml) was added to sterile distilled water (1.5 mL) and broth culture (2 mL) of *Agrobacterium tumefaciens* strains .

Controls were made in this way, DMSO (0.5 mL) and sterile distilled water (1.5 mL) were added to the tube containing broth culture (2 mL) of *Agrobacterium tumesfaciens* strains.

By using a sterile disposable pipette, one drop (0.5 mL) from these tube was used to inoculate each potato discs spreading it over the discs surface. After inoculation, petri dishes were sealed by paraffin and incubated at 27-30 °C for 14. Tumors were observed on potato discs after 14 under stereo-microscope followed by staining with Lugol's solution (5 % I_2 and 10 % KI) after 30 min and compared with control. The anti-Agrobacterium tumefaciens activity was examined by observation of crown gall produced or not.

Screening of Antiproliferative activity on Cell Lines

Antiproliferative activity of Kana-phaw and Thit-yay-gyi was studied *in vitro* using cancer cell lines (lung, cervix, breast, normal human fibroblast, liver, pancreatic, pancreas ductual adenocarcinoma) at Division of Natural Product Chemistry, Institute of Natural Medicine, University of Toyama, Japan.

The *in vitro* antiproliferative activity of the crude extracts was determined by the procedure described (Win *et al.,* 2015). Briefly, each cell line was seeded in 96-well plates (2×10^3 per well) and incubated in the respective medium at 37 °C under 5 % CO_2 and 95 % air for 24 hs. After the cells were washed with PBS (Nissui Pharmaceuticals), serial dilutions of the

tested samples were added. After 72 hrs incubation, the cells were washed with PBS and 100 μL of medium containing 10 % WST-8 cell counting kit (Dojindo; Kumamoto, Japan) solution was added to the wells. After 2 hrs incubation, the absorbance at 450 nm was measured. The concentrations of the serial dilutions of the tested samples were 100, 10, 1 μg/mL for crude extract, 100, 10, 1 μM for isolated compounds and 10, 1, 0.1 mM for positive control. Cell viability was calculated from the mean values of the data from three wells using the equation below and antiproliferative activity was expressed as the IC_{50} (50 % inhibitory concentration) value. 5-fluorouracil was used as positive control.

$$(\%)\ \text{Cell viability} = 100 \times \frac{\left\{\text{Abs}_{(\text{test samples})} - \text{Abs}_{(\text{blank})}\right\}}{\left\{\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{blank})}\right\}}$$

Extraction and Isolation of Chemical Constituents from Kana-phaw

PE extract (5 g) was mixed with silica gel. The mixture was allowed to evaporate with continuous agitation so that freely flowing of dry silica gel on which the sample was uniformly absorbed. The resulting powdered mixture was added to the column using small long necked funnel. The top of the layer was wet with solvent that had previously been allowed to remain above the gel by opening the tap. Some adsorbed gel sticking on the inner wall was washed down with the solvent. The column was then completely filled with adding the solvent system PE:EtOAc (99:1) and fraction was started. The tap was opened and the fractions were collected at the rate of one drop per seconds. Gradient elution was performed successively with PE:EtOA (99:1, 98:2, 95:5, 9:1, 5:1, 2:1, 1:1) v/v and a total of 109 fractions were collected.

The fractions which showed similar TLC behaviours were combined to give successive three main fractions $F_{I\ (1\text{-}20)}$, $F_{II\ (21\text{-}55)}$ and $F_{III\ (56\text{-}109)}$. The collected fractions were monitored by TLC behaviours. After the solvents have been evaporated, fractions F_I and F_{III} were obtained as a mixture. Fraction F_{II} was washed with acetone and purified by crystallization from pet ether to give 30 mg (0.01 %) of compound A (Kaurenoic acid) as white crystal.

Structural Identification

The structures of isolated compound was identified by modern spectroscopic techniques such as FT IR, NMR, HMBC and ESI MS. FT IR spectra of isolated compounds were recorded at Department of Chemistry, University of Yangon. ^{1}H NMR, ^{13}C NMR, HMBC and ESI MS spectra of isolated compound A were measured at Department of Organic and Biomolecular Chemistry, Georg-August University, Goettingen, Germany.

Results and Discussion

Antimicrobial Activity

The antimicrobial activity of Thit-yay-gyi and Kana-phaw was screened by agar well diffusion method on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas aeruginosa* and, *Candida albicans* (Figure 1). The resultant inhibition zone diameters are described in Table 1. The larger the inhibition zone diameters, the higher the antimicrobial activity.

According to the results, it was found that EtOAc extract of Thit-yay-gyi and Kana-phaw exhibited more potent antimicrobial activity against all test species with inhibition zone diameter between 18 mm and 35 mm for Thit-yay-gyi and 21mm and 35mm for Kaka-phaw. But Thit-yay-gyi of EtOH extract and Kana-phaw of PE extracts showed less activity.

Therefore, EtOH extract of Thit-yay-gyi and PE extract of Kana-phaw exhibited less antimicrobial activity and EtOAc extract of Thit-yay-gyi and Kana-phaw had the highest antimicrobial activity.

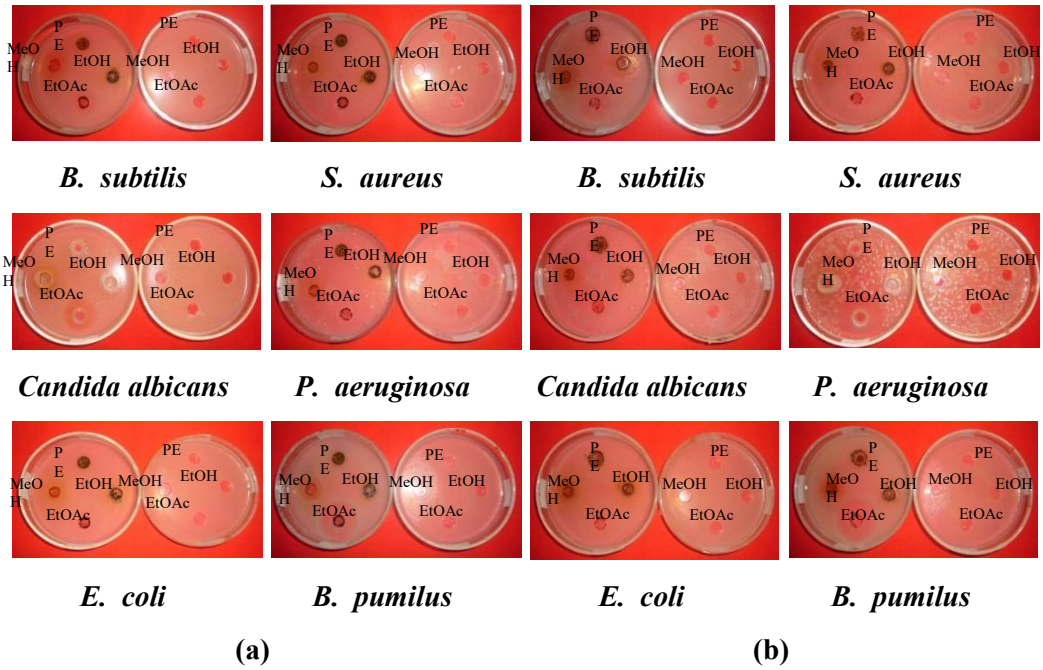

Figure 1: Antimicrobical activity of crude extracts of
(a) Thit-yay-gyi and
(b) Kana-phaw

Table 1. Inhibition Zone Diameters (mm) of Crude Extracts of Thit-yay-gyi and Kana-phaw against 6 Microorganisms

Sample	Solvent	***B. subtilis*** **(mm)**	***S. aureus*** **(mm)**	***P. aeruginosa*** **(mm)**	***B. pumilus*** **(mm)**	***Candida albicans*** **(mm)**	***E. coli*** **(mm)**
Thit-yay-gyi	PE	13 (+)	-	-	-	-	24 (+++)
	MeOH	17 (++)	32 (+++)	-	19 (++)	-	19 (++)
	EtOAc	18 (++)	30 (+++)	19 (++)	**35 (+++)**	25 (+++)	25 (+++)
	EtOH	-	-	-	-	-	-
Kana-phaw	PE	-	20 (+++)	-	-	-	-
	MeOH	20 (+++)	32 (+++)	-	21 (+++)	15 (++)	19 (++)
	EtOAc	21 (+++)	32 (+++)	30 (+++)	**35 (+++)**	14 (+)	25 (+++)
	EtOH	-	17 (++)	15 (++)	-	-	-
Control	PE	-	-	-	-	-	-
	MeOH	-	-	-	-	-	-
	EtOAc	-	-	-	-	-	-
	EtOH	-	-	-	-	-	-

Agar well - 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

Antixoidant Activiy

Antioxidant compounds in plant play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease.

The antioxidant activity of EtOH and H_2O crude extracts was evaluated by DPPH (2, 2- diphenyl-1-picrylhydrazyl) radical scavenging assay. The radical scavenging activity of crude extracts were expressed in terms of % RSA and IC_{50} (50 % inhibitory concentration). These results are shown in Table 2.

It was found that increasing the concentration of samples, the % RSA was also increased. These results were shown in Figure 2. The antioxidant activity of ethanol and water extracts of Kana-phaw (IC_{50} = 22.53 μg/mL and 40.63 μg/mL) was found to be more potent than of Thit-yay-gyi (IC_{50} = 37.75 μg/mL and 44.81 μg/mL).

Table 2: Radical Scavenging Activity (% RSA) and IC_{50} Values of Crude Extracts from Kana-phaw and Thit-yay-gyi and Standard Ascorbic Acid on Antioxidant Activity

Tested sample	%RSA±SD at Different concentration (μg/mL)					
	12.5	25	50	100	200	IC_{50} (μg/mL)
Kana-phaw (Watery)	42.90±0.80	47.30±0.20	52.41±1.00	57.5±1.41	63.35±1.21	40.63
Thit-yay-gyi (Watery)	46.05±1.03	48.87±0.62	56.87± 0.62	60.67±0.21	74.29±0.60	44.81
Kana-phaw (EtOH)	49.42±0.21	51.16±0.21	57.85±4.73	69.77±0.82	90.41 ±3.29	22.53
Thit-yay-gyi (EtOH)	37.94±0.21	48.11 ±0.41	51.45±0.21	55.67 ±0.21	61.19 ±0.31	37.75
Vitamin C	49.19±2.44	52.55±0.70	55.38±0.70	56.59±0.00	64.25±8.36	15.55

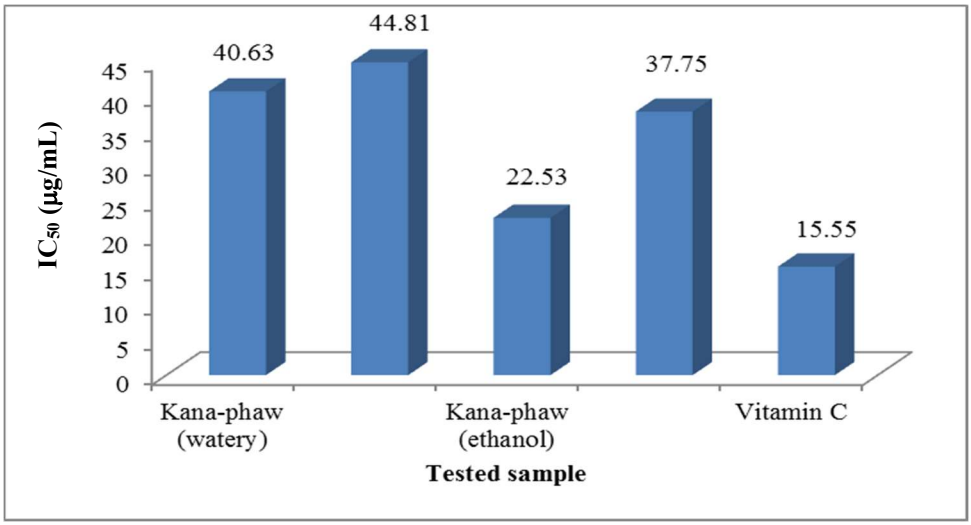

Figure 2: A bar graph of IC_{50} (µg/mL) of EtOH and watery crude extracts of Kana-phaw and Thit-yay-gyi

Cytotoxicity

The cytotoxicity of ethanol and watery of Kana-phaw and Thit-yay-gyi were evaluated by brine shrimp cytotoxicity bioassay. This bioassay is general toxicity screening for bioactive plants and their derivatives. A model animal that has been used for this purpose is the brine shrimp, *Artemia salina* (Tawaha, 2006).

The cytotoxicity of crude extracts were expressed in terms of mean ± SEM (standard error mean) and LD_{50} (50 % Lethality Dose) and the results are shown in Table 3. In this experiment, standard potassium dichromate ($K_2Cr_2O_7$) and caffeine were chosen because $K_2Cr_2O_7$ is well-known toxicity in this assay and caffeine is a natural product.

As shown in Table 3, the cytoxicity of EtOH extracts of Kana-phaw and Thit-yay-gyi were more toxic to brine shrimp than the watery extracts. The LD_{50} values of EtOH extract were 68.2 µg/mL in Thit-yay-gyi and 989 µg/mL in Kana-phaw**.** On the other hand, the LD_{50} values of watery crude extracts were 86.04 µg/mL in Thit-yay-gyi and 224.66 µg/mL in Kana-phaw. The LD_{50} values of standard $K_2Cr_2O_7$ and caffeine are 43.74 µg/mL and 1000 µg/mL respectively.

Table 3: Cytotoxicity of Different Doses of Crude Extracts of Thit-yay-gyi and Kana-phaw against *Artemia salina* (Brine Shrimp)

Sample	No. of Dead of Brine Shrimp (Mean ± SEM) in Various Concentration (µg/mL)					
	0.1	**1**	**10**	**100**	**1000**	**LD_{50} (µg/mL)**
Thit-yay-gyi(EtOH)	0	0	1.33 ± 1.15	7.00 ± 2.00	9.44 ± 2.89	**68.2**
Thit-yay-gyi(Watery)	2.60 ± 4.00	2.98 ± 1.53	3.33 ± 0.58	5.33 ± 0.58	5.67 ± 2.89	86.04
Kana-phaw(EtOH)	0.23 ± 0.58	0.82 ± 0.58	2.00 ± 0.58	2.33 ± 1.53	5±5.46	989
Kana-phaw(Watery)	0.43 ± 1.53	2.35 ± 0.58	3.34 ± 1.15	4.66 ± 1.15	7.66 ± 0.58	224.66
*$K_2Cr_2O_7$	0.52 ± 2.00	0.67 ± 0.66	2.00 ± 2.00	10.00 ± 0.00	10.00 ± 0.00	43.74
*Caffeine	0	0	2.33 ± 1.20	3.00 ± 0.58	5.00 ± 1.16	1000

* Used as Cytotoxic Standard

Antitumor Activity

In this study, tumor producing bacteria, *Agrobacterium tumefaciens* was first isolated from the gall tissues of *Sandoricum koetjape* Merr. (Thitto) leaf and cultured for use in the Patato Crown Gall (PCG) test with plant extracts. Antitumor activity of ethanol and water extracts of Thit-yay-gyi and Kana-phaw were also tested on tumor produced bacteria, *Agrobacterium tumefaciens* isolated from *Sandorium keotjape Merr*. (Thitto) leaves, using PCG (Potato Crown Gall) test. From this experiment, ethanol and water extracts of Thit-yay-gyi significantly inhibited the formation of tumor with the dose of 0.12 and 0.50 g/disc. In addition, it was observed that ethanol and water extracts of Kana-phaw showed to prevent the tumor formation with dose of 0.25 and 1 g/disc. These results are shown in Table 4.

Table 4: Antitumor Activity of Crude Extracts from Thit-yay-gyi and Kana-phaw by PCG Test

Concentrations (g/disc)	Antitumor Activity of Extracts			
	Thit-yay-gyi		Kana-phaw	
	Water extract	EtOH extract	Water extract	EtOH extract
1.00	-	-	-	-
0.50	-	-	+	-
0.25	+	-	+	-
0.12	+	-	+	+
Control	+	+	+	+

(+) Tumor appeared (-) No tumor appeared

Antiproliferative Activity

Antiproliferative Activity

Antiprolifertative activity of EtOH and H_2O extracts of Thit-yay-gyi and Kana-phaw was done by using seven human cancer cell lines. Antiproliferative activity was expressed as the IC_{50} (inhibitory concentration) value. 5-fluorouracil was used as positive control. The antiproliferative activity of crude extracts are summarized in Table 5. The H_2O extract of Kana-phaw did not show antiproliferative activity. The EtOH extract of Thit-yay-gyi and were observed to possess higher antiproliferative activity against breast (MCF 7), cervix (Hela), pancreatic (PSN-1) and pancreas ductal adenocarcinoma (PANC-1) human cancer cell lines than other extracts. In addition, H_2O extract of Thit-yay-gyi found to possess antiproliferative activity against breast human cancer cell line (MCF 7). The EtOH extract of Kana-phaw exihibited potent antiproliferative activity against liver (HePG-2), cervix (Hela) and pancreas ductal adenocarcinoma (PANC-1) human cancer cell lines. It may be concluded that Thit-yay-gyi preserve the high potentially against the breast (MCF 7), cervix (Hela), pancreatic (PSN-1) and pancreas ductal adenocarcinoma (PANC-1) human cancer cell lines than Kana-phaw.

Table 5: Antiproliferative Activity of Crude Extracts against Various Types of Cancer Cell Lines

Sample	IC_{50} (μg/mL) of crude extracts against Tested Cancer Cell Lines						
	A549	**MCF7**	**WI-38**	**Hela**	**HePG 2**	**PSN-1**	**PANC-1**
Thit-yay-gyi (EtOH-extract))	>100	48.70	>100	59.15	>100	62.32	76.45
Thit-yay-gyi (H_2O-extract)	>100	64.12	>100	>100	>100	>100	>100
Kana-phaw (EtOH-extract))	>100	>100	>100	52.06	80.73	>100	74.35
Kana-phaw (H_2O-extract)	>100	>100	>100	>100	>100	>100	>100

A 549 = human lung cancer
Hela = human cervix cancer
MCF 7 = human breast cancer
WI-38 = normal human fibroblast
HePG 2 = human liver cancer
PSN-1 = human pancreatic cancer
PANC-1 = pancreas ductal adenocarcinoma

Conclusion

The antimicrobial activity of different crude extracts from the Thit-yay-gyi and Kana-phaw was determined by agar well diffusion method. Ethyl acetate extracts of both plants samples showed the antimicrobial activity on all tested microorganisms with inhibition zone diameters in the range between 18 mm-35 mm for Thit-yay-gyi and 21 mm-35 mm for Kana-phaw. In antioxidant activity, ethanol extract of Kana-phaw [IC_{50} = 22.53 μg/mL] more potent than the other extracts. In addition, the cytotoxicity of EtOH and H_2O crude extracts from Thit-yay-gyi and Kana-phaw were evaluated by brine shrimp cytotoxicity bioassay. The cytotoxicity of Thit-yay-gyi EtOH extract (LD_{50}=68.2μg/mL) showed more cytotoxic effect than other crude extracts. In

antitumor activity of ethanol and water extracts Thit-yay-gyi and Kana-phaw significantly inhibited the formation of tumor with the dose of 0.12 g/ disc. The EtOH extract of Thit-yay-gyi was possessed the higher antiproliferative activity than other extracts. On silical gel column chromatographic separation, kaurenoic acid (A, 0.015%, m.pt. 172°C) was isolated from PE extract of Kana-phaw. The isolated compound was characterized by some physical and chemical properties and structurally identified by the combination of FT IR, NMR, HMBC and ESI MS spectroscopic methods and also by comparing with the reported data. The cytotoxicity effect of compound A (kaurenoic acid) was found on Brine Shrimp up to minimum dose of 0.00001 μg/mL.

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