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Phytochemical screening, phenolic and flavonoid contents, antioxidant and cytotoxic activities of *Graptophyllum pictum* (L.) Griff

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Abstract

The phytochemical screening of the leaves of *Graptophyllum pictum* (L.) Griff. was carried out using the standard procedures which revealed the presence of flavonoids, steroids, tannins, coumarins, saponins, anthraquinones, phenolics and sugars. *In vitro* antioxidant activity, total phenolic and total flavonoid contents of four different fractions from the ethanolic leave extract of *G. pictum* (L.) Griff. were determined using spectrophotometric methods. The ethyl acetate fraction showed the highest total phenolic content (102.57 ± 0.19 mg gallic acid equivalent/g) and the hexane fraction contained the highest flavonoid (28.21 ± 0.04 mg quercetin equivalent/g). The ethyl acetate fraction also exhibited the highest antioxidant capacities in the DPPH radical scavenging assay with the IC_{50} value of 0.78 ± 0.01 mg/mL and was found 69.19 ± 0.73 mg trolox equivalent capacity/g and 48.04 ± 0.49 mg ascorbic acid equivalent capacity/g when investigating by the ABTS radical scavenging assay. Moreover, the hexane, the ethyl acetate and the aqueous fractions exhibited significant cytotoxicity against MCF-7 cell lines with IC_{50} values of 38.66, 26.01 and 20.41 μ g/mL, respectively. All fractions were non-cytotoxic against Vero cells. The results of all experiments suggest that the leaves of *G. pictum* (L.) Griff. can be a natural candidate for rich source of antioxidants for further chemical investigation.

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Phytochemical Screening, Phenolic and Flavonoid Contents, Antioxidant and Cytotoxic Activities of *Graptophyllum pictum* (L.) Griff.

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ABSTRACT

The phytochemical screening of the leaves of *Graptophyllum pictum* (L.) Griff. was carried out using the standard procedures which revealed the presence of flavonoids, steroids, tannins, coumarins, saponins, anthraquinones, phenolics and sugars. *In vitro* antioxidant activity, total phenolic and total flavonoid contents of four different fractions from the ethanolic leave extract of *G. pictum* (L.) Griff. were determined using spectrophotometric methods. The ethyl acetate fraction showed the highest total phenolic content (102.57 ± 0.19 mg gallic acid equivalent/g) and the hexane fraction contained the highest flavonoid (28.21 ± 0.04 mg quercetin equivalent/g). The ethyl acetate fraction also exhibited the highest antioxidant capacities in the DPPH radical scavenging assay with the IC_{50} value of 0.78 ± 0.01 mg/mL and was found 69.19 ± 0.73 mg trolox equivalent capacity/g and 48.04 ± 0.49 mg ascorbic acid equivalent capacity/g when investigating by the ABTS radical scavenging assay. Moreover, the hexane, the ethyl acetate and the aqueous fractions exhibited significant cytotoxicity against MCF-7 cell lines with IC_{50} values of 38.66, 26.01 and 20.41 μ g/mL, respectively. All fractions were non-cytotoxic against Vero cells. The results of all experiments suggest that the leaves of *G. pictum* (L.) Griff. can be a natural candidate for rich source of antioxidants for further chemical investigation.

Keywords: phytochemical screening, phenolic content, flavonoid content, antioxidant activity, cytotoxicity, *Graptophyllum pictum* (L.) griff

1. INTRODUCTION

Free radicals are defined as atoms or molecules that have one or more unpaired electrons such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Generating free radicals in human bodies from endogenous (e.g. cellular metabolisms)

and exogenous (e.g. UV-light, air-pollution) sources [1]. Overproduction of free radicals or diminishing of antioxidants is called oxidative stress which damages the molecule composition of cells such as lipids, carbohydrates, DNA and proteins. The impairment of cellular structure and functions can lead to develop of various diseases, e.g. cancers, cardiovascular diseases, rheumatoid arthritis, diabetes, Alzheimer's diseases and Parkinson's diseases [2].

The secondary metabolites from plants are the potential sources of natural antioxidants such as flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignins, catechin, gallic acid, etc. Beta-carotene, ascorbic acid and vitamin E are widely used for antioxidant supplements [3].

Graptophyllum pictum (L.) Griff. (Acanthaceae) is a Papua New Guinea native shrub. In Thailand, it is commonly known as 'Bai Ngeoun'. It is cultivated in the tropics as an ornamental plant; however occasional medicinal use has been reported [4]. *G. pictum* (L.) Griff. has been used in Asian folk medicine to relieve the bowel costiveness, heal hemorrhoids, expel gall stone, assuage liver discomfort and earache. A paste of the leaves had used to heal cuts, wounds, ulcers and soothe swollen parts [5-6].

There are no reports on phytochemical screening, total phenolic and total flavonoid contents and antioxidant activities from *G. pictum* (L.) Griff. This research presents the phytochemical screening and the determination of the total phenolics, total flavonoids and antioxidant activities from the leaves of *G. pictum* (L.) Griff. using spectrophotometric methods.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All analytical grade organic solvents: hexane, ethyl acetate, butanol and acetone were purchased from RCI Labscan (Bangkok, Thailand), ethanol and methanol were obtained from Carlo Erba (France) and dichloromethane

was purchased from Fisher Scientific (UK). Sulfuric acid (H_2SO_4), hydrochloric acid (HCl), acetic acid (AcOH) and ammonia solution were purchased from Carlo Erba (France). Folin-Ciocalteu's reagent, sodium carbonate (Na_2CO_3), aluminium chloride ($AlCl_3$), ferric chloride ($FeCl_3$), sodium hydroxide (NaOH), potassium hydroxide (KOH) and potassium iodide (KI) were purchased from Merck (Darmstadt, Germany). ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), gallic acid were purchased from Sigma (St. Louis, USA). Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) and 3,5-dinitrobenzoic acid were obtained from Aldrich (Milwaukee, USA). Potassium persulfate was obtained from UNILAB (AU). DPPH (2,2'-diphenyl-1-picrylhydrazyl), ascorbic acid and lead acetate were obtained from Fluka (Buchs, Switzerland). Quercetin was purchased from Acros organics (New Jersey, USA). Bismuthsubnitrate was obtained from Sigma-Aldrich (St. Louis, USA).

2.2 Plant Material

The fresh leaves of *G. pictum* (L.) Griff. were collected from the Medicinal Plants Garden, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand, in May, 2009. The specimen was identified by Dr. J.F. Maxwell at the Herbarium of the Department of Biology, Faculty of Science, Chiang Mai University where a voucher specimen (herbarium no. N. Jiangseubchatveera 1) has been deposited.

2.3 Preparation of Plant Extracts

The leaves of *G. pictum* (L.) Griff. were washed with distilled water and dried in a hot air oven at 40°C for 24 h. Then the dried leaves (583.4 g) were ground and soaked in 95% ethanol (EtOH) (6.0 liters) for one week at room temperature with shaking day by day. The extract was filtered and evaporated to dryness under pressure using a rotary evaporator (EYELA, rotary vacuum evaporator, Tokyo Rikakikai

Co., Ltd., Japan) to obtain the ethanolic extract (66.3 g). Then this extract was partitioned with hexane, ethyl acetate (EtOAc), *n*-butanol (6 x 500 mL of each solvent), respectively. Each fraction and the residue (aqueous fraction) were concentrated to dry mass and kept at 4°C for further analysis.

2.4 Preliminary Phytochemical Screening

The preliminary phytochemical screening for the secondary metabolites in different fractions was performed using the standard procedures [7-10]. Thin layer chromatography was carried out using silica gel 60 GF254 aluminium sheets (Merck, Germany).

2.4.1 Flavonoids

(a) Shinoda's test

One milliliter of 80% ethanolic extract was added a small piece of magnesium followed by 8-9 drops of concentrated hydrochloric acid was added. The formation of pink, reddish or brown color indicated the presence of flavonoids.

2.4.2 Steroids

(a) Salkowski reaction

One milliliter of chloroform extract was slowly added a few drops of sulfuric acid by the sides of the 5-mL test tube. The formation of red color indicated the presence of steroids.

2.4.3 Tannins

A few drops of 5% w/v ferric chloride solution was added to 1-2 mL of the 80% ethanolic extract. A greenish color indicated the presence of gallotannins, while brown color indicated the presence of pseudotannins.

2.4.4 Resins

One milliliter of ethanolic extract was dissolved in acetone and the solution was poured into distilled water. A turbidity indicated the presence of resin.

2.4.5 Coumarins

(a) Dry sample 0.2 g was transferred into a 5-mL test tube. The test tube was sealed with 20% NaOH coated filtered paper, then heated on a water bath at 70°C for 5 min. The filtered paper was observed under long wavelength UV at 365 nm. The greenish blue spot indicated the presence of volatile coumarins.

(b) To the same test tube, 0.5-1.0 mL of 35% EtOH was added and heated on a water bath for 15 min. The clear solution was dropped on the 20% NaOH coated filtered paper. The greenish blue spot was observed under UV-365 nm, indicated the presence of non-volatile coumarins.

2.4.6 Saponins

One gram of dry sample was extracted with 5 mL of distilled water, heated on a water bath for 5 min and filtered. The extract was divided into 2 test tubes, (a) The test tube was shaken vigorously. (b) One milliliter of 10% H₂SO₄ was added and heated on a water bath for 5 min, cooled down and then shaking vigorously. The formation of honeycomb like froth stayed for 10 min, indicated the presence of saponins.

2.4.7 Anthraquinones

Dry sample (0.5 g) was extracted with 5 mL of methanol, heated on a water bath for 10-15 min, filtered and concentrated to 1-2 mL. The extract (20 µL) was applied on a TLC plate (3.3x10 cm) and using EtOAc: MeOH: H₂O (80:10:8 v/v) as mobile phase. The chromatogram was sprayed with KOH reagent (5% KOH in 95% EtOH) and viewed under long wavelength UV (365 nm). Yellow/ orange/ red spot indicated the presence of anthraquinones.

2.4.8 Cardiac glycosides

Dry sample (0.5 g) was extracted with 5 mL of 50% MeOH and 10% lead acetate, heated on a water bath for 10-15 min, filtered and then

extracted with 2.5 mL of CHCl_3 2-3 times and concentrated to 1-2 mL. The extract (20 μL) was applied on a TLC plate (3.3x10 cm) and using EtOAc: MeOH: H_2O 80:10:8 as mobile phase. After spraying the chromatogram with Kedde's reagent (5 mL of 3% 3,5-dinitrobenzoic acid in EtOH mixed with 5 mL of 2M NaOH). Pink/ yellow/ violet spot indicated the presence of glycosides and digoxin.

2.4.9 Phenolics

Dry sample (1 g) was extracted with 10 mL of dichloromethane, heated on a water bath, filtered and concentrated to 1 mL. The extract (25 μL) was applied on a TLC plate (3.3x10 cm) and using DCM: EtOAc: MeOH 90:5:5 as mobile phase. The chromatogram was sprayed with FeCl_3 reagent (5% FeCl_3 in EtOH). Blackish blue/ Blackish green spot indicated the presence of phenolics.

2.4.10 sugars

Dry sample (1 g) was extracted with 10 mL of distilled water, heated on a water bath for 10-15 min, filtered and concentrated to 1-2 mL. The extract (10 μL) was applied on a TLC plate (3.3x10 cm) and using MeOH: H_2O 60:40 as mobile phase. The chromatogram was sprayed with 10% H_2SO_4 reagent and heated at 100°C for 5-10 min. Blackish gray/ blackish blue spot indicated the presence of sugars.

2.4.11 Alkaloids

Dry sample (1 g) was extracted with 80% EtOH, heated on a water bath for 30-60 min, then 1 mL of 28% ammonia solution and 1 mL of CHCl_3 were added, shaken and separated the CHCl_3 layer and then concentrated to 1-2 mL. The extract (40 μL) was applied on a TLC plate (3.3x10 cm) and using EtOAc: hexane 60:40 as mobile phase. The chromatogram was sprayed with Dragendorff's reagent (A: 0.85 g of bismuthsubnitrate in 10 mL of AcOH and adjusted the volume to 50 mL with distilled

water, B: 8 g of KI in 20 mL of distilled water; mixed 1 mL of A with 1 mL of B, 4 mL of AcOH and 20 mL of distilled water). Orange spot on a yellow background indicated the presence of alkaloids.

2.5 Total Phenolic Contents Determination

Total phenolic content of all fractions was determined using the Folin-Ciocalteu assay [11]. Different concentrations of the fractions in MeOH were prepared; the hexane fraction (1.0 mg/mL), the ethyl acetate fraction (0.5 mg/mL) the butanolic fraction (1.0 mg/mL) and the aqueous fraction (1.0 mg/mL). Gallic acid (0.01 - 0.07 mg/mL) was prepared in MeOH and used as standard. Sample (300 μL) was introduced into a test tube followed by 1.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 1.2 mL of 7.5% w/v Na_2CO_3 . The mixture was allowed to stand in the dark for 30 min at room temperature. The absorbance at 765 nm was measured by a spectrophotometer (Shimadzu UV-2450, Japan). Triplicate determinations were performed. The results were expressed as milligram gallic acid equivalents (mg GAE) per gram of the extract by comparison with standard curve of gallic acid.

2.6 Total Flavonoid Content Determination

The total flavonoid content of the fractions was determined according to the method of Ramamoorthy *et.al*/with some modification [12]. Quercetin (0.001 - 0.07 mg/mL) was prepared in MeOH for standard solutions. One milliliter of 2% AlCl_3 in MeOH was mixed with 1 mL of sample (1 mg/mL). The mixture was incubated for 10 min at room temperature. The absorbance was measured using a spectrophotometer (Shimadzu UV-2450, Japan) at 415 nm against a blank sample consisting of a 1 mL sample solution with 1 mL MeOH without AlCl_3 . Triplicate determinations were performed. The calibration line was plotted between the absorbance and the concentration of quercetin.

The content of flavonoids was expressed in terms of quercetin equivalent (mg QE) per gram of the extract.

2.7 Determination of Antioxidant Activity

2.7.1 DPPH radical scavenging assay

The free radical scavenging activity of the fractions was determined according to the DPPH method [13] with some modifications. All fractions were prepared in EtOH at various concentrations; the hexane fraction (1.0 - 25.0 mg/mL), the ethyl acetate fraction (0.09 - 2.7 mg/mL), the butanolic fraction (0.6 - 6.0 mg/mL) and the aqueous fraction (3.7 - 18.4 mg/mL). Twenty microliters of each sample was added to 180 μ L of DPPH in EtOH (0.0066 % w/v) in a 96-well microtiter plate. After incubation for 30 min in the dark, the absorbance of each well was measured at 520 nm spectrophotometrically (spectrophotometer: Multimode detector, Beckman Coulter DTX 880, USA). The DPPH solution was used as a negative control. Trolox and ascorbic acid were used as reference standards. Triplicate determinations were performed. The percentage of DPPH scavenging activity was calculated as $[(A_c - A_s) / A_c] \times 100$, where A_c is the absorbance of the control and A_s is the absorbance of the sample. The IC_{50} values denote the concentration of the sample which is required to scavenge 50% of DPPH free radicals.

2.7.2 ABTS^{•+} radical scavenging activity

The antioxidant activity of the fractions was measured using the ABTS radical scavenging assay according to the method described by Re *et. al* [14] with some modifications. ABTS radical cation (ABTS^{•+}) was generated by reacting 5 mL of ABTS stock solution (7 mM) with 2.5 mL of potassium persulfate (2.4 mM) in the darkness for 16 h at room temperature. Prior to assay, the freshly prepared ABTS^{•+} solution was diluted with deionized water to obtain the absorbance at 734 nm of 0.700 \pm

0.050 determined using a spectrophotometer (Shimadzu UV-2450, Japan). The fractions were prepared in EtOH; the hexane fraction (25.0 mg/mL), the ethyl acetate fraction (9.0 mg/mL), the butanolic fraction (6.0 mg/mL) and the aqueous fraction (4.0 mg/mL). Sample 20 μ L was mixed with 2.0 mL of the ABTS^{•+} solution and incubated at room temperature for 5 min. The decrease in absorbance at 734 nm was measured. Trolox and ascorbic acid (concentration range 0.5 - 2.5 mM) were used as the standards. Triplicate determinations were performed. The percentage inhibition of free radical by ABTS^{•+} (I%) was calculated as $[(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$, where A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample) and A_{sample} was the absorbance of the test sample. The standard curves were plotted between the percentage inhibitions (I%) and concentrations of trolox and ascorbic acid. The ABTS of sample were determined from the standard curves and expressed as milligram of trolox equivalent antioxidant capacity (TEAC) per gram of the extract and milligram of ascorbic acid equivalent capacity (VCEC) per gram of the extract, respectively.

2.8 Cytotoxic Activity

The cytotoxicities of the fractions were determined against the KB (epidermoid carcinoma of oral cavity, ATCC CCL-17), MCF-7 (breast adenocarcinoma, ATCC HTB-22) and NCI-H187 (small cell lung carcinoma, ATCC CRL-5804) cancerous human-cell lines using the Resazurin microplate assay (REMA), using the method described by Brien *et. al* [15]. The fractions were first diluted to 50 μ g/mL in 0.5% DMSO and then subjected to a doubling series of dilutions. Cells at a logarithmic growth phase were harvested and diluted to 7×10^4 cells/mL for KB and 9×10^4 cells/mL for MCF-7 and NCI-H187 in fresh medium. Successively, 5 μ L of each sample solution and 45 μ L cell

suspension were added to 384-well plates, incubated at 37°C in 5% CO₂ in an incubator. After the incubation period (3 days for KB and MCF-7, and 5 days for NCI-H187), 12.5 µL resazurin solution (62.5 µg/mL) was added to each well, and the plates were then incubated at 37°C for 4 h. The fluorescence was measured using a SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA) at the excitation and emission wavelengths of 530 nm and 590 nm. The percentage inhibition of cell growth (%) was calculated as $[1 - (FU_T / FU_C)] \times 100$ where FU_T and FU_C are the mean fluorescent units from treated and untreated conditions, respectively. The IC₅₀ values were derived from the dose response curves using the SOFTMax Pro software (Molecular Devices). Ellipticine, doxorubicin and tamoxifen were used as positive controls and 0.5% DMSO was used as a negative control.

The cytotoxicity assay against Vero cells (African green monkey kidney, ATCC CCL-81) was performed using the Green Fluorescent Protein (GFP) detection method [16]. The assay was carried out by adding 45 µL of cell suspension at 3.3×10^4 cells/mL to each well

of 384-well plates containing 5 µL of test compounds previously diluted in 0.5% DMSO and then incubating for 4 days in an incubator at 37°C with 5% CO₂. The fluorescence was measured using a SpectraMax M5 microplate reader in the bottom-reading mode at the excitation and emission wavelengths of 485 and 535 nm. The percentage of cytotoxicity and IC₅₀ value of each sample were calculated. Ellipticine was used as a positive control and 0.5% DMSO was used as a negative control.

3. RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical Screening

The preliminary phytochemical screening of *G. pictum* (L.) Griff. leaves in various solvents revealed the presence of flavonoids, steroids, tannins, coumarins, saponins, anthraquinones, phenolics and sugars as shown in Table 1. The presence of flavonoids was according to the result described by Ozaki *et. al* [6].

3.2 Preparation of Plant Extract

The dried powder (leaves) 583.4 g was macerated with 95% EtOH 6.0 liters at room temperature for a week and shaken every day.

Table 1. Preliminary Phytochemical Screening of *G. pictum* (L.) Griff. leave extracts using the standard procedures.

Test	Results*
Flavonoids (in 80% EtOH)	+
Steroids (in chloroform)	+
Tannins (in 80% EtOH)	+
Resins (in EtOH)	-
Coumarins (react with 20% NaOH coated filtered paper)	+
Saponins (in water)	+
Anthraquinones (in methanol)	+
Glycosides (in 50% MeOH in the presence of 10% lead acetate)	-
Phenolics (in dichloromethane)	+
Sugars (in water)	+
Alkaloids (in 80% EtOH)	-

Note : *(+): presence, (-): absence of phytochemical

After 7 days – maceration, the extract was filtered and dried by using a rotary evaporator to receive the 95% ethanolic extract 66.3 g. This extract was further partitioned with hexane, ethyl acetate and *n*-butanol, respectively. The residue of the partition was an aqueous fraction. The partition yields of all fractions (%w/w) were 36.67, 1.36, 31.73 and 14.13%, respectively (Table 2).

3.3 Total Phenolic and Flavonoid Contents

The total phenolic content was measured by spectrophotometric method using the Folin-Ciocalteu's reagent. The principle of this method is the reduction of Folin-Ciocalteu's by phenols in a basic condition to form a blue chromophore consisted of phosphotungstic-phosphomolybdenum complex. This chromophore can be quantified by UV-Vis spectrophotometry [17]. Table 2 showed the total phenolic contents in various fractions are expressed in the term of mg gallic acid equivalent (GAE) /g of the extract (the standard curve equation: $y = 10.802x + 0.0447$, $R^2 = 0.9919$). The total phenolic contents ranged from 11.69 – 102.57 mg GAE/ g of the extract. The ethyl acetate fraction showed the highest total phenolic content (102.57 ± 0.19 mg GAE/g) whereas the hexane fraction has the lowest total phenolic content (11.69 ± 0.09 mg GAE/g).

Flavonoids are natural constituents of plants which have potential biological activities including antioxidant properties. The determination of total flavonoid contents was measured with spectrophotometric method using aluminium chloride. This method based on the formation of acid stable complex between flavonoid and aluminium chloride which have a maximum absorption at 415 nm [18]. The total flavonoid contents of all fractions are summarized in Table 2 which expressed in the term of mg quercetin equivalent (QE)/ g of the extract (the standard curve equation: $y = 26.916x - 0.0287$, $R^2 = 0.9990$). The total flavonoid contents of *G. pictum* (L.) Griff. fractions ranged from 2.02 – 28.21 mg QE/ g of the extract. The highest total flavonoid content found in the hexane fraction (28.21 ± 0.04 mg QE/ g) and the lowest total flavonoid contents was shown in the aqueous fraction (2.02 ± 0.02 mg QE/ g).

3.4 DPPH and ABTS Assays

The antioxidant activities of different fractions from *G. pictum* (L.) Griff. were determined using the DPPH and ABTS assays, respectively.

The DPPH method depends on the reduction of the purple-colored DPPH radical (DPPH[•]) by accepting electron or proton radical from antioxidant to form the reduced

Table 2. The percentage yield, total phenolic and flavonoid contents of different fractions of *G. pictum* (L.) Griff. leaves.

Fractions	%Yield	Total phenolic contents	Total flavonoid contents
	(%w/w)	(mg GAE/g extract)*	(mg QE/g extract)**
Hexane	36.67	11.69 ± 0.09	28.21 ± 0.04
Ethyl acetate	1.36	102.57 ± 0.19	22.45 ± 0.02
Butanolic	31.73	45.33 ± 0.23	9.02 ± 0.04
Aqueous	14.13	16.51 ± 0.16	2.02 ± 0.02

Note : *GAE: gallic acid equivalent, **QE: quercetin equivalent, Values are expressed as means of triplicate determinations \pm SD.

DPPH (DPPH-H). The color changes from purple to yellow after reduction which can be determined spectrophotometrically at 520 nm. The degree of discoloration indicates the scavenging potential of the antioxidant or sample by their hydrogen donating ability [18]. The inhibitory concentration at 50% (IC_{50}) of each fraction was measured and compared with the antioxidant standard; trolox and ascorbic acid (Table 3). For *G. pictum* (L.) Griff., the hexane, ethyl acetate, butanolic and aqueous fractions showed significant antioxidant activities with the IC_{50} values of 7.60 ± 0.28 , 0.78 ± 0.01 , 2.58 ± 0.08 and 18.08 ± 0.69 mg/mL, respectively. The calibration curves of trolox and ascorbic acid (the standard curve equations: $y = 528.51x + 2.0649$, $R^2 = 0.9987$ and $y = 809.1x - 1.611$, $R^2 = 0.9920$, respectively) were expressed the IC_{50} values of 0.10 ± 0.0002 and 0.06 ± 0.003 mg/mL, respectively. These results showed that the ethyl acetate fraction showed the highest antioxidant activity with the lowest IC_{50} value followed by butanolic, hexane and aqueous fractions, respectively.

The ABTS assay is a decolorization assay which starts from a generating of greenish blue

ABTS radical cation ($ABTS^{+\cdot}$) by oxidation between ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) and potassium persulfate. The $ABTS^{+\cdot}$ is reduced by donating electron or hydrogen of antioxidant. The discolorization of $ABTS^{+\cdot}$ was determined by the percentage inhibition of the absorbance at 734 nm [14]. The antioxidant of each fraction was calculated from the calibration curves of trolox and ascorbic acid (the standard curve equations: $y = 145.44x + 0.3368$, $R^2 = 0.9971$ and $y = 216.73x - 2.8094$, $R^2 = 0.9970$, respectively) and expressed in the term of mg trolox equivalent antioxidant capacity (TEAC)/g of the extract and mg ascorbic acid equivalent antioxidant capacity (VCEC)/g of the extract, respectively. The results are shown in Table 3. The different fractions from *G. pictum* (L.) Griff.; hexane, ethyl acetate, butanolic and aqueous fractions exhibited significant potential antioxidant activities with the values of 19.63 ± 0.48 , 69.19 ± 0.73 , 29.53 ± 0.16 and 4.25 ± 0.25 mg TEAC/g, respectively and showed with the values of 13.75 ± 0.32 , 48.04 ± 0.49 , 22.24 ± 0.11 and 6.48 ± 0.16 mg VCEC/g, respectively. From these results revealed the antioxidant activities

Table 3. DPPH and ABTS radical scavenging assays.

Fractions/Standards	DPPH radical scavenging assay		ABTS radical scavenging assay	
	IC_{50} (mg/mL)	(mg TEAC/g extract)	(mg VCEC/g extract)	
Hexane	7.60 ± 0.28	19.63 ± 0.48	13.75 ± 0.32	
Ethyl acetate	0.78 ± 0.01	69.19 ± 0.73	48.04 ± 0.49	
Butanolic	2.58 ± 0.08	29.53 ± 0.16	22.24 ± 0.11	
Aqueous	18.08 ± 0.69	4.25 ± 0.25	6.48 ± 0.16	
Trolox	0.10 ± 0.002	-	-	
Ascorbic acid	0.06 ± 0.003	-	-	

Note : *TEAC: trolox equivalent antioxidant capacity, **VCEC: ascorbic acid equivalent capacity, Values are expressed as means of triplicate determinations \pm SD.

in ABTS assay of four fractions in the order: ethyl acetate > butanolic > hexane > aqueous, respectively which correlated to the results of DPPH assay.

3.5 Cytotoxic Activity

The cytotoxicity of different fractions from *G. pictum* (L.) Griff. against three cancerous human-cell lines, KB (epidermoid carcinoma of oral cavity), MCF-7 (breast adenocarcinoma) and NCI-H187 (small cell lung carcinoma) were determined using the Resazurin microplate assay (REMA). The results are shown in Table 4. The hexane, ethyl acetate and aqueous fractions only inhibited the growth of MCF-7 cell lines with IC₅₀ values of 38.66, 26.01 and 20.41 µg/mL, respectively. All fractions showed non-cytotoxic against Vero cells. In this study, the preliminary phytochemical screening showed that the leaves of *G. pictum* (L.) Griff. consist of phenolics, flavonoids, tannins and coumarins. These compounds had potential anti-cancer activities [19-20]. One or all of these compounds may involve in the cytotoxicity against MCF-7 cell lines of *G. pictum* (L.) Griff.

4. CONCLUSIONS

In conclusion, the preliminary phytochemical screening of *G. pictum* (L.) Griff. leaves identified the presence of flavonoids, steroids, tannins, coumarins, saponins, anthraquinones, phenolics and sugars. These results indicated that this plant should have an antioxidant property. The ethanolic extract of the leaves was partitioned with different solvents to obtain hexane, ethyl acetate, butanolic and aqueous fractions, respectively. All fractions were determined their total phenolic and flavonoid contents, antioxidant and cytotoxic activities. The ethyl acetate fraction showed the highest total phenolic content whereas the hexane fraction exhibited the maximum flavonoid content. The antioxidant activities in this study were investigated by the DPPH and ABTS assays. The results showed that the ethyl acetate had the highest antioxidant capacities in these 2 methods. Moreover, the hexane, the ethyl acetate and the aqueous fractions exhibited significant cytotoxicity against MCF-7 cell lines and all fractions were non-cytotoxic to Vero cells. From the results of all experiments

Table 4. Cytotoxic activity of the fractions from *G. pictum* (L.) Griff.

<i>G. pictum</i> (L.) Griff. fractions/Standards	IC ₅₀ ^a (µg/mL)			
	KB	MCF-7	NCI-H187	Vero cells
Hexane	NA	38.66	NA	NA
Ethyl acetate	NA	26.01	NA	NA
Butanolic	NA	NA	NA	NA
Aqueous	NA	20.41	NA	NA
Ellipticine ^b	0.841	-	1.20	1.48
Doxorubicin ^b	0.481	8.63	0.076	-
Tamoxifen ^b	-	9.87	-	-

Note : ^a Concentration that killed 50% of cell lines; NA = No activity; ^b Anticancer drugs used as positive controls.

suggest that the leaves of *G. pictum* (L.) Griff. are rich source of antioxidants which could be beneficial to support or prevent the body from free radical overproduction. Further chemical investigations are being undertaken using bioassay-directed isolation in order to determine bioactive compounds which will be useful for new drug discovery.

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