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Determination of Antioxidant and Anticancer Activities together with Total Phenol and Flavonoid Contents of *Cleidion javanicum* Bl. and *Bridelia retusa* (L.) A. Juss.

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ABSTRACT

The antioxidant and anticancer activities of the hexane, chloroform and methanol extracts of the leaves, stems and fruits from *Cleidion javanicum* Bl. and *Bridelia retusa* (L.) A. Juss. were investigated. The antioxidant activities were evaluated by DPPH and ABTS methods. The methanol extracts of the stems and fruits from *B. retusa* and the methanol extract of the leaves from *C. javanicum* showed the highest antioxidant activity. The methanol extract of the leaves from *B. retusa* possessed significant anticancer activity against KB-Oral cavity cancer (IC_{50}=21.24 µg/mL), MCF-7 breast cancer cell (IC_{50}=17.81 µg/mL), and NCI-H 187-small cell lung cancer (IC_{50} = 24.24 µg/mL), whereas the methanol extract of the fruits exhibited the highest anticancer activity against NCI-H 187-small cell lung cancer with an IC_{50} value of 8.21 µg/mL. The hexane extract of the fruits from *C. javanicum* showed significant anticancer activity against NCI-H 187-small cell lung cancer with an IC_{50} value of 29.11 µg/mL. All of the extracts of these two medicinal plants were non-cytotoxic to primate cell line (Vero cell). Quantitative determination of phenols and flavonoids in leaves, stems and fruits extracts from *B. retusa* and *C. javanicum* was carried out using spectrophotometric methods. The methanol extract of *B. retusa* fruits and the methanol extract of *C. javanicum* stems contained the highest phenolic content. The methanol extract of *B. retusa* leaves contained maximum flavonoid, whereas the chloroform extract of *C. javanicum* leaves contained maximum flavonoid. These two plants may play important roles for the production of health supplement and might be led to the isolation of lead compounds for drug development.

Keywords: anticancer activity, antioxidant activity, total phenol and flavonoid contents, *Cleidion javanicum, Bridelia retusa*

1. INTRODUCTION

Natural products are well recognized as sources for drugs in several human ailments including cancers for examples, vincristine, innotecan etc. [1]. Despite the discovery of many drugs of natural origin, the search for new anticancer agents is still necessary, in order to increase the range available and to find less toxic and more effective drugs.

Plants interact with stressful environments by physiological adaptation and altering the biochemical profile of plant tissues and producing secondary metabolites. Secondary metabolites are of special interest to researchers because of their unique pharmacophores and medical properties. Secondary metabolites like polyphenols, flavonoids, terpenes and alkaloids have been reported to possess antimutagenic and anticancer properties in many studies.

Two medicinal plants in Euphorbiaceae family: *Bridelia retusa* (L.) A. Juss. and *Cleidion javanicum* Bl. were selected for this investigation. *B. retusa* is a medium to large deciduous tree. It is found from Africa, Australia and Asia. It is used as traditional medicine in India, such as its bark extract is as contraceptive to develop sterility [2], treatment of dysentery [3]. The paste of leaf mixed with the leaves of *Curculig orchiodes* and the oils of caster, coconut and gingelly are applied externally to cure wound [4] and treatment of skin disease [5]. The bark of *B. retusa* roots has supposed to possess anti-rheumatic properties, and is used in traditional medicine. *C. javanicum* is a large shrub. It is found in the forests at low and medium altitudes from India to New Guinea. Various parts of this plant are used medicinally in Thailand and the Philippines. Its stem has been employed as analgesic, antipyretic and diaphoretic [6]. Decoction of its leaves is reputed to cause abortion, whereas a decoction of the bark is used for treatment of stomachic. Its seeds are used for treatment of constipation [7].

The antioxidant activities of the stem bark of *B. retusa* extracts in various solvents (such as water, ethanol, methanol, 50% ethanol, 50% methanol and 70% acetone) were determined by using the DPPH method. All of the extracts possessed the antioxidant activity. The acetone extract of *B. retusa* showed higher antioxidant activity and contained higher amount of phenolics as compared to the others [8, 9].

There are no reports on antioxidant and anticancer activities and quantitative analysis of total phenolic and total flavonoid contents from *B. retusa* and *C. javanicum*. Only a few reports described the antioxidant activity and the phenolic content of the stem bark extract of *B. retusa* [8, 9].

This is the first report on antioxidant and anticancer activities, and the quantitative estimation of total flavonoid and total phenolic contents from leaves, stems and fruits of *B. retusa* and *C. javanicum* by spectrophotometric method.

2. MATERIAL AND METHODS

2.1 Apparatus

- EYELA Rotary vacuum evaporator, Tokyo Rikakikai co., LTD, Japan.
- Spectrophotometer: multimode detector, Beckman Coulter DTX880, USA.
- UV - VIS spectrophotometer, Model 7800, Jasco, Japan.

2.2 Chemicals

All chemicals used were of analytical reagent grade. Hexane, chloroform,methanol,

ethanol and Folin-ciocalteu reagent were purchased from Merck, Germany. ABTS (2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), DPPH (1,1-diphenyl-2-picrylhydrazyl), gallic acid and quercetin were purchased from Sigma (St. Louis, USA). Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid) was obtained from Aldrich (Milwaukee, USA). Potassium persulfate was obtained from UNILAB (AU). Ellipticine, Doxorubicin, Resazurin, L-glutamine, and Geneticin were purchased from Sigma (St. Louis, USA).

2.3 Plant Materials

Fresh leaves, stems and fruits of *C. javanicum* and *B. retusa* were collected from Huay Kaew Arboretum, Huay Kaew Road, Chiang Mai, Thailand in June 2010, and identified by J.F. Maxwell, Chiang Mai University, Chiang Mai, Thailand. Voucher specimens (No. 2 and No. 3) were deposited in the Herbarium of Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

2.4 Preparation of Plant Extracts

The aerial parts and fruits of *C. javanicum* and *B. retusa* were cleaned with running tap water and dried in a hot air oven at 40°C for 24 h. The dried plant was finely ground. Then 1 kg (leaves), 500 g (stems) and 200 g (fruits) of the dried plant powders were extracted successively with hexane, chloroform, and methanol (2 L, 1 L and 500 mL, respectively) for three days. For each extraction step, the solvent was removed in vacuo to give crude extract.

2.5 Determination of Antioxidant Activity

2.5.1 DPPH radical scavenging activity

The antioxidant activity of the extracts was determined by the DPPH radical scavenging assay [10, 11] with some modification. The DPPH 6.6 mg/mL (in ethanol) was prepared and stored in the dark before use. Various concentrations of Trolox standard solutions and the sample solutions were prepared using ethanol as solvent. This experiment was carried out with samples (leaves) in the concentrations of 1, 2, 3, 4, 5, 6, 7, and 10 mg/mL and 10, 20, 30, 40, 50, 60, 70 and 100 mg/mL for stems and fruits. To each well of a 96-well microtitre plate, 180 µL of ethanolic DPPH solution and 20 µL of the test sample (the extract in ethanol) were added. The total volume for each reaction mixture in each well was 200 µL. The plates were then incubated at 37 °C for 30 min to check for the colorimetric change (from deep violet to light yellow) when DPPH was reduced. The absorbance of each well was measured at 540 nm. The DPPH solution was used as the negative control. Trolox was used as the reference standard. Radical scavenging capacity was calculated by using the formula (Equation 1):

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

where Ac is the absorbance of the control and As is the absorbance of the test sample after incubation for 30 min. The values of % inhibition were obtained from Equation 1. For the 50% Inhibitory Concentration (IC_{50}) evaluation of the extract, graphs showing the concentration of the test samples (hexane extract, chloroform extract, and methanol extract) versus % Inhibition (% DPPH reduction) were plotted. A linear regression ($R^2 = 0.9984$) of standard Trolox (Figure. 1) was also used to calculate the radical scavenging capacity.

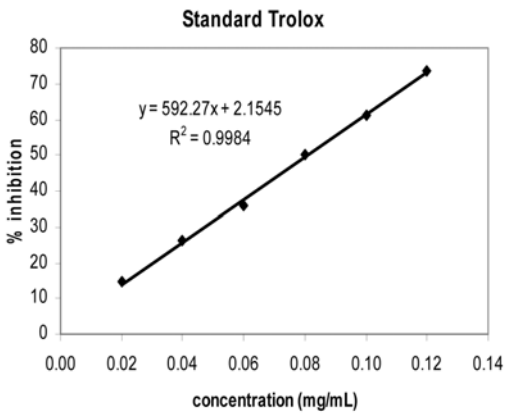

Figure 1. Calibration plot for DPPH assay.

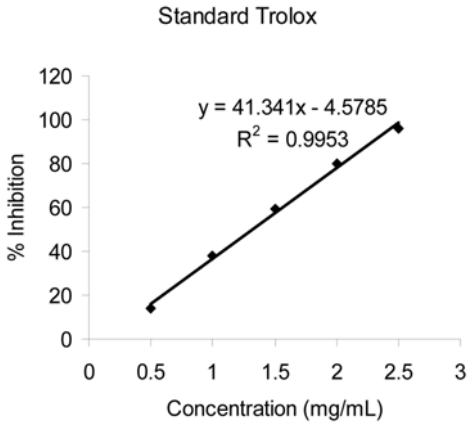

Figure 2. Calibration plot for ABTS assay.

2.5.2 ABTS$^{\bullet+}$ radical scavenging activity

The antioxidant activity of the extract was performed using the ABTS radical cation scavenging assay [12] compared with the Trolox standard. For the ABTS assay, 20 µL of the extract (0.1 g mL^{-1}) was mixed with 2.0 mL of diluted ABTS solution (A_{734nm} = 0.700 ± 0.020) and the absorbance was determined at 734 nm after incubation for 5 min. at room temperature. Appropriate solvent blank was run in each assay. All determinations were carried out at least three times, and in triplicate. The percentage inhibition of free radical by ABTS$^{\bullet+}$ (% Inhibition) was calculated by the following formula (Equation 2):

$$\% \text{ Inhibition} = [(A_{blank} - A_{sample}) / A_{blank}] \times 100 \quad \text{[Equation 2]},$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. The percentage inhibition of the absorbance at 734 nm was calculated and plotted as a function of concentration of antioxidants and of Trolox for the standard reference data (Figure 2).

2.6 Determination of Anticancer Activity

The anticancer activity of the extracts was assayed by using three cancerous human cell lines: KB cell line (epidermoid carcinoma of oral cavity, ATCC CCL-17), MCF 7 cell line (breast adenocarcinoma, ATCC HTB-22), and NCI-H 187 cell line (small cell lung carcinoma, ATCC CRL-5804). This assay was performed using the method described by Brien *et al.* [13] with some modification. In brief, cells at a logarithmic growth phase were harvested and diluted in fresh medium to 7×10^4 cells/mL for KB and 9×10^4 cells/mL for MCF-7 and NCI-H 187. Successively, 5 µL of each test sample (the hexane, chloroform, and methanol extracts) was diluted in 5% DMSO, and 45 µL of cell suspension were added to 384-well plates, incubated at 37°C in 5% CO_2 incubator. After the incubation period (3 days for KB and MCF-7; 5 days for NCI-H187), 12.5 µL of 62.5 µg/mL Resazurin solution was added to each well, and the plates were then incubated at 37°C for 4 hours. Fluorescence signal 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, respectively. The percentage inhibition of the

cell growth was calculated by the following equation (Equation 3):

$$\% \text{ Inhibition} = [1 - (FU_T / FU_C)] \times 100 \quad \text{[Equation 3]},$$

where FU_T and FU_C are the mean fluorescent unit from treated and untreated conditions, respectively. Dose response curves were plotted from six concentrations of twofold serially diluted test compounds and the sample concentrations that inhibit cell growth by 50% (IC_{50}) were derived using the SOFTMax Pro software (Molecular Devices, USA).

2.7 Determination of Cytotoxicity Test

The cytotoxicity of the extract against primate cell line (Vero) was assayed by using Green Fluorescent Protein (GFP) detection methodology described by Hunt *et al.* [14]. The GFP-expressing Vero cell line was generated in-house by stably transfecting the African green monkey kidney cell line (Vero, ATCC CCL-81) with pEGFP-N-1 plasmid (Clontech). The cell line was maintained in minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate, and 0.8 mg/mL geneticin at 37°C in a humidified incubator with 5% CO_2.

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 at 37 °C with 5% CO_2. Fluorescence signals were measured by using a SpectralMax M5 multi-detection microplate reader (Molecular Devices, USA) in the bottom reading mode with excitation and emission wavelengths of 485 nm and 535 nm, respectively. The fluorescence signal at day 4 was subtracted from the background fluorescence at day 0. The percentage of cytotoxicity was calculated by the following equation (Equation 4):

$$\% \text{ cytotoxicity} = [1 - (FU_T / FU_C)] \times 100 \quad \text{[Equation 4]}$$

where FU_T and FU_C represent the fluorescence units of cells treated with test compound and untreated cell, respectively. IC_{50} values were derived from dose-response curves, using six concentrations of twofold serially diluted samples, by the SOFTMax Pro software (Molecular device). Ellipticine and 0.5% DMSO were used as positive and negative controls, respectively.

2.8 Determination of Total Phenolic Contents

The total phenolic contents of the leaves, stems and fruits of *C. javanicum* and *B. retusa* were determined according to the method described by Singleton and Rossi [15] with some modification. An aliquot (1 mL) of the diluted extracts or standard solutions of gallic acid (0.004, 0.01, 0.02, 0.04, 0.06 and 0.08 mg/mL) was added to a 25 ml volumetric flask containing 9 ml of deionized water. A reagent blank using deionized water was prepared. One milliliter of Folin & Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added with mixing. The solution was then immediately diluted to volume (25 ml) with deionized water and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance versus prepared blank was read at 750 nm. Total phenolic contents of the extracts of *C. javanicum* and *B. retusa* were expressed as % gallic acid (w/w) of dry plant material by comparison with the gallic acid standard curve (Figure 3). All samples were analyzed

in three replications.

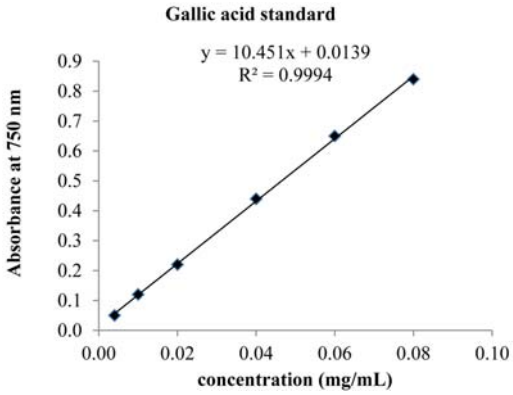

Figure 3. Calibration plot for phenolic determination.

2.9 Determination of Total Flavonoid Contents

The aluminium chloride method [16] was used for the determination of total flavonoid contents of the extracts with some modification. One milliliter of the diluted solution containing flavonoids, 5% (w/w) $NaNO_2$ (0.7 mL) and 30% (v/v) ethanol (10 mL) were mixed for 5 min, and then 10% $AlCl_3$ (w/w, 0.7 mL) was added and mixed altogether. Six minutes later, 1 mol/L NaOH (5 mL) was added. The solution was then diluted to 25 mL with 30% (v/v) ethanol. After standing for 10 min, the absorbance of the solution was measured at 430 nm with a spectrophotometer. A standard curve was plotted using quercetin as a standard. Different concentrations of quercetin were prepared in 80% ethanol and their absorbance was read at 430 nm using a spectrophotometer. The results were expressed in % quercetin (w/w) of dry plant material by comparison with the quercetin standard curve (Figure 4), which was made under the same condition.

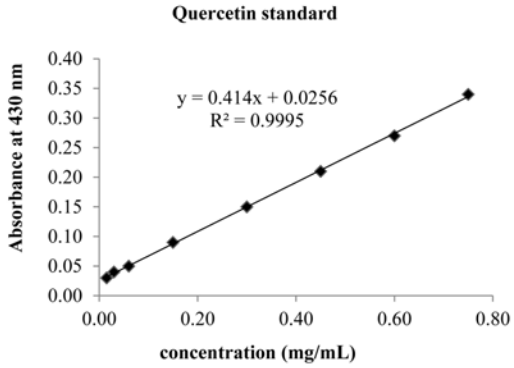

Figure 4. Calibration plot for flavonoid determination.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity

The antioxidant activities of the extracts (leaves, stems and fruits) of *B. retusa* and *C. javanicum* were evaluated by using the DPPH and ABTS assays

3.1.1 DPPH assay

In the DPPH assay, the ability of the samples act as donor of hydrogen atoms or electrons in transforming of $DPPH^{\bullet}$ into its reduced form DPPH-H was investigated. The examined sample can reduce the stable, purple-colored radical DPPH into yellow colored DPPH-H. The IC_{50} of the extracts were determined by reference to the calibration curve (Figure 1). The results were compared with the antioxidant standard, Trolox.

For *B. retusa*, the hexane, chloroform, and methanol extracts (leaves) showed significant antioxidant activities with the IC_{50} values of 5.54 ± 0.015, 6.92 ± 0.013, and 0.52 ± 0.031 mg/mL, respectively. The hexane, chloroform, and methanol extracts (stems) showed significant antioxidant activities with the IC_{50} values of 29.88 ± 0.011, 10.46 ± 0.009,

and 0.12 ± 0.003 mg/mL, respectively. The hexane, chloroform, and methanol extracts (fruits) showed significant antioxidant activities with the IC_{50} values of 36.23 ± 0.009, 29.98 ± 0.011, and 0.17 ± 0.005 mg/mL, respectively. The results are shown in Table 1. The methanol extracts of the leaves, stems and fruits of *B. retusa* showed strong antioxidant activities as compared to that of the methanol extract of the stem bark [8].

For *C. javanicum*, the hexane, chloroform, and methanol extracts (leaves) showed significant antioxidant activities with the IC_{50} values of 2.72 ± 0.015, 1.26 ± 0.001, and 0.21 ± 0.011 mg/mL, respectively. The hexane, chloroform, and methanol extracts (stems) showed significant antioxidant activities with the IC_{50} values of 22.13 ± 0.009, 11.40 ± 0.011, and 0.76 ± 0.011 mg/mL, respectively. The hexane, chloroform, and methanol extracts (fruits) showed significant antioxidant activities with the IC_{50} values of 33.33 ± 0.005, 22.5 ± 0.001,and 3.56 ± 0.005 mg/mL respectively. The results are shown in Table 2.

Table 1. Antioxidant activity of the extracts from *B. retusa* by DPPH and ABTS assays.

Test Sample	IC_{50} (mg/mL)*	
	DPPH assay	ABTS assay
Leaves		
Hexane extract	5.54 ± 0.015	16.62 ± 0.001
Chloroform extract	6.92 ± 0.013	9.85 ± 0.003
Methanol extract	0.52 ± 0.031	1.22 ± 0.003
Stems		
Hexane extract	29.88 ± 0.011	37.76 ± 0.011
Chloroform extract	10.46 ± 0.009	30.49 ± 0.021
Methanol extract	0.12 ± 0.003	0.56 ± 0.005
Fruits		
Hexane extract	36.23 ± 0.009	58.41 ± 0.035
Chloroform extract	29.98 ± 0.011	32.55 ± 0.005
Methanol extract	0.17 ± 0.005	0.29 ± 0.005
Trolox	0.08 ± 0.001	1.32 ± 0.005

Trolox was used as positive control.

*Values are given as mean ± S.D. of triplicate experiments.

Table 2. Antioxidant activity of the extracts from *C. javanicum* by DPPH and ABTS assays.

Test Sample	IC_{50} (mg/mL)*	
	DPPH assay	ABTS assay
Leaves		
Hexane extract	2.72 ± 0.015	8.53 ± 0.009
Chloroform extract	1.26 ± 0.001	3.72 ± 0.009
Methanol extract	0.21 ± 0.011	0.67 ± 0.007
Stems		
Hexane extract	22.13 ± 0.009	32.55 ± 0.005
Chloroform extract	11.40 ± 0.011	19.47 ± 0.001
Methanol extract	0.76 ± 0.011	1.41 ± 0.001
Fruits		
Hexane extract	33.33 ± 0.005	43.46 ± 0.005
Chloroform extract	22.45 ± 0.001	11.56 ± 0.001
Methanol extract	3.56 ± 0.005	5.58 ± 0.009
Trolox	0.08 ± 0.001	1.32 ± 0.005

Trolox was used as positive control.

*Values are given as mean ± S.D. of triplicate experiments.

3.1.2 ABTS assay

The antioxidant activities of the extracts were also determined by using the ABTS assay. This method is based on the inhibition by antioxidants of the absorbance of the radical cation 2,2'-azinobis(3-thylbenzothiazoline-6 sulfonate) [ABTS] which has a characteristic long-wavelength absorption spectrum showing maximum at 734 nm. The IC_{50} of the extracts were determined by reference to the calibration curve (Figure 2). The results were compared with antioxidant standard, Trolox.

For *B. retusa*, the hexane, chloroform, and methanol extracts (leaves) showed significant antioxidant activities with the IC_{50}values of 16.62 ± 0.001, 9.85 ± 0.003, and 1.22 ± 0.003 mg/mL, respectively. The hexane, chloroform, and methanol extracts (stems) showed significant antioxidant activities with the IC_{50}values of 37.76 ± 0.011, 30.49 ± 0.021, and 0.56 ± 0.005 mg/mL, respectively. The hexane, chloroform, and methanol extracts (fruits) showed significant antioxidant activities with the IC_{50}values of 58.41 ± 0.035, 32.55 ± 0.005, and 0.29 ± 0.005 mg/mL, respectively. The results are shown in Table 1.

For *C. javanicum*, the hexane, chloroform, and methanol extracts (leaves) showed significant antioxidant activities with the IC_{50}values of 8.53 ± 0.009, 3.72 ± 0.009, and 0.67 ± 0.007 mg/mL, respectively. The hexane, chloroform, and methanol extracts (stems) showed significant antioxidant activities with the IC_{50}values of 32.55 ± 0.005, 19.47 ± 0.001, and 1.41 ± 0.001 mg/mL, respectively. The hexane, chloroform, and methanol extracts (fruits) showed significant antioxidant activities with the IC_{50}values of 43.46 ± 0.005, 11.56 ± 0.001, and 5.58 ± 0.009 mg/mL, respectively. The results are shown in Table 2.

3.2 Anticancer Activity

The anticancer activities of all the extracts of *B. retusa* and *C. javanicum* were determined by Resazurin Microplate Assay using KB-oral cavity cancer, MCF-7 breast cancer cell and NCI-H 187 small cell lung cancer. Triplicate determinations were performed. The results are presented in Table 3 and Table 4. The methanol extract of *B. retusa* leaves was found to possess anticancer activity against all the cell lines as follows : IC_{50} for KB-oral cavity cancer = 21.24 µg/mL for MCF 7-Breast cancer = 17.81 µg/mL and for NCI-H 187 Small cell lung cancer = 24.24 µg/mL respectively, whereas the methanol extract of the fruits possessed anticancer activity against KB-oral cavity cancer and NCI-H 187 Small cell lung cancer with the IC_{50} values of 32.46 µg/mL and 8.21 µg/mL respectively. But the hexane and the chloroform extracts of the leaves, stems and fruits did not exhibit anticancer activity against any of the cell line.

The anticancer activities of all extracts of *C. javanicum* were also evaluated. Only the hexane extracts of the stems and the fruits possessed anticancer activities.

The hexane extract of the stems showed weak anticancer activities against KB-oral cavity cancer cell (IC_{50} = 49.53 µg/mL) and NCI-H 187 small cell lung cancer (IC_{50} = 47.72 µg/mL), but the hexane extract of the fruits showed strong anticancer activity against NCI-H 187 Small cell lung cancer with an IC_{50} value of 29.11 µg/mL.

All of the extracts of these two medicinal plants were non-cytotoxic to Vero cell.

Table 3. Anticancer activities of the extracts from leaves, stems and fruits of *B. retusa.*

Test Sample	IC_{50} (mg/mL)*		
	KB-Oral Cavity Cancer	MCF7-Breast Cancer	NCI-H187-Small Cell Lung Cancer
Leaves			
Hexane extract	NA	NA	NA
Chloroform extract	NA	NA	NA
Methanol extract	21.24	17.81	24.24
Stems			
Hexane extract	NA	NA	NA
Chloroform extract	NA	NA	NA
Methanol extract	NA	NA	NA
Fruits			
Hexane extract	NA	NA	NA
Chloroform extract	NA	NA	NA
Methanol extract	32.46	NA	8.21

IC_{50} is inhibition cell growth by 50%.
NA = No activity
*Results from three determinations.

Table 4. Anticancer activities of the extracts from leaves, stems and fruits of *C. javanicum.*

Test Sample	IC_{50} (mg/mL)*		
	KB-Oral Cavity Cancer	MCF7-Breast Cancer	NCI-H187-Small Cell Lung Cancer
Leaves			
Hexane extract	NA	NA	NA
Chloroform extract	NA	NA	NA
Methanol extract	21.24	17.81	24.24
Stems			
Hexane extract	NA	NA	NA
Chloroform extract	NA	NA	NA
Methanol extract	NA	NA	NA
Fruits			
Hexane extract	NA	NA	NA
Chloroform extract	NA	NA	NA
Methanol extract	32.46	NA	8.21

IC_{50} is inhibition cell growth by 50%.
NA = No activity
*Results from three determinations.

3.3 Total Phenol and Flavonoid Contents

All the methanol extracts of the leaves, stems and fruits from *B. retusa* and *C. javanicum* gave the highest percentage yields compared to those of the chloroform and hexane extracts.

Quantitative determination of phenols and flavonoids in the extracts of leaves, stems and fruits from *C. javanicum* and *B. retusa* was carried out using spectrophotometric methods. The total phenolic contents of the extracts were determined using the Folin Ciocalteu reagent. The aluminum chloride method was used for the determination of total flavonoid contents of the extracts. The results are summarized in Table 5 and Table 6.

Table 5. Extraction yields and total phenol, flavonoid contents of the extracts of *B. retusa* .

Extracts	Extraction yield (%)	Total phenolic content[a]	Total flavonoid content[b]
Leaves			
Hexane extract	2.19	0.26 ± 0.001	14.70 ± 0.0010
Chloroform extract	0.78	0.54 ± 0.001	15.18 ± 0.0005
Methanol extract	3.44	0.44 ± 0.002	19.06 ± 0.0012
Stems			
Hexane extract	0.09	0.26 ± 0.0005	12.78 ± 0.0005
Chloroform extract	0.46	0.94 ± 0.0020	12.30 ± 0.0023
Methanol extract	3.38	0.54 ± 0.0005	7.94 ± 0.0012
Fruits			
Hexane extract	2.55	0.20 ± 0.0001	11.32 ± 0.0021
Chloroform extract	1.63.	0.48 ± 0.0005	4.70 ± 0.0011
Methanol extract	3.36	1.40 ± 0.0015	4.70 ± 0.001

All analyses are the mean of triplicate measurements ± standard deviation (SD).
[a]: Expressed as % gallic acid (w/w) of dry plant material.
[b]: Expressed as % quercetin (w/w) of dry plant material.

Table 6. Extraction yields and total phenol, flavonoid contents of the extracts of *C. javanicum.*

Extracts	Extraction yield (%)	Total phenolic content[a]	Total flavonoid content[b]
Leaves			
Hexane extract	0.39	0.22 ± 0.001	16.64 ± 0.0012
Chloroform extract	1.02	1.08 ± 0.000	0.98 ± 0.0011
Methanol extract	4.72	3.00 ± 0.002	8.08 ± 0.002
Stems			
Hexane extract	0.15	0.38 ± 0.0000	16.64 ± 0.00051
Chloroform extract	0.25	1.20 ± 0.0005	2.78 ± 0.00201
Methanol extract	0.91	3.32 ± 0.0010	4.22 ± 0.0020
Fruits			
Hexane extract	0.19	0.68 ± 0.0005	12.78 ± 0.001
Chloroform extract	1.29	1.00 ± 0.0010	8.42 ± 0.0011
Methanol extract	2.51	1.46 ± 0.0012	3.26 ± 0.002

All analyses are the mean of triplicate measurements ± standard deviation (SD).
[a]: Expressed as % gallic acid (w/w) of dry plant material.
[b]: Expressed as % quercetin (w/w) of dry plant material.

The present study revealed the total phenolic contents of the leaves, stems and fruits of *B. retusa* in terms of %gallic acid (w/w) of dry plant material (standard plot : $y = 10.45X + 0.013$, $R^2 = 0.9994$, Figure 3). The values of methanol extracts were found between 0.44 ± 0.002 to 1.40 ± 0.0015 % gallic acid (w/w) of dry plant. The methanol extract of the fruits contained the maximum and the minimum amount of phenolic compounds. The values of chloroform extracts were found between 0.48 ± 0.0005 to 0.94 ± 0.0020 %gallic acid (w/w) of dry plant. The chloroform extract of the stems contained the maximum and the chloroform extract of the fruits contained the minimum amount of phenolic compounds. The hexane extracts contained equal small amount of phenolic compounds. Phenolics present in the leaves, stems and fruits have received considerable attention because of their potential biological activity. The methanol extract of *B.retusa* stem bark also contained reasonable amount of phenolic compounds [8].

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural

phenols .These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Using the standard plot of of quercetin ($y = 0.414X + 0.025$, $R^2 = 0.9994$, Figure 4). The flavonoid contents of *B. retusa* leaves, stems and fruits were found ranging from 7.94 ± 0.0012 to 19.06 ± 0.0012, 12.30 ± 0.0023 to 15.18 ± 0.0005 and 11.32 ± 0.002 to 14.70 ± 0.0010 %quercetin (w/w) of dry plant for methanol, chloroform and hexane extracts respectively.

The flavonoid content of the methanol extract of the leaves was quite high compared to that of the fruits amd stems (Table 5).

The total phenolic and flavonoid contents of the leaves, stems and fruits extracts of *C. javanicum* were also evaluated, using the standard plot of gallic acid (Figure 3) and the standard plot of quercetin (Figure 4). The methanol extract of the stems contained the maximum and the hexane extract of the leaves contained the minimum amount of phenolic compounds. The flavonoid content of the chloroform extract of the leaves was quite high compared to that of the stems and fruits (Table 6).

4. CONCLUSION

In conclusion, the hexane, chloroform and methanol extracts of the leaves, stems and fruits from *B. retusa* and *C. javanicum* possess antioxidant activities. The methanol extracts of the stems and fruits from *B. retusa* had the highest antioxidant capacities (IC_{50} = 0.12 ± 0.003 mg/mL and 0.17 ± 0.005 mg/mL respectively [DPPH assay]). The methanol extract of the leaves from *C. javanicum* also showed the highest antioxidant activity with IC_{50} value of 0.21 ± 0.011 mg/mL (DPPH assay). The DPPH radical scavenging activity suggests the stems and fruits from *B. retusa* and the leaves from *C. javanicum* are rich source of antioxidants, which indicate their effectiveness in diseases caused by overproduction of radicals.

The methanol extract of *B. retusa* fruits contained the highest of phenolic content, whereas the methanol extract of the leaves contained maximum flavonoid. The methanol extract of *C. javanicum* stems contained the highest phenolic content, whereas the chloroform extract of *C. javanicum* leaves contained maximum flavonoid.

The methanol extract of *B. retusa* leaves exhibited significant anticancer activity against KB-oral cavity cancer, MCF-7 breast cancer cell and NCI-H 187-Small cell lung cancer. The methanol extract of *B. retusa* fruits also possessed strong anticancer activity against NCI-H 187-Small cell lung cancer, but this extract also showed moderate anticancer activity against KB-oral cavity cancer. The hexane extract of *C. javanicum* stems possessed anticancer activity against KB-oral cavity cancer and NCI-H 187-Small cell lung cancer. The hexane extract of *C. javanicum* fruits exhibited significant anticancer activity against NCI-H 187-Small cell lung cancer. Therefore, the methanol extracts of the leaves and fruits from *B. retusa* and the hexane extracts of the stems and fruits, the methanol extracts of the leaves and stems and the chloroform extract of the leaves from *C. javanicum* may play important roles in new drug development or as health supplements. Further chemical investigations are being undertaken using bioassay-directed isolation in order to determine bioactive compounds which will be useful for discovery of new drugs.

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