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Full Length Research Paper

Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (*Boesenbergia rotunda*, *Boesenbergia pulchella* var *attenuata* and *Boesenbergia armeniaca*)

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Extracts (methanol) of the leaves, stem and rhizome of *Boesenbergia species* were studied for their phytochemical constituents, total phenolics and flavonoid contents, antioxidant as well as anticancer properties. The plants revealed the presence of polyphenols such as quercetin, kaempferol, rutin, naringin, hesperidin, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, chlorogenic acid, gallic acid, luteolin and diosmin by using High Performance Liquid Chromatographic (HPLC). It was indicated with significant composition of hesperidin and naringin in *B. pulchella* var *attenuata* (leaves and stem); quercetin and kaempferol in *B. rotunda*; luteolin in *B. armeniaca*. The results of antioxidant assessments conducted were similar to the trend of total phenolic and flavonoid contents: *B. pulchella* var *attenuata*> *B. rotunda*> *B. armeniaca*. In the cytotoxicity assay, *B. rotunda* showed the most prominent and promising result as anticancer medicinal plant. It showed positive antiproliferative effect against five cancer cell lines: ovarian ($CaOV_3$), breast (MDA-MB-231 and MCF-7), cervical (HeLa) and colon (HT-29) cancer cell lines with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay conducted. In addition, the rhizome of *B. pulchella* var *attenuata* and *B. armeniaca* shown positive result in cytotoxicity assay tested against breast cancer (MCF-7). Thus, the *Boesenbergia* species investigated would be a promising anticancer remedy for breast cancer.

Key words: Phytochemicals, antioxidant, anticancer, *Boesenbergia.*

INTRODUCTION

Strong epidemiological evidence suggests that regular consumption of fruits and vegetables can reduce cancer risk. Towards this end, the past several decades have been an explosion of research focused on the role played by antioxidant nutrients in human cancer. Phytochemicals are available in vegetables and fruits. Briefly, phytochemicals are plant chemicals or more appropriately defined as bioactive non-nutrient plant compounds in citrus fruits, vegetables, grains and other plant foods that have been linked to reduce the risks of major chronic diseases and cancers. Phytochemicals in common fruits and vegetables can have complementary and overlapping mechanisms of action, including of gene expression in cell proliferation, cell differentiation, oncogenes and tumour suppressor genes; induction of cell-cycle arrest and apoptosis; modulation of enzyme activities in detoxification, oxidation and reduction; stimulation of the immune

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system; regulation of hormone metabolism; as well as antibacterial and antiviral effects (Sun et al., 2002).

Boesenbergia belongs to a ginger family, Zingiberaceae in the order of Zingiberales. *Boesenbergia* is a genus of about 80 *Boesenbergia* species in the genus, distributed from India to South East Asia. Borneo, as one of the two distribution centres apart from Thailand, which estimated to have 25 species (Larsen, 2003). *Boesenbergia* species is extremely rare compare to other genera. Mostly, they are found in very damp, shaded areas and usually close to streams or in boggy conditions.

The genus of *Boesenbergia* has attracted more than one specialist in recent years. Many researchers have proven that the rhizome part of *Boesenbergia* spp. displayed health-benefits properties. The *B. rotunda* rhizome has been reported to contain essential oil, boesenbergin, cardamonin, pinostrobin, 5, 7-dimethoxyflavone, 1, 8-cineole, panduratin (Kirana et al., 2007). In the primary health care project of Thailand, the rhizome of this plant is used for the treatment of dyspepsia. As regards its biological activities, *B. rotunda* exhibited antibacterial, antifungal, anti-inflammatory, analgesic, antipyretic, antispasmodic, antitumor and insecticidal activities (Tewtrakul et al., 2003). The rhizome of *B. rotunda* is generally used as a culinary spice in Thailand and also has been used for the treatment of oral diseases (that is dry mouth), stomach discomfort, stomach pain, leucorrhoea, diuretic, dysentery, and inflammation. The rhizomes are used in traditional medicine as antiseptic and for the treatment of stomach ache (Hasnah et al., 1995), diarrhea, dermatitis, dry cough and mouth ulcers (Burkill, 1935; Heyne, 1987), gastrointestinal disorders and post-natal treatment (Burkill, 1935). Mahady (2005) reported new *in vitro* and *in vivo* data on two Thai plants from the Zingiberaceae, namely finger-root (*B. rotunda* (L.) Mansf.) and turmeric (*Curcuma longa* L.), both of which are used in Thailand for the treatment of gastrointestinal ailments, including peptic ulcer disease. These antioxidant and anti-inflammatory compounds have often been shown to be effective as anticancer agents.

Therefore, the objectives of the present study were to evaluate the polyphenolic compounds, antioxidant activity and cytotoxicity effects of selected *Boesenbergia* species in Sabah, Malaysia. In this study, *B. rotunda* (available in Peninsular Malaysia) was chosen as positive control.

MATERIALS AND METHODS

Sample collection and preparation

Whole plant (leaves, stem and rhizome) of *B. armeniaca*, *B. pulchella* var *attenuata* and *B. rotunda* were collected and processed in the Institute for Tropical Biology and Conservation of Universiti Malaysia Sabah, Malaysia. Voucher specimens of these plants have been deposited at BORNEENSIS, Universiti Malaysia Sabah (BORH) and the Kinabalu Park, Sabah. Authentication was done by a taxonomist in that department. The leaves and stems as well as rhizome were freeze-dried separately and were ground to 0.50 mm mesh size.

Sample extraction

Samples were extracted according to Wettasinghe et al. (2002) with slight modifications. 6 g of powdered leaves and stem as well as rhizome were extracted with 100 ml methanol in an amber container for three days. The residue was twice re-extracted with methanol under the same conditions. The resulting slurry was vacuum-filtered through a Whatman No. 1 filter paper and the filtrate was subjected to vacuum rotary evaporation at 40°C to remove methanol. The concentrated methanolic extract was put in the desiccator until the extract was free from methanol solvent. The extract was stored in the 4°C refrigerator for future usage.

Analysis of polyphenol composition by high performance liquid chromatographic (HPLC) Standards

The flavonoids standards were prepared in methanol-DMSO (v/v; 50:50) at -18°C. They were phenolic acids (caffeic acid, p-coumaric acid, ferulic acid and chlorogenic acid), flavanones (naringin, hesperidin), flavonols (quercetin, kaempferol and rutin), flavones (luteolin and diosmin) and flavanols (gallic acid). The sample preparation was done according to Schieber et al. (2001) with slight modification. 0.1 g sample was added in 1ml methanol-DMSO (v/v; 50:50) in a centrifuge tube. It followed by stirring for 10 min at room temperature. Then, it was centrifuged at 9000 rpm for 15 min at 4°C. Supernatant were top up to 2.5 ml and filtered with 0.45 µm membrane filter.

High performance liquid chromatographic (HPLC)

The HPLC system consisted of a Waters system (Milford, MA, USA) 717 autoinjector, 616 pump, and 996 PDA detectors. C_{18} column (250 x 4.6 mm, 5 µm) was used. The mobile phase consisted of 2% acetic acid (aqueous) (eluent A) and of 0.5 % acetic acid (aqueous)-acetonitrile (50:50; v/v) (eluent B). The gradient elution was at 0 time, 95:5; 10 min, 90:10; 40 min, 60:40; 55 min, 45:55; 60 min, 20:80; 65 min, 0:100 with the flow rate of 1 ml/min. The UV absorbance measured at 280 nm for phenolic acid, flavanone, flavanol and flavonol while 340 nm for flavones.

Total phenolic content determination

Total phenolics were determined using Folin-Ciocalteu's reagent as adapted from Velioglu et al. (1998). Two hundred milligrams of sample was extracted for 2 h with 2 ml of 80% methanol containing 1% hydrochloric acid at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000 g for 15 min and the supernatant decanted into 4 ml vials. The pellets were extracted under identical conditions. Supernatants was combined and used for total phenolics assay. One hundred microliters of extract was mixed with 0.75 ml of Folin-Ciocalteu's reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22°C for 5 min; 0.75 ml of sodium bicarbonate (60 g/L) solution was added to the mixture. After 90 min at 22°C, absorbance was measured at 725 nm. Result was expressed as ferulic acid equivalents.

Total flavonoid content determination

Total flavonoid was measured according to Zhishen et al. (1999). 1 ml aliquot of extract (same extraction method as total phenolic content determination) and appropriately diluted standard solution of catechin (20, 40, 60, 80 and 100 mg/l) was added into a 10 ml

volumetric flask containing 4 ml deionized water. At zero time, 0.3 ml of 10% $AlCl_3$ was added. At 6 minutes, 2 ml of 1M NaOH was added to the mixture. Immediately, the reaction flask was diluted to the volume with the addition of 2.4 ml of deionized water and thoroughly mixed. Absorbance of the mixture, pink in colour was determined at 510 nm versus prepared water blank. Total flavonoid of the samples was expressed on a dried weight as mg/100 g catechin equivalent.

Antioxidant activities

$ABTS^{\bullet+}$ decolorization assay: 2, 2'-azinobis (3-ethylbenzthiazoline)-6-sulphonic acid or ABTS free radical decolorization assay was done according to Re et al. (1999) with some modification. Briefly, the pre-formed radical monocation of ABTS was generated by reacting ABTS solution (7 mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand for 15 h in the dark at room temperature. The solution was diluted with methanol to obtain the absorbance of 0.7 ± 0.2 units at 734 nm. The plant extracts was separately dissolved in methanol to yield a concentration of 1 mg/ml. The aliquot of 200 µl of methanolic test solution of each sample was added to 2000 µl of ABTS free radical cation solution. The absorbance, monitored for 5 min was measured spectrophotometrically at 734 nm using a spectrophotometer. Appropriate solvent blanks were run in each assay. The radical-scavenging activity was expressed as the trolox equivalent antioxidant capacity (TEAC), defined as mMol of trolox per gram of sample. BHT and QCT were used as positive control.

DPPH (2, 2 -diphenyl-1-pycryl-hydrazyl) free radical scavenging action: According to Mensor et al. (2001), 1 ml from 0.3 mM methanol solution of 2, 2 -diphenyl-1-pycrylhydrazyl (DPPH) was added into 2.5 ml sample or standards. The solution was mixed vigorously and left to stand at room temperature for 30 min in the dark. The mixture was measured spectrophotometrically at 518 nm. The antioxidant activity (AA) was calculated as below:

AA%=

$$100 - [((Abs\ sample - Abs\ empty\ sample)/(Abs\ control)] \times 100$$

Where; *Abs* is absorbance.

The result was also expressed as ascorbic acid equivalent antioxidant capacity (AEAC) (Leong and Shui, 2002) using the following equations:

$AEAC = (IC_{50\ (AA)}/IC_{50\ (sample)}) \times 10^5$

Where; AA is ascorbic acid

FRAP (Ferric reducing/antioxidant power) assay: This procedure was done according to Benzie and Strain (1996) with slight modification. The working FRAP reagent was produced by mixing 300mM acetate buffer (pH 3.6), 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) solution and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio prior to use and heated to 37°C in water bath. A total of 3.0 ml FRAP reagent was added to a cuvette and blank reading was then taken at 593 nm using spectrophotometer. A total of 100 µl selected plant extracts and 300 µl distilled water was added to the cuvette. After addition of the sample to the FRAP reagent, a second reading at 593 nm was performed after 4 min. The change in absorbance after 4 min from initial blank reading was compared with standard curve. The FRAP values for the samples was taken determined using this standard curve. The final result was expressed as the concentration of antioxidant having a ferric reducing ability.

Cytotoxicity study

Culturing of cells: MCF-7 (hormone dependent breast cancer), MDA-MB-231 (non-hormone dependent breast cancer), CaOV3 and HeLa (cervical cancer), HT29 (colon cancer) cell lines were obtained from American Type Culture Collection (ATCC, USA). 3T3 (mouse fibroblast cell lines) was the normal cell line that was chosen as control. The cell lines were cultured in RPMI 1640 medium with L-glutamine. The cells were cultured in the medium supplemented with 10% of fetal calf serum, 1% penicillin streptomycin using 25 cm^2 flasks in an incubator at 37°C.

MTT Assay (Roche Diagnostic, USA): The viability of cells was determined by staining with tryphan blue. Exponential growing cells was harvested and counted by using haemocytometer. The specific medium for that particular cell line was used to dilute the cells to a concentration of 1×10^5 cells ml^{-1}. From this cell suspension, 100 µl was pipetted into a 96 well microtiter plate and incubated for 24 h in a 5% CO_2 incubator at 37°C. Sample was extracted in a range of doses and added into the plate. After adding the samples extract, new medium was added to make up the final volume of 100µl in each well. The plate was incubated in a 5% CO_2 incubator at 37°C for 72 h. Then, 10 µl of MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] reagent was added into each well. This plate was incubated again for 4 h in CO_2 incubator at 37°C. Subsequently, 100µl of solubilization solution was added into each well. The cell was then left overnight 37°C, CO_2 incubator. Lastly, the absorbance was read with the ELISA reader at 550nm (Mosmann, 1983).

$$\%\ \textbf{cytotoxicity} = \frac{\textbf{OD sample (mean)}}{\textbf{OD control (mean)}} \times 100\%$$

OD=optical density

RESULTS

High performance liquid chromatography (HPLC)

The plants revealed the presence of phytochemicals such as quercetin, kaempferol, rutin, naringin, hesperidin, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, chlorogenic acid, gallic acid, luteolin and diosmin (Table 1). The plants exhibited significant result in hesperidin and naringin. They were the major flavonoid presented in all the samples after HPLC analysis was conducted. *B. rotunda* showed the highest content of quercetin and kaempferol (Table 1). Quercetin was the major flavonols presented in *B. rotunda*, while luteolin was the major flavones in *B. armeniaca*.

Antioxidant and total phenolic and flavonoid content

The ranking of the three antioxidant assessments (Table 2) conducted were similar to the trend of total phenolic and flavonoid contents (Table 3): *B. pulchella* var *attenuata*> *B. rotunda*> *B. armeniaca*. However, the trend of total phenolic and flavonoid compound in the leaves as well as stem part were much higher than rhizome of the selected plants (Table 3).

Table 1. The polyphenols contents of *Boesenbergia* species.

Polyphenols	*B. rotunda* (Rhizome)	*B. pulchella* var *attenuata* (Rhizome)	*B. pulchella* var *attenuata* (Leaves & stem)	*B. armeniaca* (Rhizome)	*B. armeniaca* (Leaves & stem)
	mg/g dry basis				
Quercetin	0.58 ± 4.624	0.04 ± 0.001	0.04 ± 0.028	0.50 ± 0.305	0.21 ± -0.127
Kaempferol	0.61 ± 0.693	0.10 ± 0.020	0.35 ± 0.191	0.14 ± 0.573	0.17 ± 0.687
Rutin	ND	0.03 ± 0.001	0.29 ± 0.127	ND	ND
Naringin	0.693 ± 0.071	0.21 ± 0.001	23.62 ± 14.29	0.53 ± 0.085	1.23 ± 0.071
Hesperidin	11.91 ± 0.714	0.35 ± 0.001	35.58 ± 0.721	0.45 ± 0.001	1.51 ± 0.148
Caffeic acid	0.03 ± 0.014	0.04 ± 0.001	0.04 ± 0.049	0.36 ± 0.085	0.27 ± 0.014
ρ-Coumaric acid	0.03 ± 0.10	0.03 ± 0.001	0.04 ± 0.007	0.04 ± 0.007	0.04 ± 0.001
Ferulic acid	ND	0.02 ± 0.001	1.75 ± 0.410	0.46 ± 0.262	0.93 ± 0.184
Sinapic acid	ND	ND	0.001 ± 0.007	ND	ND
Chlorogenic acid	0.01 ± 0.007	0.01 ± 0.001	0.02 ± 0.001	0.01 ± 0.001	0.01 ± 0.001
Gallic acid	ND	ND	0.04 ± 0.007	0.01 ± 0.007	ND
Luteolin	ND	ND	ND	0.87 ± 0.007	0.81 ± 0.014
Diosmin	ND	0.19 ± 0.001	1.58 ± 0.962	0.73 ± 0.085	0.88 ± 0.035

Data presented are in means ± standard deviation (n = 3)
ND - not detected at the concentration tested.

Table 2. Antioxidant properties of *Boesenbergia* species.

Samples	ABTS decolorization assay[A]	DPPH free radical scavenging[B]	FRAP assay[C]
B. rotunda (Rhizome)	$4.24 \pm 0.01_a$	$4.29 \pm 0.55_c$	$1.77 \pm 0.09_d$
B. pulchella var *attenuata* (Rhizome)	$4.63 \pm 0.01_a$	$7.05 \pm 0.76_a$	$4.63 \pm 0.02_b$
B. pulchella var *attenuata* (Leaves & stem)	$4.37 \pm 0.02_a$	$5.09 \pm 0.30_b$	$5.26 \pm 0.06_a$
B. armeniaca (Rhizome)	$1.60 \pm 0.05_c$	$0.94 \pm 0.18_d$	$1.81 \pm 0.04_d$
B. armeniaca (Leaves & stem)	$3.41 \pm 0.06_b$	$4.02 \pm 0.84_c$	$2.86 \pm 0.14_c$

Values are presented in mean ± SD (n = 3) in which with different letters are significantly different at $p < 0.05$.
[A]ABTS decolorization assay was expressed as Trolox equivalent antioxidant capacity (TEAC (µg/ml)).
[B] DPPH free radical scavenging was expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC) in 1 g dry sample.
[C] FRAP was expressed as ferric reduction to ferrous in 1 g of dry sample.

Table 3. Total phenolic and flavonoid contents of *Boesenbergia* species.

Species	Total phenolics (mg GAE/g dw)[A]	Total flavonoid (mg CE/g dw)[B]
B. rotunda (rhizome)	$6.19 \pm 0.39_c$	$2.19 \pm 0.02_d$
B. pulchella var *attenuata* (rhizome)	$11.97 \pm 0.02_b$	$4.75 \pm 0.01_b$
B. pulchella var attenuata (leaves & stem)	$14.15 \pm 0.02_a$	$5.7 \pm 0.01_a$
B. armeniaca (rhizome)	$2.36 \pm 0.06_d$	$1.42 \pm 0.03_e$
B. armeniaca (leaves and stem)	$5.92 \pm 0.20_c$	$3.64 \pm 0.03_c$

Values are presented in mean ± SD (n = 3) in which with different letters are significantly different at $p < 0.05$.
[A]Total phenolic was expressed as mg gallic acid equivalent (mg GAE) in 1 g of dry sample.
[B]Total flavonoid was expressed as mg catechin equivalent (mg CE) in 1 g of dry sample.

Cytotoxicity activity

In the cytotoxicity assay, 3T3 (mouse fibroblast cell lines) was the normal cell line that was chosen as control. This proved that no cytotoxicity was observed in normal cell lines (Data not shown). *B. rotunda* showed the most prominent and promising result as anticancer medicinal plant. It showed positive antiproliferative effect against

Table 4. IC_{50} value of samples tested against several cancer cell lines by using MTT assay.

Samples	Breast cancer		Ovarian cancer	Colon cancer	Cervix cancer
	MCF-7	MDA-MB-231	$CaOV_3$	HT-29	HeLa
B. rotunda (rhizome)	51	66.50 ± 2.12	71.00 ±1.41	52.00 ± 4.24	65.50 ± 2.12
B. pulchella var *attenuata* (rhizome)	93.00 ± 2.83	ND	ND	ND	ND
B. pulchella var attenuata (leaves)	ND	ND	ND	ND	ND
B. armeniaca (rhizome)	94.50 ± 0.71	ND	ND	ND	ND
B. armeniaca (leaves)	ND	ND	ND	ND	ND
Vincristine	8.50 ± 3.41	13.50 ± 3.14	17.50 ± 0.82	<1	ND
DL-Sulforaphane	<1	3.00 ± 0.73	2.50 ± 2.47	<1	9.50 ± 5.99

Values are expressed as mean ± standard deviation (n = 2) which with different letters are significantly different at $p< 0.05$.
MTT cytotoxicity assay tested against MCF-7 was expressed in IC_{50} value (µg/ml).
ND - Not detected at concentration tested.

five cancer cell lines: ovarian ($CaOV_3$), breast (MDA-MB-231 & MCF-7), cervical (HeLa) and colon (HT-29) cancer cell lines with MTT assay conducted (Table 4). The least IC_{50} recorded was the plant extract tested against hormone dependent breast cancer (MCF-7). Vincristine and DL-Sulforaphane were used as positive control. Other than *B. rotunda*, the plant extracts that shown positive result in cytotoxicity assay tested against MCF-7 were the rhizome of *B. pulchella* var *attenuata* and *B. armeniaca* (Table 4). However, the extracts were less effective than the standards tested in all the cancer cell lines.

DISCUSSION

Flavonoids are a group of more than 4000 polyphenolic compounds that occur naturally in foods of plant origin. Flavonoids are a large class of phytochemicals which are omnipresent in human diets, found for example in fruit, vegetables, tea, chocolate, and wine, and to which a number of beneficial effects on human health, such as antioxidant, anti-inflammatory, antiallergic, antiviral, and anticarcinogenic activities; while some flavonoids exhibit potential for anti–human immunodeficiency virus functions (Yao et al., 2004). Flavonoids are important for human health because of their high pharmacological activities as radical scavengers. These compounds possess a common phenylbenzopyrone structure (C6-C3-C6), and they are categorized according to the saturation level and opening of the central pyran ring,mainly into flavones, flavanols, isoflavones, flavonols, flavanones, and flavanonols.

Phenolic compounds are known as powerful chain breaking antioxidants (Shahidi and Wanasundara, 1992), may contribute directly to antioxidative action (Duh et al., 1999). These compounds are very important constituents of plants and their radical scavenging ability is due to their hydroxyl groups (Hatano et al., 1989). The ranking of the three antioxidant assessments (Table 2) conducted were similar to the trend of total phenolic and flavonoid contents (Table 3). Phenolic compound have been reported to defend the plant against microorganisms and herbivores (Hada et al., 2001). This might explain the importance of higher phenolic compound in the leaves and stem part rather than rhizome of the plant. Moreover, high correlations were observed in antioxidant capacities and phenolic and flavonoid content of the selected plants. This finding was with the agreement of Abu Bakar et al. (2009). Thus, the antioxidant activities most probably might be contributed by polyphenols contents in the plant extracts.

The plants exhibited significant result in hesperidin and naringin (Table 1). These might be the major components contributing to antioxidant activities of the plants investigated. The ranking of flavanone contents was similar to the trend of three antioxidant assessments conducted: *B. pulchella* var *attenuata*> *B. rotunda*> *B. armeniaca*. Thus, the antioxidant activities most probably might be contributed by flavanone contents in the plant extracts. Hesperetin which was categorised as flavanone has been shown to inhibit chemically induced mammary (So et al., 1996), urinary bladder (Yang et al., 1997), and colon (Miyagi et al., 2000) carcinogenesis in laboratory animals.

Quercetin was the major flavonols presented in *B. rotunda*, while luteolin was the major flavones in *B. armeniaca* (Table 1). Thus, quercetin and luteolin might be the agent possible contributed to anticancer activities (especially MCF-7) in *B. rotunda* and *B. armeniaca* respectively (Table 4). Moreover, Huang et al. (1999) discovered that quercetin and luteolin induced apoptosis in a wide range of tumor cells such as HepG2 and MCF-7. Wei et al. (1994) reported that quercetin induced apoptosis, characterized by typical morphological changes, in certain tumor cell lines. These findings supported that the two flavonoids types might be contributing to anticancer activities.

Boesenbergia rotunda (formerly known *Boesenbergia pandurata*) (Zingiberaceae) has been used as the main ingredient of a popular traditional tonic called “jamu”

especially for women in Indonesia. The fresh rhizomes are used in cooking and traditional medicine as antiseptic and for the treatment of stomach ache (Hasnah et al., 1995), diarrhea, dermatitis, dry cough and mouth ulcers (Burkill, 1935; Heyne, 1987), gastrointestinal disorders and post-natal treatment (Burkill, 1935). Another species of *Boesenbergia*, for example, *B. stenophylla* stem part was used by local people as a charm to protect babies from ghosts in the past. The stem was tied to the baby's hammock. Villagers chew on this plant species to relieve cough, diarrhea and food poisoning. However, *B. pulchella* var *attenuata* and *B. armeniaca* are not commonly used as medicinal plant by the local people in Sabah. However, these promising findings showed that the three plants investigated have potential as an anticancer drug in future.

The potential medicinal uses of these *Boesenbergia* species are supported by the presence of the above mentioned polyphenols constituents, antioxidants and anticancer activities. Hence, the need to exploit the potentials of these plants especially in areas of traditional medicine and pharmaceutical industries arises.

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REFERENCES

Abu Bakar MF, Mohamed M, Rahmat A, Fry J (2009). Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*). Food Chem. 113: 479-483.

Benzie IFF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem. 239: 70-76.

Burkill IH (1935). A Dictionary of the Economic Products of the Malay Peninsula. London: Government of the Straits Settlements and Federated Malay State by the Crown agents for the colonies, 1: 1078-1079.

Duh PD, Tu YY, Yen GC (1999). Antioxidant activity of water extract of harn jyur (*Chyrsanthemum morifolium* Ramat). LWT-Food Sci. Technol. 32: 269–277.

Hada M, Hino K, Takeuchi Y (2001). Development of UV defense mechanisms during growth of spinach seedlings. Plant Cell Phys. 42(7): 784-787.

Hasnah MS, Shajarahtunnur J, Neelavany M (1995). Chemical constituents of *Boesenbergia* species. Chemical prospecting in the Malaysian forest. In Ghazally I, Murtedza M, Laily D (eds), Pelanduk Publications (M) Sdn. Bhd: Kuching, Malaysia.

Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara D (1989). Effects of interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chem. Pharm. Bull. 37: 2016-2021

Heyne K (1987). Tumbuhan berguna Indonesia. Jilid IV. Jakarta: Badan Litbang Kehutanan, pp 592-594.

Kirana C, Jones GP, Record IR, McIntosh GH (2007). Anticancer properties of panduratin A isolated from *Boesenbergia pandurata* (Zingiberaceae). J. Nat. Med. 61:131–137.

Larsen K (2003). The Zingiberaceae in Flora of Thailand. In: Chantaranothai, P. Larsen, K. Sirirugsa, P, Simpson D. (Eds.). Proceedings of the third symposium on the family Zingiberaceae. Khon Kaen University 1-5.

Mahady GB (2005). Medicinal plants for the prevention and treatment of bacterial infections. Curr. Pharm. Design. 11: 2405-2427.

Mensor LI, Menezes FS, Leitao GG, Reis AS, dos Santos T, Coube CS, Leitao SG (2001). Screening of Brazillian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 15: 127-130.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods. 16: 55–63.

Miyagi Y, Om AS, Chee KM, Bennink MR (2000). Inhibition of azoxymethane-induced colon cancer by orange juice. Nutr Cancer. 36: 224-9

Re N, Pellegrinni A, Proteggente A, Pannala M, Yang C, Rice-Evans (1999). Antioxidant activities applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26: 1231-1237.

Schieber A, Keller P, Carle R (2001). Determination of phenolic acids and flavonoids of apple and pear by HPLC. J. Chromatogr. A. 910: 265-273.

Shahidi F, Wanasundara PKJPD (1992). Phenolic antioxidants. Cr. Rev. Food Sci. Nutr. 32: 67-103.

So FV, Guthrie N, Chambers AF, Moussa M, Carroll KK (1996). Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices. Nutr. Cancer. 262: 167- 81.

Sun J, Chu YF, Wu X, Liu RH (2002). Antioxidant and anti-proliferative activities of fruits. J. Agric. Food Chem. 50: 7449-7454.

Tewtrakul S, Subhadhirasakul S, Puripattanavong J, Panphadung T, (2003). HIV-1 protease inhibitory substances from the rhizomes of *Boesenbergia pandurata* Holtt. Songklanakarin J. Sci. Technol. 25(4): 503-508.

Velioglu YS, Mazza G, Gao L, Oomah BD (1998). Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. J. Agric. Food Chem. 46(10): 4113 -4117.

Wettasinghe M, Bolling B, Plhak P, Parkin K (2002). Screening for phase II enzyme-inducing and antioxidant activities of common vegetables. J. Food Sci. 67: 2583-2588.

Yao LH, Jiang YM, Shi J, Tomas-baeberan FA, Datta N, Singanusong R (2004). Flavonoids in Food and Their Health Benefits. Plant Food Hum. Nutr. 59: 113–122.

Yang M, Tanaka T, Hirose Y, Deguchi T, Mori H, Kawada Y (1997). Chemopreventive effects of diosmin and hesperidin on N-butyl-N-(4hydroxybutyl)nitrosamine-induced urinary-bladder carcinogenesis in male ICR mice. Int. J. Cancer. 73: 719- 24.

Zhishen J, Mengcheng T, Jianming W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64: 555-559.