

Antioxidant and anti-proliferative activities of Sabah *Ruellia tuberosa*

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ARTICLE INFO

Article history:

Received on: 24/09/2013

Revised on: 08/11/2013

Accepted on: 06/12/2013

Available online: 30/12/2013

Key words:

Ruellia tuberosa; antioxidant activity, anti-proliferative activity.

ABSTRACT

The study was carried out to evaluate the total phenolic constituents, antioxidant and anti-proliferative activities of Sabah *Ruellia tuberosa*. The total phenolic and flavonoid contents of the plant extracts were determined by using Folin-Ciocalteu and aluminium chloride colorimetric assays, respectively. The antioxidant activity of the plant extracts was evaluated using DPPH free radical scavenging assay while the anti-proliferative activity was evaluated using MTT assay against the human breast cancer (MCF-7) and cervical cancer (HeLa) cell lines. The methanol leaf extract was found to possess the highest total phenolic content (82.67 ± 2.09 mg GAE/g) while the ethyl acetate leaf extract was found to possess the highest total flavonoid content (152.77 ± 4.68 mg Cat/g). The ethyl acetate leaf possessed the highest radical scavenging activity, with IC_{50} of 720 μ g/ml. Meanwhile, the methanol stem extract showed the highest anti-proliferative activity against MCF-7 cancer cells, with IC_{50} of 22 μ g/ml but none of the extracts exhibited strong anti-proliferative activity against the HeLa cancer cell lines. Significant correlation was found between the total phenolic/flavonoid contents with the total antioxidant activity while weak correlation was found between the total phenolic/flavonoid contents with the inhibition of MCF-7 cell proliferation. Our findings indicate that Sabah *Ruellia tuberosa* could be a potential source for natural antioxidant as well as chemo-preventive agent against breast cancer in future. Thus, further isolation and characterization of the respective bioactive compounds from the plants are necessary.

INTRODUCTION

Ruellia tuberosa L. (Acanthaceae) is a perennial plant where its native range is in Central America but presently has become naturalized in many countries of tropical South and Southeast Asia. It has different names such as Minnie Root, Fever Root, Snapdragon Root and Sheep Potato. In Sabah, Malaysia, the plant is called by the local as "Cracker Plant" as the seeds burst when contact with water. The plants can be easily found in the shady and moisture place such as side drain and have been used by the local peoples as diuretic, antipyretic and anti-hypersensitive agents. According to Cragg and Newman (2000), over 50% of the drugs in clinical trials for anticancer activity were isolated from natural sources or are related to them. There are several drugs used in chemotherapy nowadays are derived from plant species. For example, vincristine and vinblastine which are isolated from *Catharanthus roseus*, taxol and taxanes which are isolated from *Taxus* species, and camphothecin which is isolated from *Camphotheca acuminata*, and so on (Costa-Lotufo et al., 2005). To date, few chemical constituents and pharmacological activities

have been reported for *Ruellia tuberosa* from Taiwan and India. The whole plant has been shown to possess antimicrobial activity (Arirudran et al., 2011) and the stem has also been shown to possess antioxidant activity (Chen et al., 2004). Besides, three flavonoids (cirsimarín, cirsiliol 4'-glucoside and sorbifolin) isolated from the aerial parts of the plants also possess anticancer activities against the tested epidermoid carcinoma (KB) and hepatoma (HepG2) cell lines (Lin et al., 2006). Due to its reported anticancer effect and also good sustainability in Sabah, the plant is considered to be one of the potential sources for the development of chemopreventive drugs in future. Therefore, this study was carried out to investigate the antioxidant and anti-proliferative activity of *Ruellia tuberosa* from Sabah. Since there is no report of the phenolic and flavonoid contents from this plant yet, the total phenolic and flavonoid contents of the plant were also quantified and the correlation with antioxidant and anti-proliferative activity were also studied.

MATERIALS AND METHODS

Chemicals and reagents

2,2-diphenyl-1-picryl-hydrazyl (DPPH), α -tocopherol, ascorbic acid, gallic acid, (+)-catechin, butylated hydroxytoluene

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(BHT), aluminium chloride, sodium nitrite and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). All reagents used (methanol, ethyl acetate, and dimethyl sulfoxide/DMSO) were of analytical grade and obtained from Fisher Scientific (Pittsburgh, PA, USA). RPMI-1640 medium, ferum bovine serum, trypsin-EDTA and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Nacalai Tesque (Kyoto, Japan). The human cervical cancer (HeLa) and breast cancer (MCF-7) cell lines were purchased from the American Type Culture Collection (ATCC) (Rockville, MD, USA).

Plant materials

The leaves and stems of *Ruellia tuberosa* were collected in July, 2012 at the side drains of Kingfisher Park, Kota Kinabalu, Sabah, Malaysia. The plants were identified by botanist from Universiti Malaysia Sabah. The specimen voucher (ACRT01/2013) was deposited at the herbarium of the university. The plants were washed and freeze-dried before pulverized and stored at -80°C for further use.

Preparation of plant extracts

For absolute methanol and ethyl acetate extractions, 12 g of powdered leaves or stems were extracted in a Soxhlet apparatus with 300 ml of solvent at 60°C for 13 hours. The extracts were filtered and evaporated to dryness under reduced pressure at 40°C in a rotary evaporator. The crude extracts were kept at -80°C and re-dissolved in appropriate solvents before they were used for the subsequent assays.

Determination of total phenolic content

The total phenolic contents in the four methanol and ethyl acetate extracts were determined with Folin-Ciocalteu colorimetric assay as described by Slinkard and Singleton (1977) but with some modifications. The standard gallic acid solutions were prepared with different concentrations (ranged between 0.05 mg/ml to 0.4 mg/ml) whereas the plant extracts were prepared in 0.625 to 10 mg/ml. Briefly, 0.1 ml of aliquot of the extracts or standard were added with 4.5 ml of distilled water. Then, 0.1 ml of Folin-Ciocalteu reagent (previously diluted 3-fold with distilled water) and 0.3 ml 2% Na₂CO₃ solution were added to the mixture and vortexed. The mixture was left to stand in dark condition at room temperature for 2 hours. The absorbance of the mixture was measured at 760 nm by using a visible spectrophotometer (Thermo Scientific, USA), against a blank containing 0.1 ml of solvent used to prepare the extracts. The amount of total phenolic compounds was calculated as mg of gallic acid equivalents (GAE) from the calibration curve of gallic acid standard and expressed as mg GAE/g dry weight (DW) of the plant material. The data were presented as the average of triplicated analyses.

Determination of total flavonoid content

The total flavonoid content of the plant extracts was determined by using a procedure described by Sakanaka *et al.*,

(2005), with some modifications. Briefly, 125 µl of the extracts (prepared from 0.625 to 5 mg/ml) or (+)-catechin standard solution (prepared from 15 to 250 µg/ml) was mixed with 625 µl of distilled water in a test tube, followed by the addition of 40 µl of a 5% (w/v) sodium nitrite solution. After 6 min of incubation at room temperature, 75 µl of a 10% (w/v) aluminium chloride solution was added and the mixture was allowed to stand for another 5 min before 250 µl of 1 M NaOH was added. The mixture was made up to 1.5 ml with distilled water and mixed well. The absorbance was measured immediately at 510 nm with a visible spectrophotometer. The results of triplicate analyses were expressed as mg Cat/g dry weight of the plant material.

DPPH free radical scavenging assay

The free radical scavenging activity of the plant extracts was measured as according to Oszoy *et al.*, (2008). Briefly, 0.1 ml aliquot of each extract (0.625-5 mg/ml), gallic acid (0.016-0.125 mg/ml) and positive controls (BHT, ascorbic acid, α-tocopherol, from 0.016-0.5 mg/ml) in absolute methanol was added to 3.9 ml of 6 X 10⁻⁵ M absolute methanolic solution of DPPH. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. The decrease in absorbance of the resulting solution was then measured at 517 nm against methanol using a spectrophotometer. All measurements were made in triplicate. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = (1 - absorbance of sample at 517 nm / Absorbance of control at 517 nm) X 100%

Anti-proliferation assay

The human cervical cancer (HeLa) and breast cancer (MCF-7) cell lines were used for this study. Both of the human cancer cell lines were maintained in RPMI media supplemented with 10% FBS. Cells were maintained at 37°C and 5% CO₂. Routine observation for cell lines viability was performed under phase contrast inverted microscopy. Cell numbers were determined by trypan blue exclusion method and counted in a hemocytometer. The MTT assay was performed by using Cell Proliferation Kit (Roche, Switzerland) according to manufacturer's instruction with some modifications. Briefly, cells were plated in 96-well plates with 10⁵ cells/well and incubated at 37°C and 5% CO₂ for 24 hours. After 24 hours, the extracts (15 - 90 µg/ml) dissolved in DMSO were added to each well and incubated for 3 days (72 hours). The negative control received the same amount of DMSO instead of extracts. At the end of 72 hours of incubation time, the medium in each well was replaced by fresh medium (200 µl) containing 10 µl of labelling reagent and incubated. Growth of cancer cells was quantified by the ability of living cells to reduce the yellow dye, MTT to a purple formazan product. After 4 hours of incubation, the formazan product of MTT reduction was solubilized using solubilisation reagent and incubated for 24 hours, followed by measurement at 540 nm by the microplate reader (Molecular Devices, USA). The 50% growth inhibitory concentration (IC₅₀) was defined as the plant extract concentration

(units in mg/ml) required to cause a 50% inhibition and was used as the basis for comparing anti-proliferative activity of different samples.

Statistical analysis

Statistical analysis was performed using SPSS Statistic version 20 (IBM Corporation, USA). Correlations between various parameters were also investigated. Significance was determined at $p < 0.05$. All data were reported as the mean \pm SD of three replications.

RESULTS

Total phenolic and flavonoid contents of the plant extracts

The total phenolic contents of the four types of *Ruellia tuberosa* extracts are shown in Table 1. Total phenolic contents were expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg GAE/g DW) of the part of plants. The methanol leaf extract had the highest amount of phenolic content (82.67 ± 2.09 mg GAE/g DW), followed by ethyl acetate leaf extract (73.67 ± 0.58 mg GAE/g DW), methanol stem extract (61 ± 2.65 mg GAE/g DW) and ethyl acetate stem extract (60.33 ± 1.53 mg GAE/g DW).

Meanwhile, the flavonoid contents were expressed as milligrams of catechin equivalents per gram of fresh weight (mg Cat/g DW) of the part of plants. The ethyl acetate leaf extract had the highest amount of flavonoid content (152.77 ± 4.68 mg GAE/g DW), followed by methanol leaf extract (140.29 ± 2.86 mg Cat/g DW), methanol stem extract (113.71 ± 1.88 mg Cat/g DW) and ethyl acetate stem extract (52.39 ± 1.20 mg Cat/g DW).

Table 1: Total phenolic content (as gallic acid equivalents) and total flavonoid content (as catechin equivalent) in the *Ruellia tuberosa* leaf and stem extracts.

Extract	Total Phenolic Content (mgGAE/g DW)	Total Flavonoid Content (mgCat/g DW)
Methanol leaf	82.67 ± 2.09	140.29 ± 2.86
Methanol stem	61 ± 2.65	113.71 ± 1.88
Ethyl acetate leaf	73.67 ± 0.58	152.77 ± 4.68
Ethyl acetate stem	60.33 ± 1.53	52.39 ± 1.20

Values were the means of three replicates \pm standard deviation (SD).

DPPH free radical scavenging activity of the plant extracts

The total antioxidant activity, determined by DPPH free radical scavenging assay, is shown in Figure 1. Ascorbic acid (vitamin C) and α -tocopherol (vitamin E) were used as standard drugs in this study. The percentage of inhibitory activity of free radicals by 50% (IC_{50}) was used as parameter to measure the antioxidant activity. In Figure 1, all the standard drugs and four types of plant extracts have significantly reduced the DPPH radicals with increasing concentrations. From the four types of *R. tuberosa* extracts, the ethyl acetate leaf possessed the highest radical scavenging activity, with IC_{50} of $720 \mu\text{g/ml}$. This was followed by methanol leaf extract (IC_{50} of $800 \mu\text{g/ml}$), methanol stem extract (IC_{50} of $1050 \mu\text{g/ml}$) and ethyl acetate stem extract (IC_{50} of $1640 \mu\text{g/ml}$).

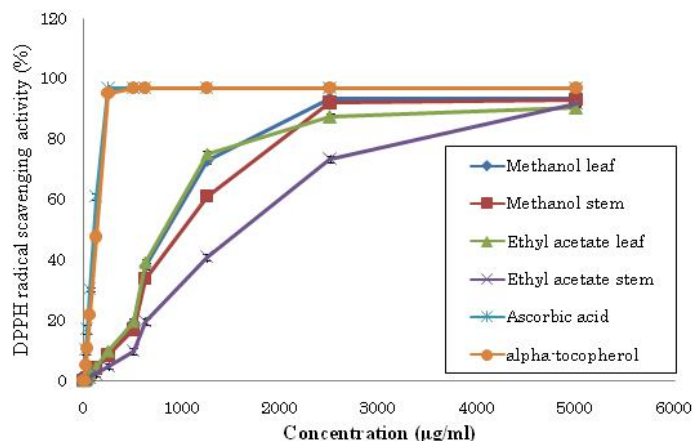


Fig. 1: DPPH radical scavenging activities of the four *R. tuberosa* extracts in comparison with ascorbic acid and α -tocopherol. Results are means \pm SD (n = 3).

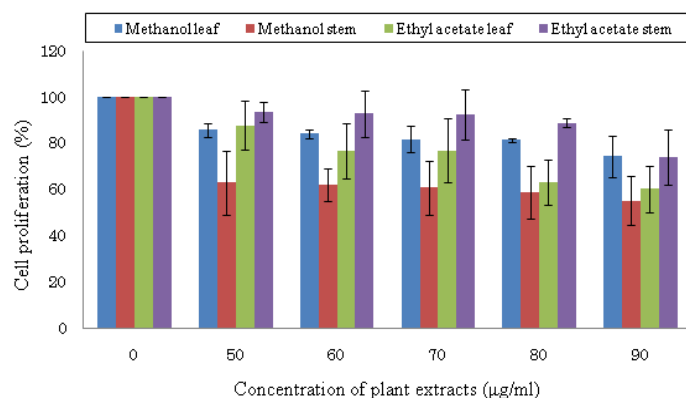


Fig. 2: Percent inhibition of HeLa cell proliferation by the four *R. tuberosa* extracts. All the extracts used were from 50 to 90 $\mu\text{g/ml}$. Results are means \pm SD of duplicate analysis of three replications.

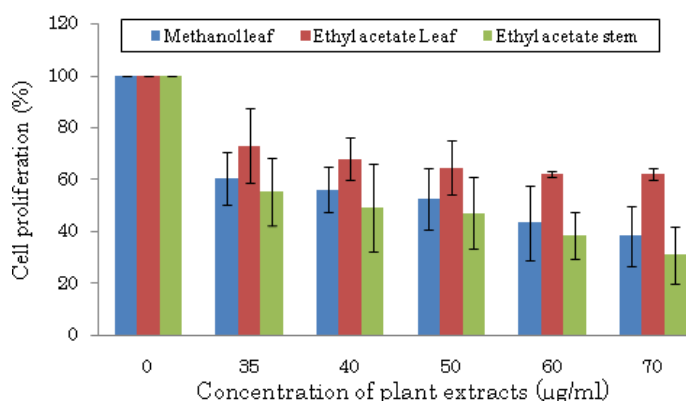


Fig. 3: Percent inhibition of MCF-7 cell proliferation by the four *R. tuberosa* extracts. (a) Extracts used were from 35 to 70 $\mu\text{g/ml}$ and.

Anti-proliferative activity of the plant extracts

Anti-proliferative activities of the four *R. tuberosa* extracts on the growth of human cervical cancer (HeLa) cell lines are summarized in Figure 2. The results were expressed as the IC_{50} (50% growth inhibitory concentration), with a lower IC_{50} value indicating a higher anti-proliferative activity. Among the four tested extracts, all of them have shown slight inhibitory activity on the HeLa cell growth, all with IC_{50} values more than $90 \mu\text{g/ml}$.

The methanol stem extract possessed the highest anti-proliferative activity, followed by ethyl acetate leaf extract, methanol leaf extract and ethyl acetate stem extract.

Meanwhile, the anti-proliferative activities of the four *R. tuberosa* extracts on the growth of human breast cancer (MCF-7) cell lines are summarized in Figure 3 (a) and (b). Among the four extracts, the methanol stem extract possessed the highest anti-proliferative activity, with IC₅₀ value of 22 µg/ml, followed by ethyl acetate stem extract (40 µg/ml), and methanol leaf extract (55 µg/ml). The ethyl acetate leaf extract showed minor inhibitory effect on the cell growth with IC₅₀ value more than 70 µg/ml.

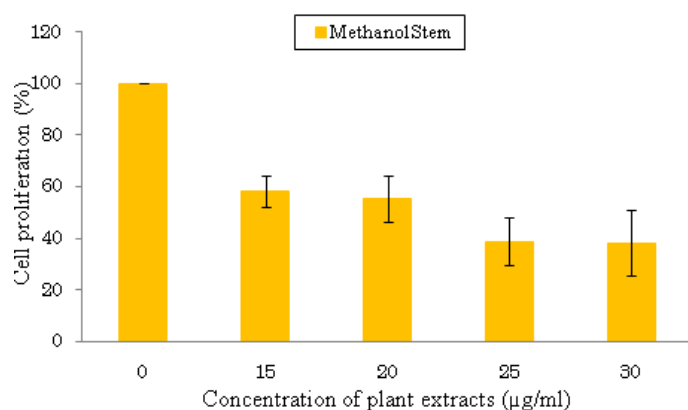


Fig. 3b: (b) 15 – 30 µg/ml. Results are means ± SD of duplicate analysis of three replications.

Correlations of the total phenolic/flavonoid contents with the plants' antioxidant and anti-proliferative activities

The correlations between phytochemical contents with total antioxidant and anti-proliferative activities are summarized in Table 2. A significant linear relationship was found between total phenolic content and total antioxidant activity ($R^2 = 0.756$, $p < 0.01$), as well as between flavonoid content and total antioxidant activity ($R^2 = 0.966$, $p < 0.01$). Positive correlation indicates that the higher the total phenolic/flavonoid content, the higher the total antioxidant activity. Besides, the relationship between the total phenolic/flavonoid contents with the anti-proliferative activity against MCF-7 cell lines was also examined. There were weak correlations between the inhibition of MCF-7 cell proliferation with phenolic content ($R^2 = 0.667$, $p < 0.05$) and flavonoid content ($R^2 = 0.576$, $p < 0.05$), respectively.

Table 2: Correlation analysis of phytochemical content, antioxidant and anti-proliferative activity.

	Antioxidant Activity	Anti-proliferative Activity, MCF
Phenolics	0.756**	0.667*
Flavonoids	0.966**	0.576*

*Significantly different, $p < 0.05$, ** Significant different $p < 0.01$.

DISCUSSION

Phenolic compounds are secondary plant metabolites which possess an aromatic ring that bearing one or more hydroxyl groups and have been reported to possess antioxidant and

anticancer activities (Balasundram *et al.*, 2006; Chu *et al.*, 2002). Flavonoids, a subgroup of phenolic compounds, are especially important antioxidants and potential anticancer agents due to their high redox potential, which allows them to act as reducing agents, hydrogen donor and singlet oxygen quenchers (Tsao and Yang, 2003).

In this study, the total phenolic and flavonoid contents of the leaf and stem of Sabah *R. tuberosa* were determined. The leaves were found to possess higher phenolic and flavonoid contents than the stems (Table 1), disregard extracted by polar solvent, methanol or less polar solvent, ethyl acetate. This indicates that both the polar and less polar phenolic compounds are present and accumulated in the leaves compared to the stems.

Next, the antioxidant activities of the four leaf and stem extracts were evaluated using DPPH free radical scavenging activity. DPPH are stable organic nitrogen radicals that have a UV-vis absorption maximum at 515 nm. The purple color of the solution is faded due to the radical scavenging activity of antioxidant agent such as phenolic compounds and flavonoids (Huang *et al.*, 2005). In this study, both of the leaf ethyl acetate and methanol extracts possessed higher free radical scavenging activity than the stem extracts. A strong positive correlation between the total phenolic/flavonoid contents of the extracts with the DPPH free radical scavenging activity was also observed. This finding was agreeable with Chen *et al.* (2006) in which they reported that the antioxidant activity of the plant might be related to its flavonoid contents. Besides, the antioxidant activity of the leaf and stem extracts were also comparable to the standards used such as ascorbic acid and alpha-tocopherol. Therefore, the *R. tuberosa* plants which have good sustainability in Sabah could have the potential to be developed as antioxidant nutraceutical products in the future.

The anti-proliferative activity of the Sabah *R. tuberosa* was evaluated using MTT assay. MTT assay is the most common assay used to evaluate the growth/proliferation of living cells. A mitochondrial enzyme in living cells, succinate dehydrogenase cleaves the tetrazolium ring of MTT, converting the MTT to an insoluble purple formazan which was measured at 540 nm. Therefore, the amount of formazan produced is directly proportional to the number of viable cells (Mosmann, 1983). In this study, the plant extracts were found to inhibit the growth of breast cancer cell lines (MCF-7) but very slight inhibitory effects on cervical cancer cell lines (HeLa). The concentration of the methanol stem extracts which showed the strongest inhibitory effects against the MCF-7 cell lines has to be set much more lower (Figure 3b) compared to other extracts (Figure 3a) as high concentration of this extract showed almost 100% of inhibition and thus IC₅₀ value could not be determined accurately. In this study, a weak correlation between the total phenolic and flavonoid contents with the anti-proliferative activity against MCF-7 cell lines was observed. This suggests that the inhibition of human breast cancer cell by the extracts could not be explained solely by their phenolic/flavonoid contents. Lin *et al.* (2006) has reported the isolation of three flavonoids, they are cirsimarin, cirsiolol 4'-

glucoside and sorbifolin which show anti-proliferative activity against KB and HepG2 cell lines but Arun *et al.* (2008) also reported the isolation a phenanthrene alkaloid, tylocrobrine from the plant which possesses anticancer effect as well. Therefore, it is speculated that the anti-proliferative activity of the plant extracts against MCF-7 cells was not only due to the presence of phenolic/flavonoid compounds but may be due to the presence of other classes of compounds as well. Further investigation to identify the respective anti-breast cancer compounds especially from the stem part as well as study against more variety of cancer cell lines are necessary in future before the plants could be developed as any potential anticancer drugs.

CONCLUSION

In conclusion, our findings indicate that Sabah *Ruellia tuberosa* which have good sustainability and high phenolic/flavonoid content could be a potential source for natural antioxidant as well as chemo-preventive agent against breast cancer in future.

ACKNOWLEDGEMENTS

The authors thank Ministry of Higher Education (MOHE), Malaysia for providing the RAGS research grant (grant code: RAG0003-SG-2012) to support this research.

REFERENCES

- Arirudran B, Saraswathy A and Krishnamurthy V. Antimicrobial activity of *Ruellia tuberosa* L. (whole plant). *Pharmacognosy Journal*, 2011; 3: 91-95.
- Arun S, Giridharan P, Suthar A, Kulkarni-Almeida A, Naik V, Velmurugan R, Ram V. *et al.* 2008. Isolation of tylocrobrine from *Ruellia tuberosa* through bioassay directed column chromatography and elucidating its anti-cancer and anti-inflammatory potential. *Book of Abstracts, 7th Joint Meeting of GA, AFERP, ASP, PSI and SIF, Athens, Greece.* p.25.
- Balasundram N, Sundram K and Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, 2006; 99: 191-203.
- Chen FA, Wu AB, Shieh PC, Kuo DH and Hsieh CY. Evaluation of the antioxidant activity of *Ruellia tuberosa*. *Food Chemistry*, 2006; 94: 14-18.
- Chu YF, Sun J, Wu XZ and Liu RH. Antioxidant and anti-proliferative activities of common vegetables. *Journal of Agricultural and Food Chemistry*, 2002; 50: 6910-6916.
- Costa-Lotufo LV, Hassan Khan MT, Ather A, Wilke DV, Jimenez PC, Pessoa C, Moraes MEA and Moraes MO. Studies of the anticancer potential of plants used in Bangladeshi folk medicine. *Journal of Ethnopharmacology*, 2005; 99: 21-30.
- Cragg GM and Newman DJ. Antineoplastic agents from natural sources: achievements and future direction. *Expert Opinion on Investigational Drugs*, 2000; 9: 1-15.
- Daya LC, Patel MB, Mishra SH and Vaghasiya HU. Review on *Ruellia tuberosa* (Cracker plant). *Pharmacognosy Journal*, 2010; 2: 506-512.
- Huang DJ, Ou BX and Prior RL. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 2005; 53: 1841-1856.
- Lin CF, Huang YL, Cheng LY, Sheu SJ and Chen CC. Bioactive flavonoids from *Ruellia tuberosa*. *Journal of Chinese Medicine*, 2006; 17: 103-109.
- Mbaebie BO, Edeoga HO and Afolayan AJ. Phytochemical analysis and antioxidant activities of aqueous stem bark extract of *Schotia latifolia* Jacq. *Asian Pacific Journal of Tropical Biomedicine*, 2012; 118-124.
- Merrill RP and Weed DL. Measuring the public health burden of cancer in the United States through lifetime and age-condition rich estimates. *Annals of Epidemiology*, 2001; 11: 547-553.
- Moongkarndi P, Kosem N, Kaslungka S, Luanratana O, Pongpna N and Neungton N. Antiproliferation, antioxidation and induction of apoptosis by *Garcinia mangostana* (mangosten) on SKBR3 human breast cancer cell line. *Journal of Ethnopharmacology*, 2004; 90: 161-166.
- Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 1983; 65: 55-63.
- Ozsoy N, Can A, Yanardag R and Akev N. Antioxidant activity of Smilax excels L. leaf extracts. *Food Chemistry*, 2008; 110: 571-583.
- Raja Kannan RR, Arumugam R and Anantharaman P. *In vitro* antioxidant activities of ethanol extract from *Enhalus acoroides* (L.F.) Royle. *Asian Pacific Journal of Tropical Medicine*, 2010; 898-901.
- Sakanaka S, Tachibana Y and Okada Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chemistry*, 2005; 89: 569-575.
- Slinkard K and Singleton VL. Total phenolic analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 1997; 28: 49-55.
- Tsao R and Yang R. Optimization of a new mobile phase to know the complex and real polyphenolic composition: Towards a total phenolic index using high-performance liquid chromatography. *Journal of Chromatography A*, 2003; 1018: 29-40.
- Xu BJ and Chang SKC. Phytochemical profiles and health-promoting effects of cool-season food legumes as influenced by thermal processing. *Journal of Agricultural and Food Chemistry*, 2009; 57: 10718-10731.
- Yang LL, Lee CY and Yen KY. Induction of apoptosis by hydrolysable tannin from *Eugenia jambos* L. on human leukemia cells. *Cancer Letters*, 2000; 157: 65-75.

How to cite this article:

Bo Eng Cheong, Mohd. Zulkarnain Waslim, Fui Fui Lem, Peik Lin Teoh. Antioxidant and anti-proliferative activities of Sabah *Ruellia tuberosa*. *J App Pharm Sci*, 2013; 3 (12): 020-024.