RESEARCH ARTICLE

Antioxidant value and Antiproliferative Efficacy of Mitragynine and a Silane Reduced Analogue

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Abstract

Background: To investigate the antioxidant value and anticancer functions of mitragynine (MTG) and its silane-reduced analogues (SRM) *in vitro*. Materials and Methods: MTG and SRM was analyzed for their reducing power ability, ABTS radical inhibition and 1,1-diphenyl-2-picryl hydrazylfree radicals scavenging activities. Furthermore, the antiproliferation efficacy was evaluated using MTT assay on K 562 and HCT116 cancer cell lines versus NIH/3T3 and CCD18-Co normal cell lines respectively. Results: SRM and MTG demonstrate moderate antioxidant value with ABTS assay (Trolox equivalent antioxidant capacity (TEAC): 2.25±0.02 mmol trolox / mmol and 1.96±0.04 mmol trolox / mmol respectively) and DPPH (IC_{50}=3.75±0.04 mg/mL and IC_{50}=2.28±0.02 mg/mL respectively). Both MTG and SRM demonstrate equal potency (IC_{50}=25.20±1.53 and IC_{50}= 22.19±1.06 respectively) towards K 562 cell lines, comparable to control, betulinic acid (BA) (IC_{50}24.40±1.26). Both compounds showed concentration-dependent cytototoxicity effects and exert profound antiproliferative efficacy at concentration > 100 µM towards HCT 116 and K 562 cancer cell lines, comparable to those of BA and 5-FU (5-Fluorouracil). Furthermore, both MTG and SRM exhibit high selectivity towards HCT 116 cell lines with selective indexes of 3.14 and 2.93 respectively compared to 5-FU (SI=0.60). Conclusions: These findings revealed that the medicinal and nutitional values of mitragynine obtained from ketum leaves that growth in tropical forest of Southeast Asia and its analogues does not limited to analgesic properties but could be promising antioxidant and anticancer or chemopreventive compounds.

Keywords: Mitragynine related analogues - antioxidant value - antiproliferative efficacy

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Introduction

Mitragynine dominated *M.speciosa* Korth (Ketum) leaves has widely recognised in Southeast Asia as an effective analgesic medicinal agents owing to its opioid-like effects (Matsumoto et al., 2004; Adkin et al., 2011). Mitragynine was used as as an alternative to opium to combat craving and opioid withdrawal symptons (Matsumoto et al., 2004) and for pain management (Nelson et al. 2010; Vicknasingam et al., 2010).

Hydroxyl (–OH), and peroxyl radicals (ROO–) have been associated with carcinogenesis, coronary heart disease, and many other health issues related to advanced age (Gupta et al., 2009; Fu-Qiang et al., 2013; Jing-A et al, 2013; Simay et al., 2013; Wesen et al., 2013; Joanna and Ewa, 2014). Therefore, exogeneous supply of antioxidants is essential as a complimentary and replacement of depleted endogeneous antioxidants due to aging (Sirinya et al., 2014). The antioxidant might be used in prevention of aging-associated diseases, including cancer, by suppressing the formation of ROS and scavenging free radicals (Fu-Qing et al., 2013; Jing-A et al., 2013; Simay et al., 2013; Joanna and Ewa, 2014). Antioxidant activity is involved in cancer prevention at the initiation stage (chemopreventive) while antiproliferative activity is targeting cancer cells at the promotion and progression stage (Ahmad and Normaliza, 2013; Sami et al., 2013; Nejib et al., 2013; Eduardo et al., 2013; papavadee et al., 2013; Puthuparampil et al., 2013; Tarika et al., 2014). Antioxidant activity had been reported for M. Speciosa extracts (Suhanya et al., 2009) but no research on antioxidant activity of the pure mitragyine had been published.

The natural plant-based polyphenolic antioxidants such as flavanoids, flavanol, anthocyanins, rand rosmarinic acid and etcare very common (Supakit et al., 2013; Sami et al.,2013; Nejib et al., 2013; papavadee et al., 2013; Eduardo et al., 2013; Sadegh et al., 2013). Herraiz et al. (2003 and 2004) has reported the discovery of non-phenolic antioxidant based on β-carboline and indolic scaffold and elaborate in detail the anti-oxidative mechanism. These results suggest that indole alkaloid related analogues in general may conform to a family of radical scavengers and antioxidants other than the polyphenolic group that

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might act as potential protectants and free radical sinks at a physiological pH. This has prompted us to search and evaluate pure compounds other than polyphenolic which might have antioxidant value.

Mitragynine was found to be hepatotoxic and this necessitate further evaluation of its toxicology and pharmacological activities such as cytotoxicity. (Saidin, 2008; Saidin et al, 2008) had discovered that mitragynineexhibited dose-dependent inhibition of cell proliferation in the cell lines (HepG2, HEK 293, MCL-5, cHol and SH-SY5Y) at concentration>100 μg/ml with substantial cell death at 1000 μg/ml.

MTT assay was performed on mitragynine analogues in vitro to screen for their potential cytotoxic effect as anticancer drugs that inhibit the proliferation and the growth of the cell (Cytostatic) leading to the programmed cell death (apoptosis) (Zafer and ayse, 2013). Screening on anti-proliferation functionality of mitragynine related analogues on other cell lines is prudent since MTG demonstrate potent cytotoxicity activities against SH-SYS5 at high dose (Saidin, 2008).

Mitragynine could be promising alternatives for future pain management treatments (Matsumoto et al., 2004). However the potential cytotoxicity of mitragynine and its derivatives is still not fully documented in literature. Therefore, this prompted us to evaluate the cytotoxicity of mitragynine and its silane reduced derivative, which could be safer analogues due to its reduced indole double bond, on human cell lines in this study. Antoine et al. (2008) reported that reduced indole bond (indoline) might help to improve anti-tumour activity. Hence, the other objective is to evaluate whether the reduction of indole double bond in MTG to its analogue indoline, SRM, contribute any further enhancement in cytotoxicity and anti- proliferation functionality to human cancer cell lines as compared to MTG..

Materials and Methods

Materials

MTG was obtained from ketum leaves using the published purification protocol (TeikBeng et al., 2011). SRM was obtained using the published synthetic method (TeikBeng et al., 2013). Quercetin, BHT, trolox and all the other reagents used were of analytical grade and purchased from Sigma-Aldrich.

DPPH radical-scavenging assay

DPPH free radical scavenging activity of MTG, SRM, quercetin and BHT was measured using the method of (Chang-Hao et al., 2013; Supakit et al., 2013).Sample and standard (100 μL) at various concentrations (0.032, 0.0625, 0.125, 0.25, 0.5, and 1.0 mg/mL) were mix with 2 mL of 0.1 mM DPPH solution. After a 30 min incubation at room temperature (25 °C) in the dark, the reduction of the DPPH free radical was measured at 517 nm against 2 mL of DPPH solution in 100 μL of methanol as control. The radical-scavenging activity (RSA) was calculated using the following formula:% $\boldsymbol{RSA=[(A0-A1)\div A0]\times 100}$

where A0 is the absorbance of the control; and A1 is the absorbance of samples after 30 min.

ABTS antioxidant assay

The TEAC were determined by the ABTS antioxidant assay (Pinchuk et al., 2012, Chang Hao et al., 2013). ABTS.+ was produced by reacting 2 mM ABTS in H_2O with 2.45 μM potassium persulphate, stored in dark at 25 °C for 12-16 h before use. The ABTS+ solution was diluted to an absorbance of 0.70±0.02 at 734 nm in water. Then, 10 μL stock solutions of MTG and SRM, BHT, quercetin and Trolox at concentration 0.5-4 mM were added to 2 mL of ABTS.+ solution. The absorbance was measured at 734 nm, 6 min after addition of Trolox and samples. The TEAC values of the samples were calculated by relating the decrease in absorbance to that of the Trolox solution on a molar basis.

Measurement of reducing capacity using the FRAP assay

The ferric-reducing antioxidant power assay (FRAP) was employed with minor modification (Chang-Hao et al., 2013). Briefly, 1 mL of five different concentrations of MTG. SRM, quercetin and BHT (0.0625, 0.125, 0.25, 0.5, and 1.0 mg/mL) in methanol was mixed with 2.5 mL of PBS (pH 7.4) and 2.5 mL of 1% potassium hexacyanoferrate (lll).The solution was then incubated for 20 min in a 50°C water bath before 2.5 mL of 10% trichloroacetic acid (TCA) was added. Each solution was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.1% $FeCl_3$ (0.5 mL), and the absorbance was measured at 700 nm.

Cell culture

Normal colon fibroblast (CCD-18-Co), normal mouse fibroblast (NIH/3T3), erythroidleukaemic (K 562) and colon carcinoma cell lines (HCT 116) were purchased from the American Type Culture Collection (Rockville, MD) and were cultured in Dulbecco's modified Eagle's medium (Gibco/Invitrogen) supplemented with 10% foetal bovine serum, 100 unit/mL penicillin and 100 μg/mL streptomycin at 37°C under 5% CO_2.

MTT cell antiproliferation assay

MTG, SRM, betulinic acid (BA) and 5-fluorouracil (5-FU) were dissolved in dimethyl sulphoxide (DMSO) to form 20 mMstock solution and were kept at -20°C. Prior to the assay, the stock solutions were diluted in the complete culture medium to produce test solutions (range from 6.5 to 200.0 μM). Solutions of 0.1% DMSO in the complete culture medium were used as the vehicle control.To evaluate whether the anti-proliferative action was specific to tumour cells, these pure compounds were also tested on non-tumourigenic cell lines: NIH/3T3 and CCD-18Co. Briefly, the cells were seeded in 96-well micro-titre plates and treated with various concentrations of MTG, SRM, 5-FU and BA (range from 6.5-200 μM). The plates were incubated for 24 hbefore the test samples and the vehicle control were added, followed by incubation at 37°C for 72 h. Subsequently, 20 μl of 5 mg/mL MTT (Acros Organics, Belgium) was added to each well and incubated for another 4 h before the media were removed by aspiration.

The formazan product generated from the viable cells was dissolved in DMSO and measured at 570 nm

against the reference wavelength at 650 nm. The growth inhibitory effect of the test samples was determined by comparing the optical density of the treated sample to the optical density of the control. The results were expressed as the percentage of cell viability [OD sample/OD control x 100%] (Zafer and Ayse, 2013). A dose-response curve was plotted, and the concentrations of the compounds required to inhibit 50% (IC_{50}) of the cells' viability was either determined by interpolating the curve or calculated using Probit Analysis (SPSS Version 12.0.1, Chicago, IL, USA). All the experiments were carried out in triplicate.

Morphological changes of the untreated, cancer and normal cells treated with 6.5, 12.5, 25, 50, 100 and 200 μM MIT and SRM for 24, 48 and 72 h were viewed under an inverted light microscope.

Results

Antioxidant activity

DPPH-scavenging activity: The DPPH radical scavenging activity for MTG and SRM is shown in Figure 1a. The order of inhibition was as follows: quercetin>BHT>SRM>MTG. The antioxidant potencies, as determined by the IC_{50} value, both MTG and SRM indicate their good antioxidant activity. The IC_{50} values of MTG and SRM were 3.75±0.02 and 2.28±0.04 mg/ml, respectively (Table 1). The antioxidant activity of VAN was lower relative to quercetin (IC_{50}=0.20±0.00 mg/ml) and BHT (IC_{50}=0.28±0.00 mg/ml) (Table 1); however, the antioxidant activity of SRM and MTG, measured by the IC_{50} value, was higher than those of edible fruits, whose IC_{50} values have been reported by earlier researchers: orange (IC_{50}=5.40±1.30 mg/ml) and star fruit (IC_{50}=3.80±2.10) (Pin-Der-Duh, 1998).

Trolox equivalent antioxidant capacity: The TEAC antioxidant capacity values of MTG (1.96±0.02 mmol trolox/mmol) and SRM (2.25±0.03 mmol trolox/mmol) were much higher than the value for Trolox, which was used as a reference, but lower than the values for quercetin (4.86±0.02 mmol trolox/mmol) and BHT (3.64±0.03 mmol trolox/mmol) (Figure 1b). Thus, the ABTS radical-scavenging activities of all the tested samples were in the following order: quercetin>BHT>SRM>MTG.

Reducing capacity: Figure 1c compares the reducing capabilities of MTG and SRM to those of quercetin and BHT. The reducing powers of these mitragynine related analogues and standard compounds had the following order: quercetin>BHT>SRM>MTG. The ferric-reducing abilities of the compounds were weaker than those of quercetin and BHT, but these results indicated that this compounds are mild electron and hydrogen donors and might be able to terminate radical chain reactions by converting free radicals to more stable products via donating a hydrogen atom or by electron transfer reactions from indole nucleus (Herraiz et al., 2003; Herraiz et al., 2004).

Antiproliferation activity

Antiproliferation efficacy: All of the mitragynine

Table 1. IC_{50} (Inhibitory Concentration at 50%) of pure MTG and SRM Versus Standard Antioxidant (BHT, quercetin). Each Value is Expressed as the mean±SD (n=3)

Type of Standard/Pure Compound	IC_{50} (mg/ml)	IC_{50} (μM)
Quercetin	0.20±0.00	0.66
MTG	3.75±0.04	5.72
SRM	2.28±0.02	4.90
BHT	0.28±0.00	1.27

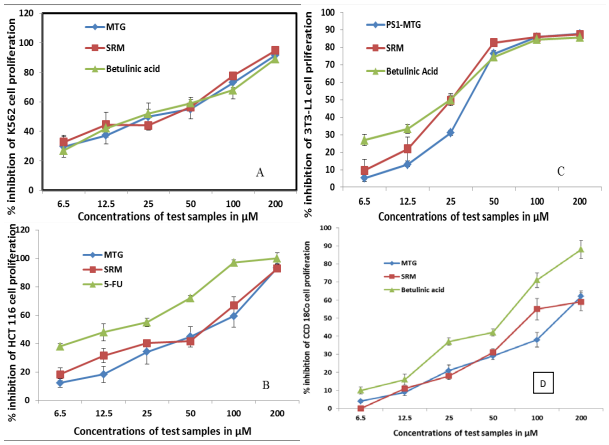

Figure 2. Effect of MTG, SRM on A) K 562, B) HCT 116 on Human Tumor Cell Lines Inhibition (%) and C) NIH/3T3, D) CCD 18 Co Normal Cell Line Inhibition (%) at Concentrations of 6.5, 12.5, 12.5, 25, 50, 100 and 200μM. Betulinic acid (selective to leukemia) and 5-Fluorouracil (selective to colon cancer) was used as the reference compound. The MTG and SRM show inhibition against cells' proliferation in dosedependent manner. Values are means of three experiments (n=3), error bars equal±SD

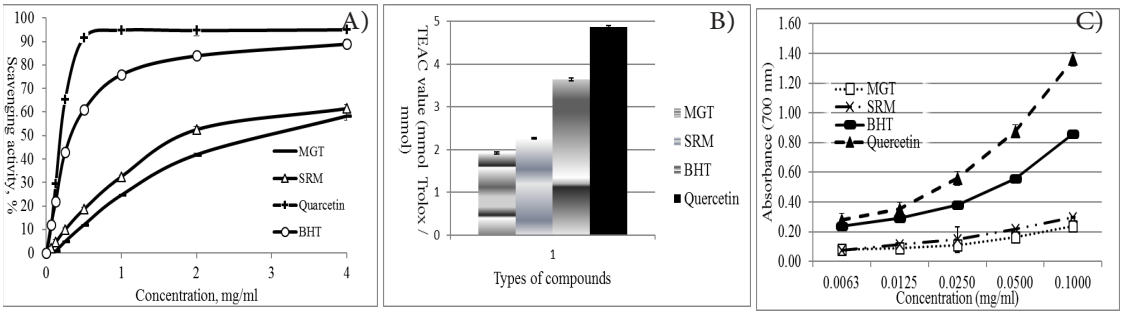

Figure 1. [a]DPPH Scavenging Activity (%) Versus Concentration of MTG, SRM, BHT and Quarcetin; [b]TEAC (Trolox Equivalent antioxidant capacity) value (mM) of MTG, SRM compared to BHT and Quercetin; [c]Reducing Power of MTG and SRM compared to BHT and Quercetin

related analogues (MTG and SRM) were assayed for anti-proliferative activities against 2 cancer cell lines (K 562 and HCT 116) and 2 normal cell lines (NIH/3T3 and CCD-18Co), using the MTT assay (Aditya et al., 2013; Alvin et al., 2014) after 48 hours of incubation in comparison with BA and 5-FU. It was interesting to note that both MTG and SRM exhibit equivalent and potent cytotoxic effects on the Leukaemia cancer K562 with IC_{50}=(25.20±1.53 μM) and (IC_{50}=22.19±1.06 μM) respectively compared to BA (IC_{50}=24.40±1.26 μM) (Table 2). However, the selective indexes is only 1.42 and 1.26 for MTG and SRM respectively compared to BA (SI=3.82) which is two time higher.

Both MTG and SRM displayed the moderate potency with IC_{50}=47.10±4.93 μm and 38.20±4.57 μm respectively against HCT 116 cell lines compared to 5-FU (7.32±0.63) (Table 2). However, from the dose concentration curve (Figure 2), at concentration above 100 μM, MTG and SRM demonstrate comparable percentage of inhibition (>80%) or efficacy, compared to BA and 5-FU.In conclusion, MTG and SRM generally exhibited potent cytotoxic effect and anti-tumour properties toward the colon cancer cell lines HCT 116 as well as the leukaemia cancer cell line K 562 at high dose (Figure 2). In this study, MTG related analogues selectively exhibited more potent cytotoxic activity toward the K562 leukimia cancer cell lines than HCT 116 colon cancer cell lines. The leukaemia cell lines were more susceptible and sensitive than colon cell lines toward these MTG analogues. The SI data listed in Table 1 indicate both MTG and SRM exhibited high selectivity toward HCT 116 with respect to CCD18-Co with selective indexes of 3.14 and 2.93 respectively compared to standard anticancer drugs for colon carcinoma, 5-FU, which had selective indexes of 0.60.

Table 2. Cytotoxicity Activity Presented as Median Inhibitory Concentration (IC_{50}) in μm and selectivity index (SI=IC_{50} Normal Cell Lines/IC_{50} Cancerous cell Lines) of MIT and SRM on Cancer Cell Lines and Normal Cell after 48 h. of Treatment, Results are Shown as Average±SD (n=3)

	IC_{50} (μM), SI (IC_{50} normal cell lines/IC_{50} cancerous cell lines) Cell Lines			
Compounds	HCT 116	CCD-18Co	K 562	NIH/3T3
MTG	47.10±3.47, 3.14	148.00±4.93	25.20±1.53 1.42	35.80±1.05
SRM	38.20±2.06 2.93	112.00±4.57	22.19±1.06 1.26	27.90± 1.18
BA	-	-	24.40± 1.26 3.82	93.27±3.18
5-FU	14.00±0.86 0.52	7.32±0.63	-	-

*Betulinic Acid (Selective to Leukemia) and 5-Fluororacil (Selective to Colon cancer) was used as the reference compound. Values are express as mean±standard deviation of three independent experiments (STD). NIH/3T3 and CCD-18Co was used as normal cell for comparison. Selectivity index (SI) of MIT and SRM on cancer cell lines above was calculated based on IC_{50} of NIH/3T3,IC50 of CCD-18Co and IC_{50} of Samples with SI value greater than 3 were considered to have high selectivity

The in vitro anti-cancer activity of MTG and SRM at different concentrations against K562 ,HCT116, NIH/3T3 and CCD-18Co after 48 h of treatment was plotted respectively to obtain a dose response curve as shown in Figure 2. It was observed that, in comparison to the positive control, BA and 5-FU, both compounds caused significant ($p<0.05$) dose-dependent cell death to a similar extent. Both MTG and SRM which reduced cell viability to below 20% of the control value at 200 μM (Figure 2). The present study clearly demonstrates the cytotoxic effect of MTG and SRM towards HCT116 and K562 cells at high dose (> 100 μM). The viability of cells as measured by the MTT assay was significantly decreased by incubation with increasing concentrations of MTG related analogues (Figure 2).In conclusion, MTG and SRM exhibited similar efficacy compared to the positive controls, BA and 5-FU at high dosage (> 100 um).

Cells morphology and apoptosis: As shown in the photograph in Figure 3, all of the compounds displayed early prominent biochemical hallmarks of programmed cell death (apoptosis): signs of nucleus shrinkage and compaction, cytoplasmic condensation, blebbing of cells,

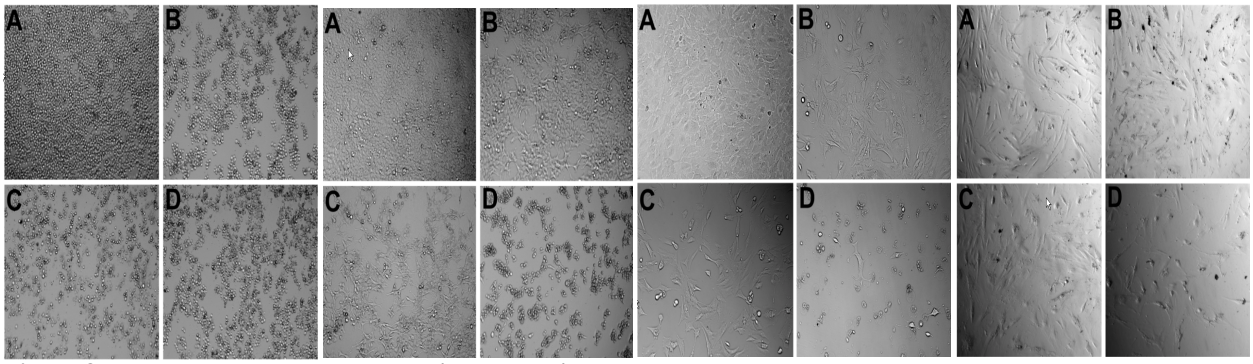

Figure 3. Human Eerythromyeloblastoid Leukemia. K562 (1), NIH/3T3 (2), colon cancer carcinoma HCT 116 (3), Human normal colonic fibroblast cell line CCD-18Co (4) cell images were taken under an inverted phase-contrast microscope at × 200 magnification with a digital camera at 48 h. after treatment with the samples, respectively. A-D in (1)-(4) indicates effects of two compounds MTG and SRM on K 562,NIH/3T3, HCT 116 and CCD-18Co cell lines respectively compared to control (A) and standard anti-cancer drugs, betulinic acid [(1D) and (2D)] and 5-FU [(3D) and (4D)]. Photomicrograph (1A), (2A), (3A) and (4A) depicts the K562, NIH/3T3, HCT116 and CCD18-Co cells from the control group formed a compact confluent layer (1-4A). Photomicrograph depicts the cytotoxic effect of the compound MTG (1-4B). It can be clearly seen that the population of cells reduced moderately after 48 h. of treatment. Photo depicts the treatment with SRM demonstrated significant cytotoxic effect on K562 cell proliferation (1-4C). Treatment with betulinic acid showed marked inhibition in cell proliferation (1-2D). The picture (3-4D) revealed that the population of the cells is reduced drastically with 5-FU (3-4D)

apoptotic cell debris separated from mother cells and the formation of vacuoles (autophagy)) (Boland et al., 2013). Therefore, MTG and SRM are apoptosis-inducing agents with differing potency against colon and leukaemia cancer cell lines.

Discussion

The DPPH assay is a reliable method to to determine the radical-scavenging activity of antioxidant, which are known to be a major factor in the biological damage caused by oxidative stress (Supakit et al., 2013; Sami et al., 2013; Vazhapilly et al., 2013). The DPPH assay is based on the gradual quenching of the stable DPPH• (2,2-diphenyl-1-picrylhydrazyl) free radical by the proton or electron donated from the antioxidant.The radical scavenging activity (Yang et al., 2014) of mitragynine related analogues could be partly attributed to its hydrogen- donating ability (Herraiz et al., 2003; Herraiz et al., 2004), whilst its ß-carboline nuclei are free radical inhibitors that react promptly with DPPH free radicals (Herraiz et al., 2003; Herraiz et al., 2004).

The antioxidant capacity (expressed as mM TEAC) is a simple and reliable measure of the antioxidant activity (Kriangsak et al., 2006; Herraiz et al., 2003; Herraiz et al., 2004). Moreover, the antioxidant activity measured in terms of mM TEAC Trolox is more meaningful and descriptive than the IC_{50} value (Kriangsak et al., 2006). Mitragynine related analogues are good antioxidants and radical scavengers,as they exhibited greater reactivity than Trolox. This result was not unexpected because β-carboline has been reported to possess great capacity to quench free radicals (ABTS•+) (Herraiz et al., 2003; Herraiz et al., 2004; Sami et al., 2013).

The effectiveness of the quenching is dependent on the molecular weight, number of aromatic rings and nature of the hydroxyl group substitution (Herraiz et al., 2003; Herraiz et al., 2004). In general, the evaluation of these compounds showed that all of the compounds with a indolic nuclear moiety exhibited antioxidant activity against the ABTS•+ at a physiological pH.

The reducing power of pure compounds may be used as a significant indicator to predict their potential antioxidant properties and reducing capability (Siddhuraju and Becker, 2003; Vazhapilly et al., 2013). Various studies have demonstrated the positive relationships between antioxidant activity and reducing power (George et al., 2012; Sami et al., 2013; Vazhapilly et al., 2013). The absorbance values expresses the reducing power of the sample studied.A stronger absorbance value indicates higher antioxidant activity. Antioxidants are reductants that inactivate oxidants via a redox reaction (Siddhuraju and Becker, 2007).In this assay, the Fe^{3+}/ferricyanide complex is used as an indicator of electron-donating activity. The capability of these pure compounds to act as reducing agents caused the reduction of the Fe^{3+}/ ferricyanide complex to its ferrous form, which was measured at 700 nm.

The result from a single antioxidant experiment must be cautiously interpreted because it merely provides a deductive suggestion and estimation regarding the antioxidant properties. Therefore, a multiple assay approach is crucial to determine conclusively the antioxidant properties (Herraiz et al., 2003; Herraiz et al., 2004). The ABTS and DPPH methods are the most popular spectrophotometric methods for determining the radical-scavenging activity. This study indicated that indole skeleton without phenolic group possess moderate antioxidant capacities and could play a crucial role in providing potential antioxidants and free radical scavengers. These results suggest that indoles, in general, may conform to a family of radical scavengers and antioxidants other than the polyphenolic group that might act as potential protectants and free radical sinks at a physiological pH.

Both MTG and SRM showed significant and selective anti-proliferation at high dosage and merit further investigation on other cell lines. This result verified with what was reported by (Saidin, 2008) that mitragynine exhibit marked cytotoxicity at higher concentration. However, interestingly, there are no significant difference in anti-proliferation efficacy for both MTG and SRM. Based on the results, we can conclude that reducing the indole double bond on MTG to indoline does not contribute to any significant enhancement in the inhibition of proliferation against both HCT 116 and K 562 cell lines.

The SI for 5-FU, which is widely used as the backbone for chemotherapy against colon cancer, was below 2 (0.60) against the HCT 116 cell line (Sasipawan et al., 2011; Ramesh et al., 2004; Wiratchanee et al., 2010). Therefore, it was generally toxic toward cells. MTG related analogues showed higher SI value against HCT 116 cell lines andmight be safer drugs for cancer treatment compared with conventional drugs such as 5-FU since they are relatively safe for non-cancerous human cells such as CCD18-Co. One important criterion for a cancer therapeutic drug is that it must have minimal or no side-effects on normal cells in patients undergoing chemotherapy. Therefore, anticancer drugs should have high selective index (Sasipawan et al., 2011; Ramesh et al., 2004; Wiratchanee et al., 2010). The SI value describes the selectivity of the pure compound for cancerous cell lines relative to that for normal cells (Sasipawan et al., 2011; Ramesh et al., 2004; Wiratchanee et al., 2010). The SI of the compounds against K 562 cells was calculated based on the IC_{50} of NIH/3T3, while the SI of HCT 116 was calculated based on the IC_{50} of CCD-18Co. An SI value below 2 indicates that the pure compound is generally toxic (Sasipawan et al., 2011; Ramesh et al., 2004; Wiratchanee et al., 2010).

The selectively induced apoptosis suggests that these mitragynine related analogues might be potent lead compounds for the development of future cancer therapeutics. Apoptosis is a better method for destroying damaged cells than necrotic cell death; thus, agents that can induce apoptosis are desirable for cancer treatment (Boland et al., 2013, Sadegh et al., 2013; Nejib et al., 2013, Zafer and Ayse, 2013).

Mitragynine analogues had moderate antioxidant values based on the DPPH assay, ABTS assay and reducing power in comparison to BHT and quercetin. SRM and MTG showed TEAC value of 1.96 mmol trolox/

mmol and 2.25 mmol trolox/mmol respectively. These activities were much higher than those of the water-soluble vitamin E analogue Trolox and of edible fruits that are considered to have good antioxidant properties. The MTG and SRM exhibit potent anti-tumour properties toward the leukaemia cell line K 562 but showed only moderate cytotoxic effect on HCT 116 cell lines. However, both MTG and SRM demonstrate marked anti-proliferative efficacy at concentration above 100 μM. Based on the in vitro experimental results, we can conclude that both MTG and SRM are comparable in potency and efficacy towards K 562 cell lines than existing standard drug for leukemia.cancer, BA. However, BA are more selective than MTG and SRM. Both MTG (SI=3.14) and SRM (SI=2.93) exhibit high selectivity towards HCT 116 cell lines compared to 5-FU (SI=0.60), although 5-FU is more potent than MTG and SRM. Therefore, MTG related analogues could be a safer option for cancer treatment. There are no significant difference in anti-proliferation efficacy and potency for both MTG and SRM. The leukaemia cell lines were more susceptible than colon cell lines toward these mitragynine analogues.

Mitragynine and its analogue exhibit potent and selective cytotoxic effect via apoptotic pathway, and high anti-proliferation efficacy at high dose (>100 um) towards K 562 and HCT 116 cancer cell lines. The results of this study also showed that mitrgynine related analogues exhibited moderate antioxidant and free radical scavenging activities. The hydrogen donating ability of these compounds has been proven through the assessment of reducing power ability and radical scavenging activities. MTG and SRM are a class of antioxidant and radical scavenger in the ABTS assay with TEAC value two folds better than Trolox in antioxidant activities. This confirms the values of mitragynine analogues as a new source of antioxidant to prevent the progress of various oxidative stress-induced diseases including cancer. Furthermore, this study justifies the efficacy of mitragynine related analogues as potential anticancer and chemo-preventive medicine. This study supports the biological relevancy and popular traditional use of mitragynine obtained from Mitragyna Speciosa Korth (Ketum or Biak-Biak) that growth in tropical forest of Southeast Asia.

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