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Original Article

TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES OF *Corchorus capsularis* AND *Stevia rebaudiana* EXTRACTS

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ABSTRACT

The purposes of this study were to determine total phenolic content (TPC) and antioxidant activities of C.capsularis and S.rebaudiana. Methodology: C.capsularis was extracted successively with methanol and chloroform methanol separately while S.rebaudiana was extracted using methanol and chloroform solvent. The plant extracts were used to conduct various antioxidant assays. TPC assay using Folin- Ciocalteu method was used to assess the presence of phenolic compounds in each sample. The extracts samples were also subjected to assess their potential antioxidant activities through DPPH, ABTS and FRAP assay. The present study showed that both methanol extract of C.capsularis and S.rebaudiana had highest TPC, followed by chloroform extract of S.rebaudiana and chloroform methanol extract of C.capsularis. Next, the DPPH radical scavenging assay was found to be higher in the methanol extract of S.rebaudiana at the concentration of 154.67µg/ml. The cation radical scavenging activity, measured by ABTS assay was shown that methanol extract of S.rebaudiana (278µg/ml) had the lowest EC_{50} as compared to the other samples. Interestingly, among the various samples, the methanol extract of S.rebaudiana demonstrated a very significant antioxidant activity in FRAP assay ($p<0.05$). The results of the present study showed that all the extract samples contain significantly high phenolic compounds with superior antioxidant activity as evidence by scavenging of free radical including DPPH and ABTS. In conclusion, it is conceivable that the C.capsularis and S.rebaudiana have shown potential as sources of natural antioxidants.

Keywords*: C.capsularis, S.rebaudiana, TPC, antioxidant activity*

INTRODUCTION

Free radical can be defined as any species that capable of independent existence that contains one or more unpaired electron and this unpaired electron will seek for other electrons in order to become stable [1]. The production of free radicals such as hydrogen peroxide and nitric oxide in our body can cause severe damage to cells. Even though human cells can defend themselves against free radicals via mechanisms of antioxidant systems, the defence mechanisms may not be adequate to protect against excessive free radicals, known as oxidative stress [2]. To compensate this severe effect, World Health Organization recommended the use of natural antioxidants.

Antioxidants can be defined as a substance that may protect cell from damage caused by unstable free radicals by stabilizing the free radicals and prevent some damage that free radicals might cause [3]. Recently, antioxidant from natural sources has gained attention among consumers and the scientific community because of potential and beneficial towards human health. Previous study reported that the consumption of antioxidant constituents reported could protect against oxidative damage which induce degenerative and pathological processes such as ageing and cancer [4]. Thus, antioxidant molecules from medicinal plants have become a major area of interest in scientific research [5].

In Malaysia, *Stevia rebaudiana* Bertoni, commonly known as sugarleaf or sweet leaf, was used traditionally as a source of natural sweetener. *S. rebaudiana* Bertoni is herbaceous perennial plant of the sunflower family (Asteraceae). The herb had been practiced for centuries by Guarani as a cardiac stimulant, for obesity, hypertension and lowered the uric acid level [6]. The leaves and callus of *S. rebaudiana* are commonly used in scientific research as they believed that it contains therapeutic value. In previous study, flavonoids, alkaloids, amino acids, lipids and trace elements have been discovered in the dry extract of leaves of *S. rebaudiana* [7] which are important compounds involving in antioxidant activities.

Corchorus capsularis is a plant belongs to the family of *Tiliaceae,* also konwn as 'kancing baju' among Malays [8]. Its stem, seeds and leaves have a potential medicinal properties which able to be remedied for certain health problems. The leaves are vital component as stimulant, laxative, appetizer. Meanwhile, its infusion helps in treating fevers, constipation, dysentery, liver disorder and dyspepsia [8]. The leaves of this natural

antioxidant have shown the presence of phytochemicals such as flavanoids and tannin [8]. Researchers found that these natural components in *C.capsularis* have therapeutic potential, which possess defence mechanisms to protect against the degenerative diseases associated with marked oxidative damage [8].

Therefore, the aim of this study is to determine the total phenolic content and antioxidant activities of methanol, chloroform and chloroform-methanol extracts of *C.capsularis* leaves. A possible correlation between total phenolic content and antioxidant activities of both plant extracts also have been evaluated. Therefore, several assays such as total phenolic content, DPPH, FRAP and ABTS assays have been used to estimate the phenolic content and antioxidant activities of *S. rebaudiana* and *C.capsularis* leaves.

MATERIALS AND METHODOLOGY

Plant material

The leaves of *S.rebaudiana* were collected from Taman Pertanian Universiti Putra Malaysia, Selangor, Malaysia. *C.capsularis* (SK 1936/11) leaves were obtained from nursery in Pahang. Both plants were identified in Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM) and the voucher specimens have been deposited in Herbarium of Laboratory of Natural Product, UPM.

Chemicals

Sodium carbonate ($NaHCO_3$) was purchased from Systerm®. Ferric chloride ($FeCI_3$) and ferrous sulphate ($FeSO_4$) were purchased from HmbG® Reagent Chemicals. Hydrochloric acid (HCI) was purchased from R & M Chemicals. Both ethanol (C_2H_6O) and chloroform ($CHCI_3$) were purchased from Merck (Darmstadt, Germany). Methanol (CH_4O) was purchased from Friedemann Schmidt (Francfort, Germany). Gallic acid ($C_7H_6O_5$), Folin-Ciocalteu reagent, 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)-ABTS, ammonium persulfate ($(NH_4)2S_2O_8$), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), were purchased from Sigma Aldrich (Germany). Dimethyl sulfoxide (DMSO) ($(CH_3)2SO$) and glacial acetic acid (CH_3CO2H) were purchased from Fisher Scientific Chemical.

Plant extraction

The leaves of *S.rebaudiana* and *C.capsularis* were dried for 72 hours in oven at 40^0C [9]. The dried leaves were ground to get fine powder. For methanol extract of *C.capsularis*, 1 kg of powder was soaked in methanol in the ratio of 1:20 w/v [8]. For chloroform methanol extract of *C.capsularis*, 1 kg of powder was soaked in chloroform methanol solution (2:1 v/v) in the proportion of 1:20 w/v [10]. The process was repeated three times with a fresh volume of solvent. The supernatant of each plant extract was filtered using Whatman filter paper No.1 (Whatman Ltd., England). The pooled extracts of both plants were concentrated using a rotary vacuum evaporator (BUCHI Rotavapor R-220) at 40^0C. Then, it was dried and stored at 4^0C [9].

Determination of total phenolic content

Total phenolic content of the extracts was evaluated by using Folin-Ciocalteu phenol The principle of this method is the ability of reducing the phenol functional group. The reduction of fosfotungstat-fosfomolibdenum complex (Folin-Ciocalteu reagent) by phenolat ion was taken place at basic condition. As the oxidation and reduction reaction of phenolat ion occurred, the yellow colour of reagent was turning into blue. The reduction of this complex reagent was increased when the extracts contain more phenolic compounds [11]. The amount of total phenolic in both plant extract was evaluated as described by method from Ismail *et al*. [12] with minor modification. 1 mg/ml of stock standard solution of gallic acid was prepared. Various concentrations ranged between 0.01 and 0.05 mg/ml were prepared. 1 mg/ml of plant extracts were prepared. Then, 100 µl of plant extract and ascorbic acid were mixed with 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with deionised water). The mixture was allowed to stand at room temperature for 5 minutes. 0.75 ml of 6% (w/v) sodium carbonate was added and mixed gently. Again, the mixture was left at room temperature for 90 minutes. The absorbance was read at 725 nm using T60 UV/Vis spectrophotometer and the standard calibration curve of gallic acid was plotted. Ascorbic acid was used as standard.

1, 1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging activity

In DPPH assay, DPPH radical was reacted with an antioxidant compound which that can donate hydrogen, and get reduced. DPPH, when acted upon by an antioxidant, was converted from diphenyl picrylhydrazyl to diphenyl picrylhydrazine. This can be identified by the conversion of purple to light yellow color [13]. The method of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity was estimated as previously described by Blois [14]. The stock solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was prepared by dissolving 2.1 mg of DPPH in 50 ml methanol. It was then incubated for 2 hours in the dark room. Ascorbic acid and plant extracts were prepared at various concentrations (1000 to 15.625 µg/ml). Then, 50 µl of the standard reagents and each concentration of plant extracts were added into 96-well plate. After that, 195 µl of 0.1 mM DPPH solution was added. The reaction mixture was incubated for one hour in the dark condition. Then, the absorbance was measured at 517 nm using microtitre plate reader (Thermo® Multiscan Ascent plate reader). The percentage scavenging activity of the sample on DPPH radical was calculated using the

following equation:

% scavenging activity = [(Abs $_{control}$ – Abs $_{sample}$) / Abs $_{control}$] x100

2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) scavenging activity

ABTS was converted to its radical cation by the addition of ammonium persulfate. This radical cation was blue in colour and absorbed light at 745 nm. During the reaction, the blue ABTS radical cation was converted back to its colorless neutral form [15]. ABTS assay was performed according to the method described by Re *et al.*[16]. The oxidant is generated by persulfate oxidation of 2, 2'-azino -bis (3-ethylbenzthiazoline-6-sulphonic acid) – ($ABTS^{2-}$). ABTS solution (7 mM) was mixed with 2.45 Mm ammonium persulphate to generate ABTS radical cation ($ABTS^{+}$). Then, the mixture was left in dark condition for 12-16 hours. Different concentration (15.625-1000 µg/ml) of plant extract (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with ethanol until 1 ml. The absorbance was analysed at 745 nm using T60 UV/Vis spectrophotometer (Tg9 Instrument). Ascorbic acid was used as a standard. The percentage scavenging activity of the sample on ABTS radical scavenging activity was calculated using following equation:

% scavenging activity = [(Abs $_{control}$ – Abs $_{sample}$) / Abs $_{control}$] x100

Ferric –reducing power (FRAP) assay

The principle of the assay was assessed by antioxidant ability to reduce FRAP reagent. It possessed the reducing potential of the antioxidants to react with a ferric trioyridyltriazine (Fe III TPTZ) complex and produced ferrous tripyridyltriazine (Fe II TPTZ) [17]. The FRAP (Ferric reducing antioxidant power assay) method was followed as described by Benzie & Strain,[17] with minor modification. Briefly, a stock solution of FRAP reagent was prepared. Then, FRAP reagent was warmed at 37^0C in water bath (Memmert), before being used. An aliquot of 50 µl plant extract and ascorbic acid of different concentrations were allowed to react. After that, 150 µl of FRAP reagent was added. The absorbance of the mixture at 590 nm was measured using a microtitre plate reader (Thermo® Multiscan Ascent plate reader). Ferrous sulfate ($FeSO_4$, 0-1000 µM in distilled water) was used as standard. The results obtained were shown as the concentration of antioxidants having a ferric reducing ability equivalent to that of µM FeSO4/ mg of dry sample weight.

Statistical analysis

All analyses were performed in triplicate (n = 3). The results were presented as mean ± standard error of mean (SEM). SPSS was used to test any difference in antioxidant activities resulting from this method. Duncan's multiple range test was used to determine significant differences, with $p < 0.05$. Correlations among data obtained were calculated using Pearson's correlation coefficient (r).

RESULTS

The amount of total phenolics, measured by Folin-Ciocalteu method, varied widely in these two plant species and was expressed as Gallic Acid Equivalent (GAE)/100 g dry weight (Table 1). The highest level of phenolic content was found in methanol extract of *S.rebaudiana*, while the lowest was in chloroform

Table 1: TPC and EC_{50} value of *Corchorous capsularis* and *Stevia rebaudiana extracts*

Samples	**TPC (mg GAE/100g of dry weight)**	**EC_{50} of DPPH (µg/ml)**	**EC_{50} of ABTS (µg/ml)**	**FRAP (µm $FeSO_4$/mg of dry weight)**
Corchorus capsularis				
Methanol	5155.56 ± 408.52^{a}	293.33 ± 108.99^{d}	483.33 ± 92.79^{g}	614.78 ± 74.07^{i}
Chloroform methanol	2475.56 ± 100.39^{b}	763.33 ± 85.70^{e}	-	418.15 ± 15.30^{j}
Stevia rebaudiana				
Methanol	13709.00 ± 585.10^{c}	154.67 ± 8.33^{f}	278.00 ± 5.00^{gh}	1127.86 ± 3.04^{k}
Chloroform	3160.33 ± 51.13^{b}	-	-	239.97 ± 3.85^{l}

Values represent as mean of three replicate determination ± SEM (standard error of mean). Different superscript in a column are significantly different at $p<0.05$.

TPC, total phenolic content; GAE, Gallic acid equivalent; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; ABTS, 2, 2'-Azinobis (3-ethylbenzothiazoline-6-sulphonicacid); FRAP, Ferric reducing antioxidant power; EC_{50}, effective concentration which scavenging 50% of free radicals; $FeSO_4$, ferric sulphate.

methanol extract of *C.capsularis*. As compared between the plant species, methanol extract of *C.capsularis* had the highest TPC than chloroform methanol extract. For *S.rebaudiana*, methanol extract also had higher TPC, and followed by chloroform extract. From the result, it was found that methanol extract was more efficient solvent for extracting the phenolic constituens in both plant species rather than other solvents. Hence, the order of TPC is; methanol extract of *S.rebaudiana* > methanol extract of *C.capsularis* > chloroform extract of *S.rebaudiana* > chloroform methanol extract of *C.capsularis*. ANOVA analysis (Table 1) showed significant difference ($p<0.05$) between TPCs of the samples.

The free radical scavenging effect of both *C.capsularis* and *S.rebaudiana* was assessed using the DPPH assay. Based on the result (Fig.1.), it showed that in both plant species, methanol extracts of *C.capsularis* and *S.rebaudiana* had considerably high DPPH radical scavenging activities as compared to the other extracting solvent. For EC_{50}, the lowest concentration was shown by methanol extract of *S.rebaudiana* (154.67 ± 8.33 µg/ml), followed by methanol extract of *C.capsularis* (293.33 ± 108.99 µg/ml) and chloroform methanol extract of *C.capsularis* (763.33 ± 85.70 µg/ml). There was significant difference ($p<0.05$) between the plant extracts by analysis of ANOVA.

ABTS assay (Fig.2.) was based on the antioxidant ability to react with ABTS radical cation generated in the assay system. Based on the result, it demonstrated that methanol extraction of both plant species had significant antioxidant activity in scavenging ABTS radicals. This was corresponded that high molecular weight phenolics have more ability in quenching ABTS radicals. Comparing between these two plants, *S.rebaudiana* showed more powerful in proving the significant effect of scavenging activity with the lowest

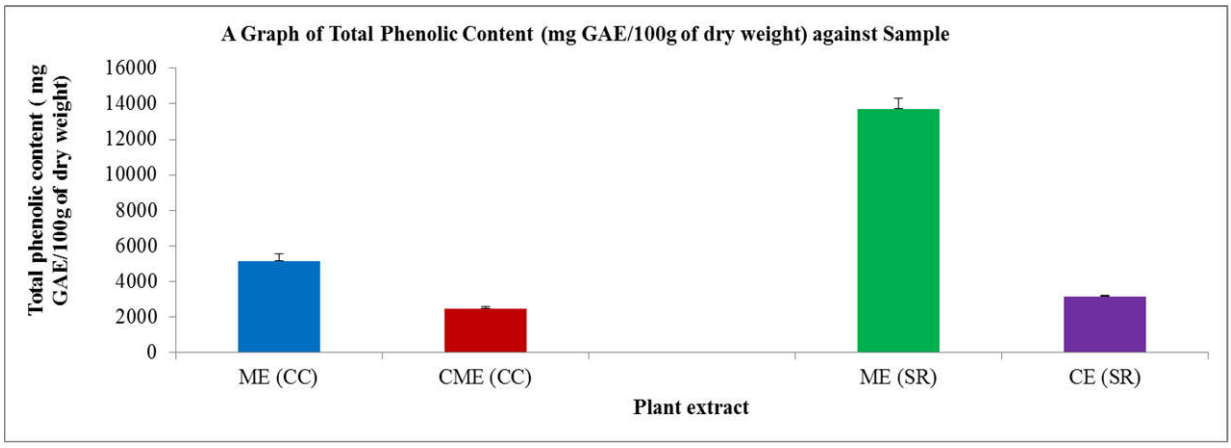

Figure1: Total phenolic content (TPC) assay of two plant species (mg GAE/100g of dry weight). ME (CC), methanol extract of *Corchorus capsularis*; CME (CC), chloroform methanol extract of *Corchorus capsularis*; ME (SR), methanol extract of *Stevia rebaudiana*; CE (SR), chloroform extract of *Stevia rebaudiana*.

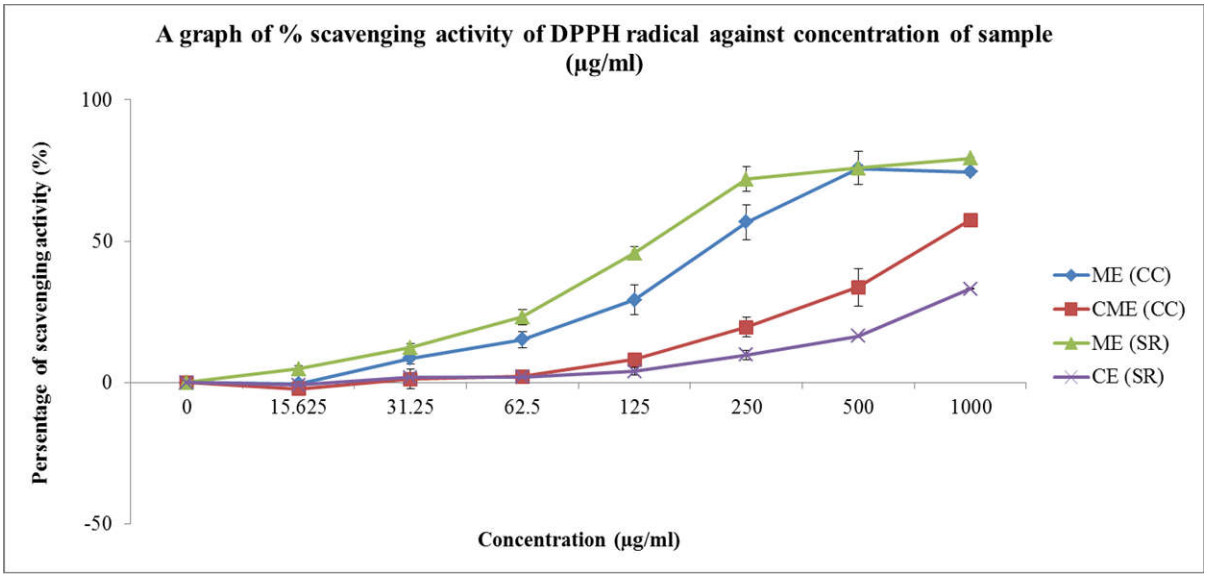

Figure 2: DPPH free radical scavenging activity of two plant species. ME (CC), methanol extract of *Corchorus capsularis*; CME (CC), chloroform methanol extract of *Corchorus capsularis*; ME (SR), methanol extract of *Stevia rebaudiana*; CE (SR), chloroform extract of *Stevia rebaudiana*.

EC_{50} at 278µg/ml. It was continuance to the significant correlation between TPC and ABTS with R^2= 0.475. This was followed by methanol extract of *C.capsularis* with EC_{50} at 483.33µg/ml. In fact, the lowest EC_{50} represents as the good antioxidant. From this study, it showed that analysis of ABTS assay was insignificantly different between these two plant species.

In the FRAP assay, it was measured the antioxidant effect of any substances or extracts in the reaction medium as reducing ability. The antioxidant capacities of both plant extracts were varied significantly, proved by highly positive correlation between TPC and FRAP on both plant extracts. Methanol extract of *S.rebaudiana* showed the highest FRAP antioxidant activity, while chloroform extract of *S.rebaudiana* had the lowest activity. However, comparing on both plant species showed that *S.rebaudiana* was found better to have more ferric reducing or antioxidant power than *C.capsularis*. The order of FRAP activity of respective plant extracts is as follow; chloroform extracts of *S.rebaudiana* > chloroform methanol extract of *C.capsularis* > methanol extract of *C.capsularis* > methanol extract of *S.rebaudiana*. Statistically, these plant extracts were significantly difference in the FRAP activity.

DISCUSSIONS

Nowadays, a lot of plants have become a source of natural antioxidants. The leafy part of plants is believed to contain multiple types of phenolic compounds. These phenolic compounds found in the leafy part are believed to exhibit the antioxidant effect and radical scavenging activity. Besides, phenolic compound also plays important role in preventing several chronic diseases related to oxidative stress and involved in cellular processes such as a redox reaction [18].

In this study, it was found that *S.rebaudiana* and *C.capsularis* plant exhibited antioxidant properties, particularly the leafy part. From the results obtained, it showed that methanol extract of *S.rebaudiana* possessed higher phenolic content, followed by methanol extract of *C.capsularis*, chloroform extract of *S.rebaudiana* and chloroform methanol extract of *C.capsularis*. Methanol extraction has more polarity and is able to extract more polar active compounds. For the chloroform methanol, it is an aqueous solvent, which is able to extract polar and non-polar active compound. While chloroform extraction are able to extract more non-polar active compound.

According to Hsu *et al.* [19], the differences in the phenolic content may be affected by availability of extractable components which depend on functional group that present in the compound. Besides that, the solubility of the antioxidant compounds in the plants also depends on the solvent polarity, extraction time and temperature as well as samples to solvent ratio [20,21]. ANOVA analysis showed that there is a significant difference between TPC's samples ($p < 0.05$). These results suggested that TPCs are varied significantly from one sample to another. Previous study also showed that the methanolic extract of *S.rebaudiana* leaves was rich in flavonoids, alkaloid and tannins [22]. While, the leaves of *C.capsularis* have shown the presence of flavanoids, tannins, steroid, saponin and triterpenes but no alkaloids [8].

The quenching activity of DPPH radicals can be ascertained by the hydrogen-donating ability of antioxidant molecules such as flavanoids, tannins and other active compounds [23]. Statistically, methanol extract is significantly difference from chloroform and chloroform methanol extract, is due to the different ability in scavenging free radical and donating hydrogen to form a stable DPPH molecule. However, some factors such as reaction time, solvent, concentration and used wavelength might influence the reaction of DPPH scavenging activity [24] . Incubation time of DPPH solution might also be one of the factors involved, because short incubation time has been frequently not sufficient to achieve the steady state of the reaction. From the results, it showed that the methanol extract of both plants has high DPPH radical scavenging activity compared to chloroform methanol extract of *C.capsularis* and chloroform extract of *S.rebaudiana*. The methanol extract of *S.rebaudiana* also exhibited low EC_{50} value compared to others. These findings, were similarly in agreement with Misuthisakul *et al*. [25] who found that, low EC_{50} obtained correspond to the highest antioxidant activity of *S.rebaudiana*. In addition, Berg *et al.* [26] reported that the secondary metabolite of *C.capsularis* such as the triterpene saponin extract is known to deliver an antioxidant activity, which exhibit the capability of DPPH radical scavenging activity. The antioxidant activity in the plant reflects the amount of phenolic compounds. This is because phenolic compounds have the ability to absorb and neutralizing free radicals as well as decompose the peroxides [27]. Hence, high phenolic content in the plant reflects the ability in scavenge DDPH radical.

ABTS radical scavenging activity

ABTS assay has the same purpose with DPPH assay as they are evaluating the antioxidant activity by scavenging free radical. Furthermore, the result showed statistically significant and indicates that antioxidant activities of both plant extracts are almost same for methanol extract. Methanol extract of plant were an effective scavenger of ABTS radical as indicated in previous study [16]. However, no EC_{50} value was reported for chloroform methanol of *C.capsularis* and chloroform of *S.rebaudiana*. Therefore, in order to achieve EC_{50} value for both plant extracts, a higher concentration might be needed.

In present study, however, there are several limitations. Firstly, it has to be taken into account that the ABTS assay is in vitro models and do not measure all of antioxidant activities in natural products. Secondly, the antioxidant capacity of plant extract and standard may differ depending on the solvent used. Previous study also reported that methanolic extract of *S.rebaudiana* leaf showed significantly ($p < 0.05$) high antioxidant activity [28]. Hence, from the result obtained, it showed that the methanolic extract of *S.rebaudiana* and *C.capsularis*

exhibited maximal percentage of inhibition activity of free radicals which is a more potent extract compared to chloroform extract of *S.rebaudiana* and chloroform extract of *C.capsularis*.

Ferric reducing ability power

In FRAP assay, the reductant acts by stopping the free radical chain by donation of hydrogen atom, and then responding with the peroxide precursor, thus preventing peroxide formation. The phenolic compounds found in plant may act in a similar activity as reductant. They may donate electrons and reacting with free radicals to convert them to more stable products, thus stop the free radical chain reaction [23]. FRAP assay will assess the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in the presence of antioxidants, which are reductant with half-reaction reduction potentials above Fe^{3+}/Fe^{2+}. It is a hydrophilic antioxidant assay and not well responding to lipophilic antioxidants.

Antioxidant will exert their effect by donating a hydrogen atom to ferric complex and will immediately break the radical chain reaction [29]. Furthermore, previous study reported that ferric reducing bioactive compounds are correlated to antioxidant activity [30]. From the result, it showed that methanol extract of *S.rebaudiana* exhibited higher FRAP value, compared to methanol extract of *C.capsularis*. This showed that methanol extracts of *S.rebaudiana* are more better in reducing the ferric iron to ferrous iron. The ability to reduce Fe (III) is due to donation of a hydrogen atom from phenolic compounds of *S. rebaudiana* leaves which also correlate with the presence of reductant agent [31]. While the FRAP value for chloroform extract of *S.rebaudiana* and chloroform methanol of *C.capsularis* has low FRAP value. This may be due to the less hydrophilic contain of antioxidant properties that is needed in reducing ferric iron.

Correlation between total phenolic content and antioxidant activities

In this study, the total antioxidant activity of *C.capsularis* and *S.rebaudiana* increased with increasing concentration of extracts, indicating the potential of both plants as antioxidants. Relatively, highest total antioxidant activity in plant extracts showed a significant correlation with phenolic contents. This showed that the phenolic contents act as potential antioxidant biomolecules.

There is a highly positive correlation between total phenolic content and FRAP assay of *C.capsularis* (R^2=0. 797). This showed that phenolic contents carried more hydrophilic properties that exhibit antioxidant activity. The significant correlation proved that total phenolic contents act as strong reducing power in FRAP assay. However, there is some lipophilic phenolic content in *C.capsularis* that exhibit antioxidant activities. As the present of lipophilic properties of phenolic content, it acts as a free radical scavenger in DPPH assay and ABTS assay. This is because DPPH and ABTS assay are lipophilic antioxidant assays and increased the antioxidant activities with lipophilic phenol content. Due to that reason, it proved that strong correlation between DPPH and ABTS assay with R^2=0.729. The negative or weak correlation between total phenolic content and DPPH and ABTS assay may be proved that *C.capsularis* extracts carried more hydrophilic phenol as compared to lipophilic phenol content. Hence, the correlation coefficient analysis showed that hydrophilic and lipophilic phenolic content were responsible for antioxidant activities in *C.capsularis*.

As for *S.rebaudiana*, from the result, ABTS and DPPH assays exhibited the highest positive correlation among others (R^2= 0.994). ANOVA result determined that there is significant correlation between DPPH and ABTS ($p < 0.01$). Instead of that, positive correlations also determined from total phenolic content and FRAP value (R^2 =0. 779) which there is a significant correlation ($p < 0.05$). Nevertheless, weak positive correlations were indicated between DPPH and FRAP, TPC and ABTS, TPC and DPPH, as well as ABTS and FRAP. But there is no negative correlation reported.

According to Shukla *et al.* [32], there is a significant and linear relationship between antioxidant activity and phenolic content in *S.rebaudiana* leaf extracts. This indicated that phenolic content is responsible for antioxidant activities of *S.rebaudiana*. In addition, Jahan *et al*. [33] reported that over 100 phytochemical such as tannins and alkaloids had been found in *S.rebaudiana* leaves. This showed that as the total phenolic content increased, the antioxidant activities also increased. Thus, phenolic content reflects the antioxidant activity of the plant that showed a therapeutic potential of antioxidant, which possess the mechanisms to protect against the degenerative disease associated with marked oxidative damage [8].

CONCLUSIONS

From this study, it can be concluded that methanol extract of *S.rebaudiana* and *C.capsularis* possessed high phenolic content that reflects high antioxidant properties. Thus, it is believed that both plants have significant potential to be used as one of the natural antioxidant found in Malaysia as it is believed to exhibit therapeutic potentials in the future. In addition, for further investigation of the plant properties, a method such high performance liquid chromatography (HPLC) can be done to analyse more details related to the active compound involved. Several different assays can also be done to evaluate more about the antioxidant properties of the plants.

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