



ANTIOXIDANT POTENTIAL OF RED AND WHITE CABBAGE: A COMPARISON

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ABSTRACT

This study aimed to compare the antioxidant activity of white cabbage (*B. oleracea* var. *capita* f. *alba*) and red cabbage (*B. oleracea* var. *capita* f. *rubra*) in vitro. Dried cabbages were extracted by Soxhlet extraction using chloroform as solvent. The extracts were screened for phytochemicals followed by the antioxidant capacity measurement through free radical scavenging activity (DPPH and ABTS). The results showed that red cabbage extract (RCE) significantly had stronger antioxidant activity than white cabbage (WCE) ($P < 0,05$). The IC₅₀ values of RCE were $350,80 \pm 5,27 \mu\text{g/ml}$ (DPPH) and $87,03 \pm 1,92 \mu\text{g/ml}$ (ABTS) while these of WCE were $613,75 \pm 8,76 \mu\text{g/ml}$ and $114,57 \pm 0,41 \mu\text{g/ml}$, respectively. Moreover, a higher level of phytoconstituents was found in RCE based on thin-layer chromatography analysis. These findings support that red cabbage has higher antioxidant activity than white cabbage which may be correlated with its phytoconstituents concentration.

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Introduction

Antioxidant substances have many benefits to prevent health disorders caused by the elevation of free radical agents. Oxidative stress is a root and target treatment of various diseases such as Parkinson's disease (PD) (Kryl'skii et al., 2021), nonalcoholic fatty liver disease (NAFLD) (Chen et al., 2020), cancer (Hayes et al., 2020) and many other chronic pathologies (García-Sánchez et al., 2020). Oxidative stress is one of the factors that destroy dopaminergic neurons directly or by activating proteins in the cell death pathway like c-Jun N-terminal kinases, NFκB (Zhou et al., 2008). Many drugs used in PD treatment affect increasing antioxidant potential (e.g Memantine) or reducing the

formation of reactive oxygen species (ROS) (e.g Levodopa) (García-Sánchez et al., 2020). The role of natural antioxidants in NAFLD management was demonstrated (Salomone et al., 2016). DNA damage caused by the elevation of ROS possibly increases the risk of cancer development (Cooke et al., 2003; Hayes et al., 2020). The substances found in plant can react to remove oxidizing elements in the cell have been used as a weapon against tumors (Kooti et al., 2017).

Brassica oleracea var. *capita* L. (cabbage) is a member of Brassicaceae, widely planted in the north extratropical part of the world. While mainly used as a vegetable, people also recognize the medicinal properties of cabbage leaves very

early (Leike, 1988). White cabbage (*Brassica oleracea* var. *capita* f. *alba*) possess strong antioxidant activity (Kusznierewicz et al., 2008), anti-inflammatory (Rokayya et al., 2014), and liver protection properties (Morales-López et al., 2017). Other type of cabbage (red cabbage - *Brassica oleracea* var. *capita* f. *rubra*) also shows strong biological activities such as antioxidant (Chun et al., 2004), antibacterial (Hafidh et al., 2011; Waghulde et al., 2019), ameliorating hypercholesterolemia, and hypertriglyceridemia (Cruz et al., 2016). To have those activities, both contain vast amount of bioactive compounds such as phenolic acid, anthocyanidins, isothiocyanates, phenolics, flavonoids, and folic acids (Hafidh et al., 2011; Liang et al., 2019). In the market, the commercial price of red cabbage is much higher than the other. Unfortunately, there supporting evidence on the nutrition of two cabbages is limited to consumers. This study aimed to compare antioxidant activity as well as phytoconstituents between red and white cabbage extract using chloroform as extracting solvent. The result could support useful information to consumers in the selecting cabbage for their daily meal.

Materials and Methods

Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl) was from Alfa-UK; ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid and Potassium persulfate, vanillin were from Sigma-Aldrich, Germany; ethanol, methanol, hexane, chloroform were from Fisher, UK; sulfuric acid, hydrochloric acid, acetic acid, sodium hydroxide, ferric chloride were from Himedia, India.

Plant extraction

Red and white cabbage planted in Da Lat, Lam Dong, Viet Nam were purchased from a local market. Shade dried cabbage leaves were ground using the blender. Twenty – five grams of leaf powders were extracted with hexane to eliminate non-polar compounds before being extracted with chloroform using

the Soxhlet apparatus. The chloroform extract was concentrated using rotary evaporator until dry (humidity less than 20%). Red cabbage extract (RCE) and white cabbage extract (WCE) were kept at 4 °C until use.

Phytochemical screening

The presence of phenols, tannin, alkaloids, glycosides, saponins, flavonoids, and steroids was determined using the chemical test following the methods described previously (Sharma & Paliwal, 2013; Yadav & Agarwala, 2011).

Thin-layer chromatography

Chemical constituents of RCE and WCE were tested by thin-layer chromatography (TLC Silica gel 60 F₂₅₄, Merck). Samples were prepared in chloroform at an equal concentration (8 mg/ml) and 5 µl of each sample was spotted onto the TLC plate. The selected mobile phase was chloroform: methanol (9:1). After spot development in TLC chamber, the plate was air-dried and first visualized in UV light chamber with λ=254 nm and 365 nm. Then, the plate was stained with Vanillin-Sulphuric acid (VS). All R_f values were calculated.

DPPH scavenging activity

DPPH assay was performed as the previous report with modifications (Doan et al., 2018). One milliliter of RCE or WCE at different concentrations (0-1000 µg/ml) was added into 2 ml of 0.1 mM methanolic DPPH tube and left in the dark at room temperature for 30 min. Then, the optical density value (OD) was measured at λ=517 nm. The value of IC₅₀ was used to express the concentration of extract required for 50% of scavenging of free radical. The proportion of DPPH inhibition was calculated using the equation below. Vitamin C was used as a standard antioxidant.

$$\% \text{ inhibition} = \frac{[(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}) / \text{OD}_{\text{control}}] \times 100}{1}$$

ABTS scavenging activity

Antioxidant activity of RCE and WCE against ABTS free radicals was determined as

previously described with some modifications (Kumkrai et al., 2015). ABTS radical cation (ABTS^{•+}) was prepared by adding 14 mM ABTS solution to 4.9 mM potassium persulfate solution (1:1; v/v) for 16 h in the dark at room temperature. After adjusting to OD = 0.70 ± 0.02 (λ=734 nm), 150 µl of CE at various concentrations (0 - 250 µg/ml) was added to 2850 µl of diluted ABTS solution, mixed well and left in the dark for 6 min. the optical density value (OD) of the reaction mixture was measured at λ=734 nm. The value of IC50 was used to express the concentration of extract required for 50% of scavenging of free radical. The proportion inhibition was calculated using the equation below. Vitamin C was used as a standard antioxidant.

$$\% \text{ inhibition} = \left[\frac{(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}})}{\text{OD}_{\text{control}}} \right] \times 100$$

Statistical analysis

The result values were expressed as mean ± SEM of 3 repeated experiments. The comparisons between means were analyzed by Graphpad Prism 7.0. A value of P < 0.05 was considered as a statistically significant difference.

Results and Discussion

Phytochemical screening

Phytochemical screening and yield results of the chloroform extract of red cabbage and white cabbage were presented in Table 1. By soxhlet extraction with hexane,

non-polar compounds, as well as latex, were removed properly. After that, red and white cabbage gave a similar yield with chloroform (1.32% and 1.40% for RCE and WCE, respectively). Both RCE and WCE showed the presence of saponin, steroids, terpenoids, flavonoids, and glycosides but phenols, alkaloids, and tannin.

Whilst we found the absence of phenols, tannin, and alkaloids in red cabbage extract in this study, other reported the appearance of alkaloids and tannin in cabbage species planted in India (Chauhan et al., 2016). Glycosides, saponin, flavonoids, phenols, tannin, carbohydrates, amino acids, and proteins in the chloroform extract of cabbage (*Brassica oleracea* L. var. capitata) were identified. The result also showed the negative of steroids, terpenoids, and alkaloids in this fraction. By successive extraction, ethanol fraction applied after chloroform could remove 8 phytochemicals from cabbage including alkaloids, steroids, and terpenoids, glycosides, flavonoids, phenols, tannin, and carbohydrates, amino acids, proteins (Ahmed et al., 2012). The difference may occur because of the different growth environments, soil conditions, and storage procedures after harvest between India and Viet Nam (Podsedek et al., 2006). It is due to the active compounds produced in cabbages are influenced by different growing methods and agricultural conditions (Giordano et al., 2018).

Table 1. Phytochemical screening and extraction yield of red and white cabbage

Test	RCE	WCE
Phenols, Tannin	-	-
Alkaloids	-	-
Glycosides	+	+
Saponins	+	+
Steroids	+	+
Terpenoids	+	+
Flavonoids	+	+
Yield (%/dried weight)	1.32	1.40

+ : presence, - : absence

TLC analysis

TLC is a simple and common method used to analyze phytoconstituents in herbal research (Gocan & Cimpan, 2004) which is known as simple, cheap, and rapid technique in phytochemical identify and purification (Sasidharan et al., 2011). An equal amount of RCE and WCE (40 µg) were subjected to TLC as a single spot. From the preliminary, the mixture of chloroform: methanol (9: 1) was selected as the only mobile phase in this analysis due to clear separating spots obtained. Both extracts showed 5 separating spots with retention factor (R_f) values 0.88,

0.54, 0.48, 0.44, and 0.31 under UV 254 nm whereas 6 spots recorded with R_f values 0.81, 0.69, 0.53, 0.49, 0.38 and 0.16 under UV 365 nm. TLC plate treated with VS revealed 7 spots with R_f value 0.86, 0.73, 0.65, 0.54, 0.48, 0.35 and 0.26 (Figure 1). Although both extracts showed the same spots after developing in the chamber, the spots size separated from RCE were greater in size and/or deeper than spots of WCE. The result suggests that RCE contains a higher concentration of phytochemical compounds compared to WCE. This result is useful for further isolation processes.

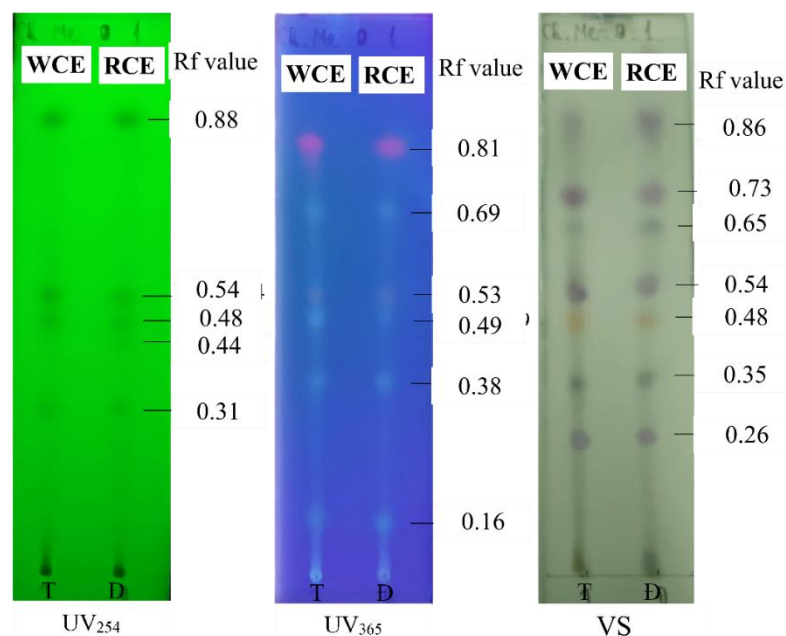


Figure 1. Thin-layer chromatography analysis of RCE and WCE

Antioxidant activity

To measure the antioxidant activity, DPPH and ABTS scavenging assays were conducted. To each extract, the DPPH and

ABTS inhibition capacity showed dose-dependent effects. RCE exhibited stronger activity than WCE in both assays significantly.

Table 2. Antioxidant activity of the chloroform extract of red and white cabbage
 IC_{50} (µg/ml)

Method	IC_{50} (µg/ml)		
	Vitamin C	RCE	WCE
DPPH	5.12 ± 0.21^a	350.80 ± 5.27^b	613.75 ± 8.76^c
ABTS	2.07 ± 0.06^a	87.03 ± 1.92^d	114.57 ± 0.41^e

* The values are expressed as mean \pm SEM, n=3. The different letters present the significant difference between groups ($P < 0.05$) compared by two-way ANOVA followed by Sidak as *post hoc* test.

In DPPH assay, RCE had inhibition activity higher than WCE at all concentrations ($P < 0.05$). At the same concentration of extracts, RCE always exhibited greater activity than WCE significantly. Moreover, RCE showed maximum inhibition effect up to $92.08 \pm 0.66\%$ while that value was lower for WCE ($60.46 \pm 0.48\%$) at the highest

concentration used. Remarkably, the free radical quenching action of RCE at $400 \mu\text{g/ml}$ was the same as WCE at $800 \mu\text{g/ml}$ or above ($P > 0.05$) (Figure 2). The IC_{50} of RCE was 1.8 times smaller than IC_{50} of WCE ($P < 0.05$) and much greater than IC_{50} of vitamin C. These values were demonstrated in Table 2.

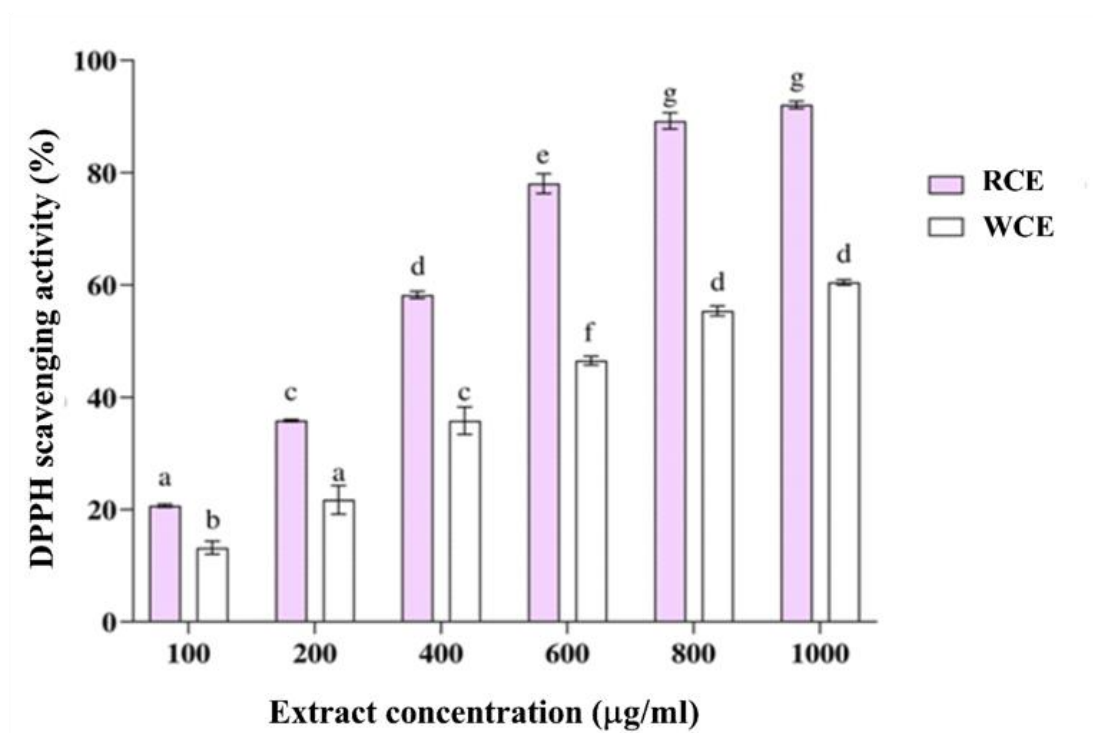


Figure 2. DPPH result of RCE and WCE. The different letters present the significant difference between groups ($P < 0.05$) compared by two-way ANOVA followed by Sidak as post hoc test. The values are expressed as mean \pm SEM, $n = 3$.

In ABTS assay, RCE displayed strong activity on ABTS free radical quenching compared to WCE ($P < 0.05$). At the same concentration, the scavenging effect of RCE was greater than that of WCE starting from $50 \mu\text{g/ml}$. RCE showed maximum inhibition effect up to $87.86 \pm 0.16\%$ while that value

was $74.73 \pm 0.23\%$ for WCE at $250 \mu\text{g/ml}$ ($P < 0.05$) (Figure 3). The IC_{50} of RCE was 1.3 times lower than IC_{50} of WCE ($P < 0.05$) and much greater than IC_{50} of vitamin C, they were 87.03 ± 1.92 , 114.57 ± 0.41 , and 2.07 ± 0.06 , respectively (Table 2).

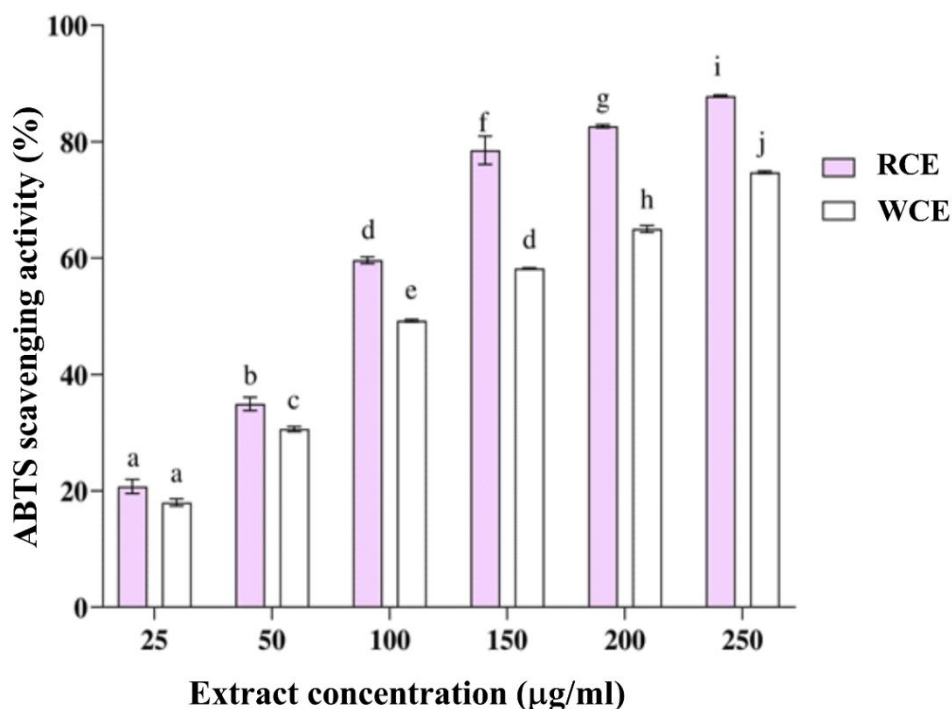


Figure 3. ABTS result of RCE and WCE. The different letters present the significant difference between groups ($P < 0.05$) compared by two-way ANOVA followed by Sidak as post hoc test. The values are expressed as mean \pm SEM, $n=3$.

To the antioxidant potential of RCE and WCE, our findings agree with Upadhyay et al. (2016). According to this report, methanol extract of red cabbage had antioxidant activity higher than green cabbage, cauliflower, and broccoli using DPPH test. Their study also found that total phenolic contents and total flavonoid contents were highest in red cabbage extract. Raw, steamed, and boiled red cabbage showed the DPPH IC₅₀ value were 35.84 ppm, 56.83 ppm, and 80.07 ppm compared to white cabbage 161.23 ppm, 217.51 ppm, and 304.59 ppm, respectively (Sugiasuti et al., 2011).

ABTS scavenging capacity of red cabbage extract was proved to be higher than that of green cabbage extract (Jacob et al., 2011). This observation is coincident with our ABTS outcomes. The study also discovered the antioxidant mechanism of the extracts. It works as a scavenging agent for oxygen-free radicals and hydroxyl-free radicals. The antioxidant of red and green cabbages was highly correlated to total phenolic and total flavonoid contents. kaempferol, quercetin, and apigenin were determined as the major

flavonoids in cabbages (Chun et al., 2004). Many publication said that terpenoids, glycosides, steroids and saponins had potential antioxidant via free radical scavenging process (Ashraf et al., 2013; González-Burgos & Gómez-Serranillos, 2012; Ma et al., 2013). In the comparison with our findings, the antioxidant activity of RCE likely occurs due to high concentrations of phytochemicals, especially flavonoids.

Podsedek et al. (2006) concluded that red cabbage and Brussels sprout were the highest antioxidant-rich sources compared to white cabbage. Water-soluble components were the major antioxidant constituents. Anthocyanins were found to be the predominant compounds in red cabbage whereas hydroxycinnamic acids were the most found in the rest. Red cabbage planted in China also revealed the highest level of phenolics and flavonoids contents which explained for its strongest antioxidant potential by using DPPH, ABTS, and ferric ion reducing antioxidant power (FRAP) assays (Liang et al., 2019). This evidence is in line with our study results.

The important role of antioxidants on health such as physiological functions of liver, kidney, cardiovascular diseases prevention, and cancer initiation has been recognized. Antioxidants are candidates for the treatment of a neurologic disorder (García-Sánchez et al., 2020), non-alcoholic fatty liver disease (NAFLD) management (Salomone et al., 2016), reducing the risk of tumorigenesis (Cooke et al., 2003; Hayes et al., 2020). Dietary intake of vegetables has a beneficial effect on inflammation and our endogenous antioxidant defense system in the body (Wilson et al., 2017). Vegetables contain an abundance of compounds that possess antioxidant activity, especially vegetables in *Bassica* genus (Podsek et al., 2006).

Our results indicate that RCE has higher antioxidant activity compared to WCE. However, the crude extract contains abundant phytochemical compounds that cause difficulty to identify which compounds are the most active compound. Therefore, further investigation is necessary to project for isolation and identification of the effective compounds in RCE and WCE.

Conclusions

Chloroform extract red and white cabbage possesses high antioxidant activity, where red cabbage is predominant. Both types of cabbages contain antioxidant compounds such as flavonoids, terpenoids, steroids, glycosides, and saponin. TLC analysis shows the bigger and darker spots in RCE than WCE indicating that RCE has a higher amount of phytoconstituents than WCE. The results suggest that consuming red cabbage may bring more benefits than white in the antioxidants aspect.

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