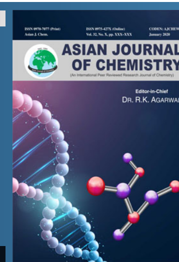
Asian Journal of Chemistry; *Vol. 32, No. 9 (2020), 2303-2307*

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22792



Variation in Phytochemical Constituents and Antioxidant Potential of Extracts Derived from Leaves of *Sansevieria trifasciata* var. *Laurentii* and *Sansevieria trifasciata* var. *Zeylanica* (Asparagaceae)

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Received: 22 April 2020; *Accepted*: 11 June 2020; *Published online*: 20 August 2020; AJC-20030

Sansevieria is an ornamental plant that has many hybrids and varieties make them difficult to distinguish. The most common varieties used for medicinal purposes are *Sansevieria trifasciata*m which is known for cure of many diseases. However, little attention is given to this plant in proving it medicinal worth and capability as an antioxidant agent. This study was initiated to set up a metabolite classes profile and the potential enzymatic antioxidant of the variations of these plants. Crude extracts of *S. trifasciata* var. *Laurentii* and *S. trifasciata* var. *Zeylanica* were prepared from their leaves, and solvent used has different polarities. Qualitative phytochemical analysis was carried out using the extracts. Phytochemical screening suggested both of these samples contain carbohydrates in all extracts. It also show that flavonoid was found in hexane and ethyl acetate extracts while did not observed in the methanol extracts for both samples. However, alkaloid, phenol and tannin were positive in all of the methanol extracts except for hexane and ethyl acetate extracts. For the biological activity, all extracts were selected for the determination of enzymatic antioxidant activity test using catalase (CAT) assay and guaiacol peroxidase (gPOD) assay using UV-VIS spectrophotometer. Based on the results, CAT specific activity was the highest in methanol extract of *S. trifasciata* var. *Laurentii* (3.15 ± 0.50 units/mg protein) compared to *S. trifasciata* var. *Zeylanica* (2.20 ± 0.05 units/mg protein). For gPOD specific activity, ethyl acetate extract of *S. trifasciata* var. *Laurentii* shows the highest activity which is 1.46 $\times 10^{-2}$ ± 0.02 units/mg protein compared to the other crude extracts.

Keywords: ***Sansevieria trifasciata* var. *Laurentii*, *Sansevieria trifasciata* var. *Zeylanica*, Phytochemistry, Enzymatic antioxidant.**

INTRODUCTION

The genus *Sansevieria* consists of over 70 species, commonly known as flowering plants which means they often produce seeds and live on land. It is an ornamental plant with fairly high economic value. It has many hybrids and varieties which make them difficult to identify. Among *Sansevieria*, the most commercialized species is *Sansevieria trifasciata*. Generally, *Sansevieria* was used as a decorative plant due to its beautiful appearances and only required a small maintenance. However, Orang Asli in Perak, Malaysia used it as a traditional cure for ear pains, swellings, boils and fevers [1].

Phytochemicals are substances found in plant's secondary metabolites that were derived from distinct biosynthetic pathways in plants [2]. Instead of quantitative phytochemical screening test, this study is focusing more on the qualitative phytochemical screening test. Qualitative phytochemical analysis is a more extensive and useful method for the fast general compounds detection. The results gained from the present study will be very useful for the determination and standardization of herbal drugs, to explain the potential of a plant in medicinal scope and to determine the toxicity levels in plants [3].

Reactive oxygen species (ROS) is a byproduct metabolism produced under normal condition and its toxicity are common in all plants. However, the production of ROS depends on the condition during the metabolic operation and initiation of environmental agitation. The type and amount of ROS production is also different due to wide dispensation of metabolic

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processes throughout different cell compartment [4]. The excessive production of ROS may lead to oxidative stress [5]. It is a serious imbalance between the production of ROS and antioxidant defenced which can cause damage to the cellular macromolecules [6]. However, antioxidative properties of these plants are able to balance the amount of ROS and keep the plant at non-toxic level. The generation of ROS especially H_2O_2 give signal to activate the defence mechanism of plant under biotic and abiotic stress. High concentration of H_2O_2 can damage the host plant. On the other hand, plant has developed ROS detox systems that include enzymatic and non-enzymatic antioxidant properties [7]. The accumulation of ROS can be neutralized by enzymatic antioxidant [8].

In this study, the variation in phytochemical constituents and antioxidant activity of extracts derived from leaves of *Sansevieria trifasciata* var. *Laurentii* and *Sansevieria trifasciata* var. *Zeylanica* were investigated.

EXPERIMENTAL

Sample preparation: The leaves of *Sansevieria trifasciata* var. *Laurentii* and *Sansevieria trifasciata* var. *Zeylanica* were washed under running tap water, and shade dry at room temperature for five days before pulverized into a fine powder. The plant powder was then stored in sealed container at 4 ºC for further analysis.

Sample extraction: The leaves of *Sansevieria trifasciata* var. *Laurentii* and *Sansevieria trifasciata* var. *Zeylanica* samples were extracted in three different solvents with increasing polarity (hexane, ethyl acetate and methanol) at room temperature using ultrasonic sonication technique (Elma D-78224 Singen Htw, Germany). The extracts were then concentrated using rotary evaporator under reduced pressure at 35-40 ºC. The crude extracts obtained were weighed, labelled and stored in chiller (4 ºC).

Phytochemical analysis: Primarily qualitative phytochemical screening tests were assessed to evaluate the classes of metabolite in the leaves sample of *Sansevieria trifasciata* var. *Laurentii* and *Sansevieria trifasciata* var. *Zeylanica* plants by using the following standard methods [9,10].

Alkaloid test (Wagner's test): Wagner's reagent was added into crude extracts. The formation of reddish brown precipitate indicates the presence of alkaloids.

Carbohydrate test (Fehling's test): The crude extracts were hydrolyzed using dil. HCl. Then, it was neutralized using NaOH and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of carbohydrates in the crude extract.

Flavonoid test (alkaline reagent test): The crude extracts were treated with a few drops of NaOH solution. Formation of intense yellow colour, which becomes colourless on addition of dil HCl, indicates the presence of flavonoids.

Phenol test (ferric chloride test)*:* The crude extracts were dissolved in 5 mL of distilled water and 2-3 drops of 10% ferric (III) chloride solution was added into the mixture. Then, the existence of phenolic content was determined when the solution changed to blue or green colour.

Tannin test: Aqueous extracts (1 mL) was added to 1 mL of water containing 1-2 drops of dilute ferric chloride solution. A dark green or blue green colouration indicated the presence of tannins in the crude extracts.

Protein test (Biuret's test): Sodium hydroxide solution (40%) and 2 drops of 1% copper sulphate were added into the crude extract. The formation of violet colour proved the presence of protein.

Saponin test (Froth test): The crude extracts was shaken with distilled water and heated until boiled. The formation of bubbles determined the presence of saponins.

Steroid/triterpenoid test: Chloroform along with a few drops of conc. H_2SO_4 was added to each of the extracts. The mixture was shaken and kept aside for a few minutes. The formation of red colour in the lower layer shows the presence of steroids while the formation of yellow colour indicated the presence of triterpenoids.

Catalase (CAT) assay: CAT specific activity assays was adapted from Claiborne method [11]. Approximately 0.15 g of samples were grounded with 1.0 mL of 50 mM phosphate buffer (pH 7.4) and clean sand in a pre-chilled mortar and pestle at 0-4 ºC. The mixture was then centrifuged at 10, 000 rpm at 4 ºC for 10 min. The reaction mixture containing 3 mL of buffer (19 mM hydrogen peroxide in 50 mM phosphate buffer, pH 7.0) and 100 µL of enzyme extract was added. The rate of change in the absorbance of the reaction mixture was monitored at 240 nm for 3 min using spectrophotometer (UV-1800 Shimadzu). CAT specific activity was expressed in µmoles of hydrogen peroxide consumed per minute per mg protein. The specific activity of catalase (CAT) assay was calculated as below:

$$\text{Specific activity}\left(\frac{\text{units}}{\text{mg protein}}\right)=\frac{\Delta A\text{ / minute}\times 1000}{E\times\text{mg protein/mL reaction mixture}}$$

where E is the molar extinction coefficient of peroxide (43.6 M^{-1} cm^{-1}).

Guaiacol peroxidase (gPOD) assay: Approximately 0.15 g of samples were grounded with 1.0 mL of 50 mM phosphate buffer having pH 7.4 and clean sand in a pre-chilled mortar and pestle at 0-4 ºC. The mixture was then centrifuged at 10000 rpm at 4 ºC for 10 min [12]. The reaction mixture containing 1 mL of 50 mM phosphate buffer (pH 7.5), 1 mL of 20 mM guaiacol and 1 mL of 30 mM hydrogen peroxide was added to 200 µL enzyme extract (supernatant). After that, the rate of changes in absorbance of the reaction mixture was monitored at 470 nm for 3 min. The specific activity of gPOD was calculated as below:

$$\text{Specific activity}\left(\frac{\text{units}}{\text{mg protein}}\right)=\frac{\Delta A\text{ / minute}\times 1000}{E\times\text{mg protein/mL reaction mixture}}$$

where, E is the molar extinction coefficient of peroxide (43.6 M^{-1} cm^{-1}).

Determination of protein content: Coomasie Brilliant Blue G-250 (100 mg) was dissolved in 50 mL of 95% ethanol. Then, 100 mL of conc. phosphoric acid was added and the mixture was diluted to 1.0 L. The solution was then filtered and stored at room temperatures in light-proof bottle. Enzyme extract (100 µL) was added to 3 mL of Bradford's reagent and

the absorbance was measured at 595 nm after 10 min. The protein concentration was calculated according to a standard curve prepared with various concentration of BSA (bovine serum albumin) in the range of 0-100 mg/L BSA [13].

Statistical analysis: Data was analyzed using Statistical Package for Social Sciences (SPSS) software version 22.0. All data recorded were reported as means ± standard error. The data of the specific enzymatic activities was analyzed using Welsch F method with One-Way ANOVA to evaluate the differences in activity of enzymatic antioxidants in *S. trifasciata* var. *Laurentii* and *S. trifasciata* var. *Zeylanica*. All recorded data were tested and mentioned as mean ± standard deviation.

RESULTS AND DISCUSSION

There were no significant differences between both plants as most of the phytochemicals detected in these plants is almost the same except for the existence or absence of a few phytochemical compounds in the crude extracts. However, three extracts from different solvent polarities of each plant revealed the presence of various phytochemicals. The extractions using methanol as the solvent gave positive reaction towards alkaloid test. Alcohol such as methanol can dissolved both free alkaloids and salt alkaloids [14].

Carbohydrates were found in all of the crude extracts as they showed the desired changes. The results presented in Table-1 also shows that the flavonoid compounds were presented in both hexane and ethyl acetate extracts but not in methanol extracts. The presence of flavonoid can be seen when the formation of intense yellow colour become colourless on the addition of HCl. The result is comparable with a study by Berame *et al.* [15] which reported that ethanolic extracts of the leaves and roots of *S. trifasciata* var. *Laurentii* were lacked in flavonoid compound.

When 2-3 drops of $FeCl_3$ solution was added into the mixture of crude extracts, the solution changed its colour to blue or green colour indicated the presence of phenol in the extracts. As a result, phenol exists in methanol extracts of both samples but absence in hexane and ethyl acetate extracts. Changes of desired colour were observed in ethyl acetate extracts of both samples for the observation of protein. However, there were no significant changes in hexane and methanol extracts. This result was supported by the preliminary phytochemical screening which stated that the extracts of these species showed the presence of protein [16].

The presence of saponin was confirmed in both hexane and ethyl acetate crude extracts of *Sansevieria trifasciata* var. *Laurentii*. Meanwhile, there were no traces of saponin in both of the sample's methanol crude extracts and ethyl acetate crude extract of *Sansevieria trifasciata* var. *Zeylanica*. The absence of saponin in alcoholic extracts was compared with the results of Berame *et al.* [15] which stated that the ethanolic extract of *S. trifasiata* var. *Laurentii* was absent in saponin. However, the presence of saponin was also detected in the root extract of this plant [17]. The slight difference in data was due to the different extraction method and solvents used.

A dark green or blue green colouration indicated the presence of tannins in the crude extracts. Based on the results, only the methanol extracts from both samples showed the presence of tannins while in hexane and ethyl acetate crude extracts the presence of tannin was not observed. These findings were supported by Berame *et al.* [15] and Ikewuchi *et al.* [17]. The presence of steroids was indicated by the formation of red colour in the lower layer of the mixture while the formation of yellow colour indicated the presence of triterpenoid. Results showed that methanol crude extracts formed a yellow colour in both species which indicates the presence of triterpenoid. While for ethyl acetate and hexane crude extract of *Sansevieria trifasciata* var. *Laurentii* showed the formation of red colour in the lower layer indicating the presence of steroid. Only the hexane and ethyl acetate crude extracts of *S. trifasciata* var. *Zeylanica* showed negative result of both steroid and triterpenoid. These results were in idential with the research by Teponno *et al.* [18], which confirmed the presence of several types of steroidal sapogenins in the plant.

The results (Table-1) showed that among the three solvents used, methanol show its potential to extract the most phytochemical compounds in the leaves of *Sansevieria trifasciata* var. *Laurentii* and *Sansevieria trifasciata* var. *Zeylanica* including alkaloids, carbohydrates, phenols, tannins and triterpenoids/steroids. Meanwhile, solvent that obviously yielded the least phytochemical compounds such as carbohydrates, flavonoids, saponins and triterpenoid/steroid is hexane. This information indicates that non-polar solvent yielded the least phytochemical compounds than solvent with higher polarities such as methanol.

Catalase specific activity: The specific activities of CAT in all of the crude extracts of *Sansevieria trifasciata* var. *Laurentii* and *Sansevieria trifasciata* var. *Zeylanica*. The range of the CAT specific activities for the crude extracts of *S. trifasciata*

TABLE-1
SUMMARY OF THE PHYTOCHEMICAL SCREENING ANALYSIS IN ALL CRUDE EXTRACTS*

	He.L	Ea.L	Me.L	He.Z	Ea.Z	Me.Z
Alkaloids test	-	-	+	-	-	+
Carbohydrates test	+	+	+	+	+	+
Flavonoids test	+	+	-	+	+	-
Phenol test	-	-	+	-	-	+
Protein test	-	+	-	-	+	-
Saponin test	+	+	-	+	-	-
Tannin test	-	-	+	-	-	+
Triterpenoid/Steroid test	+	+	+	-	+	+

+ = Positive result in 3 replicates; .L = *S. trifasciata var. Laurentii*; He = Hexane; – = Negative result in 3 replicates; .Z = *S. trifasciata var. Zeylanica*; Ea = Ethyl acetate; Me = Methanol

var. *Laurentii* lies in between 1.69 ± 1.03 and 6.06 ± 1.73 units/mg protein while the CAT specific activities for the crude extracts of *S. trifasciata* var. *Zeylanica* were in the range of 0.33 ± 0.13 to 6.17 ± 0.09 units/mg protein (Fig. 1). Based on the statistical analysis, there was no significant difference detected between each crude extract that used the same solvent.

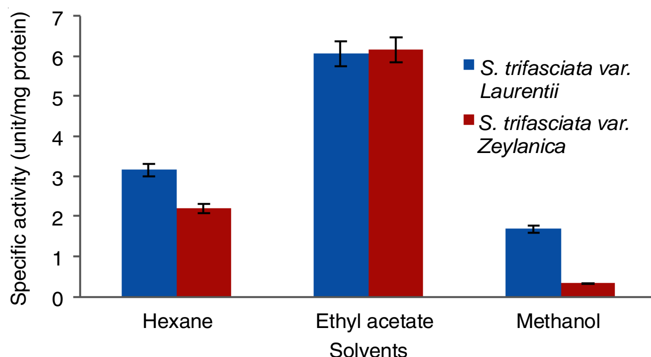

Fig. 1. Catalase (CAT) specific activities in the crude extracts of *Sansevieria trifasciata var. Laurentii* and *Sansevieria trifasciata var. Zeylanica*. Data are means ± standard deviation (n = 3)

Generally, *S. trifasciata* var. *Laurentii* possessed higher CAT specific activities in the overall result as its hexane and methanol extracts shows higher specific activity with the values of 3.17 ± 0.35 and 1.69 ± 1.03 units/mg protein, respectively. Whereas, the values for the specific activity in hexane and methanolic extracts of *S. trifasciata* var. *Zeylanica* are 2.19 ± 0.04 and 0.33 ± 0.13 units/mg protein, respectively. However, the ethyl acetate extract for *S. trifasciata* var. *Zeylanica* shows higher specific activity compared to *S. trifasciata* var. *Laurentii* with the values of 6.17 ± 0.09 and 6.06 ± 1.73 units/mg protein.

Guaiacol oeroxidase (gPOD) specific activity: The gPOD specific activity in the hexane extracts of *Sansevieria trifasciata* var. *Laurentii* and *Sansevieria trifasciata* var. *Zeylanica* has no significant difference with the value of 0.00035 ± 0.0006 units/mg protein and 0.0048 ± 0.0007 units/mg protein, respectively (Fig. 2). In ethyl acetate extracts of both *S. trifasciata* var. *Laurentii* and *S. trifasciata* var. *Zeylanica*, the gPOD specific activities were significantly different and the values were 0.0158 ± 0.0022 and 0.0018 ± 0.0008 units/mg protein, respectively. While the gPOD activities for the methanolic extracts of both species were 0.0020 ± 0.0008 and 0.0021 ± 0.0006 units/mg protein, respectively, there were no significant difference in the extracts. Generally, *S. trifasciata* var. *Zeylanica* shows higher gPOD specific activities in both of its hexane and methanol extracts while lower activity was detected in its ethyl acetate extract when compared to *S. trifasciata var. Laurentii*. Amongst all six extracts, *S. trifasciata* var. *Laurentii* shows the highest gPOD specific activities in its ethyl acetate extract with the value of 0.0158 ± 0.0022 units/mg protein.

Conclusion

Phytochemical screening analysis revealed that methanol is the most suitable solvent to extract various phytochemical compounds in *Sansevieria trifasciata* var. *Laurentii* and *Sansevieria trifasciata* var. *Zeylanica*, although, some of the chemical compounds were not detected in methanol crude

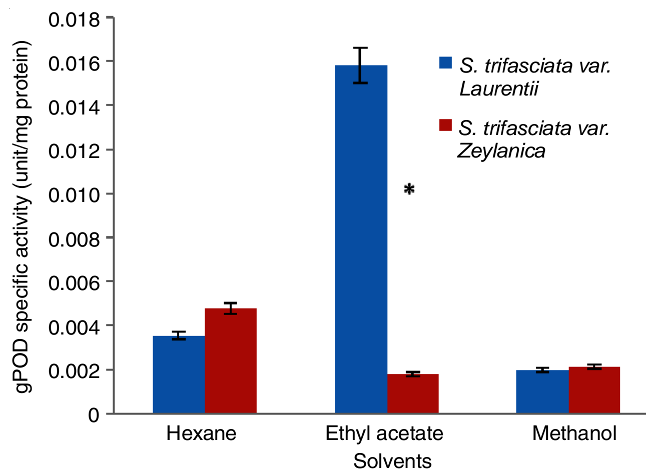

Fig. 2. Guaiacol peroxidase (gPOD) specific activities in the crude extracts of *Sansevieria trifasciata var. Laurentii* and *Sansevieria trifasciata var. Zeylanica*. Data are means ± standard deviation (n=3). * represent significant difference at $p < 0.05$

extracts. While the least phytochemical compounds extracted were in the crude extract using hexane as the extraction solvent. It was concluded that the total chemical compounds extracted with regards to different solvent used for extraction were as follow: hexane < ethyl acetate < methanol. The enzymatic antioxidant such as CAT and gPOD analysis were successfully assayed from the crude extracts of *S. trifasciata* var. *Laurentii* and *S. trifasciata* var. *Zeylanica*. Based on the results, it was concluded that the enzymatic antioxidant activities were observed higher in the analysis of CAT specific activities than in the analysis of gPOD specific activities. In terms of tolerance level, CAT seems to be the major tolerant in both variations since it shows the highest activity in all crude extracts when compared to the activities shown for gPOD analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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