# In vitro antioxidant activity and total phenolic content of Sansevieria trifasciata (Snake plant) crude ethanolic and aqueous leaf extracts

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**Abstract:** Sansevieria trifasciata is a widely used ornamental plant in the Philippines with proven anti-inflammatory, thrombolytic, antipyretic, analgesic and anti-diabetic properties. However, its antioxidant potential has not been studied. This study determined the antioxidant property of S. trifasciata crude ethanolic and aqueous leaf extracts by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and phosphomolybdenum method for reducing property as well as total phenolic content by Folin-Ciocalteu method. Results of the study revealed that both extracts exhibited antioxidant activity. The total phenolic content of the ethanolic extract (0.474 mg GAE/g) was found to be higher than that of the aqueous extract (0.285 mg GAE/g) and exhibited better reducing activity (2.417 mg AAE) than the aqueous extract (0.999 mg AAE) at 100 mg/mL. However, the aqueous leaf extract at 1000 μg/mL exhibited higher percentage inhibition (84.41 ± 0.49) than the ethanolic leaf extract (80.07 ± 0.65). Results of the study revealed the antioxidant potential of S. trifasciata with dose-dependent activity in higher concentrations.

**Keywords:** Sansevieria trifasciata, DPPH, antioxidant, phosphomolybdenum, Folin-Ciocalteu

## INTRODUCTION

The world is currently being threatened by a discreet pandemic of chronic diseases. These diseases are gradually replacing infectious and parasitic diseases as the chief cause of morbidity and mortality globally. In 2008, approximately 57 million deaths occurred worldwide; 36 million (63%) were attributed to


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non-communicable diseases, chiefly cardiovascular diseases, chronic respiratory diseases, diabetes and cancer (Alwan et al., 2010). Cancer mortality is anticipated to escalate from 7.4 million in 2004 to 11. 8 million in 2030 while deaths from heart and vascular system disorders are expected to rise from 17.1 million in 2004 to 23.4 million in 2030. Ischemic heart disease, cerebrovascular disease, chronic obstructive pulmonary disease and lower respiratory tract infections are foreseen to be the four leading causes of death worldwide in 2030. (WHO, 2008). In the Philippines, majority of the leading causes of mortality are chronic degenerative diseases such as heart and vascular system disorders, malignant neoplasm and diabetes mellitus. More than 80% (408,390) of the 498,486 deaths in 2011 were due to these chronic diseases (Department of Health, 2011). Undoubtedly, the aforementioned situations present challenges in the health sector. This has led various health and research agencies all over the world to spearhead programs directed towards disease management and prevention. It is believed that four amendable health risk behaviors contribute to the development of chronic diseases – lack of exercise, tobacco use, excessive alcohol consumption and poor nutrition (Centers for Disease Control and Prevention, 2009). Aside from these behaviors, an increasing number of studies have focused on the involvement of free radicals in the pathogenesis of diseases. Numerous researchers have studied several substances, known as antioxidants, which can combat free radicals. However, the notion that the use of antioxidants as preventive measure for such diseases seems to raise a lot of questions (Pham-Huy, He & Pham-Huy, 2008).

Recent studies indicate that the buildup of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) play a major role in the development of chronic and degenerative illnesses (Pham-Huy, He & Pham-Huy, 2008). Consequently, several researchers have focused on the discovery of natural products as potential dietary antioxidants that work by neutralizing free radicals. Aside from vitamins, minerals and fibers acquired from fruits and vegetables, majority of studies have shown they are also good sources of polyphenols, flavonoid, anthocyanins and carotenoids. Polyphenols are known to protect fatty acids from oxidative decay and defend against oxidative stress due to the accumulation of free radicals (Brewer, 2011; Kasote, Katyare, Hegde & Bae, 2015; Pham-Huy et al., 2008). Hence, polyphenols may play an essential role in preventing coronary heart disease, obesity, gastrointestinal disorders, colon


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cancer and in lowering the risk of diabetes. It was revealed that plants with high concentration of this bioactive compounds demonstrated high antioxidant activity. These studies, however, only utilized fruits and vegetables proven to be safe for human consumption (Ignat, Volf & Popa, 2011; Kevers et al., 2007; Lako et al., 2007; Lin & Tang, 2007; Rezaeizadeh et al., 2011). Other studies delved on the possible antioxidant property of leaves of atypical plants (Alabri, Al Musalami, Hossain, Weli & Al-Riyami, 2014; Chavan, Gaikwad, Kshirsagar & Dixit, 2013; Gopalakrishnan & Rajameena, 2013; Nur Alam, Roy, Mohammad Anisuzzaman & Rafiquzzaman, 2012; Phatak & Hendre, 2014; Yuan, Zhou, Zhou, Yang & Zhang, 2011). However, there are only few studies which used ornamental plants as sources of antioxidant compounds (Banerjee et al., 2011; Chinsamy, Finnie & Van Staden, 2014; Erel et al., 2012; Zemmouri et al., 2014)

Sansevieria trifasciata, a native plant of India has no recorded medicinal use in the Philippines. It is customarily used as an ornament and air purifier. Current studies revealed that S. trifasciata has significant anti-inflammatory, thrombolytic, antipyretic, analgesic and antidiabetic properties. These studies were performed on laboratory rats and no clinical trial was conducted. It has been reported that the aqueous and ethanolic extracts of the plant yielded saponins, flavonoids, alkaloids, glycosides terpenoids, proteins, tannins and carbohydrates upon phytochemical screening (Dey, Bhattacharjee, Mitra, Singla & Pal, 2014; Qomariyah, Sarto & Pratiwi, 2012; Sikder et al., 2011; Sunilson et al., 2009). However, there are no published studies showing the antioxidant property of S. trifasciata ethanolic and aqueous leaf extracts.

**Figure 1. Sansevieria trifasciata A. leaves and B. rhizomes**

In this study, the potential in vitro antioxidant activity of S.trifasciata aqueous and ethanolic leaf extracts was determined using DPPH assay for free radical scavenging activity and phosphomolybdenum assay for reducing activity. Furthermore,


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the total phenolic content of both extracts was evaluated using the Folin-Ciocalteau method. The concentration of the extracts which exhibited the highest percentage inhibition of the DPPH radical and the reduction of the phosphomolybdenum was determined. This study showed the difference between the antioxidant activity of S.trifasciata aqueous and ethanolic leaf extracts. Based on the results, this plant could be used as a natural source of antioxidant compounds which could be effective against neurodegenerative and cardiovascular diseases.

## MATERIALS AND METHODS

### Collection of plant samples

Fully mature, disease-free, succulent leaves of Sansevieria trifasciata with horizontal bands of light grayish-green, bordered with creamy yellow margins were harvested in and around the vicinity of Batangas City and Lemery, Batangas on December 2015. Plant materials were submitted to the Herbarium of University of the Philippines-Los Baños for authentication.

### Preparation of samples

The leaves were washed thoroughly with tap water. The samples were divided into two portions, 1.0 kg leaf samples were dried under shade at 25 °C for 3 days while 1.0 kg fresh samples were sliced into tiny pieces for the extraction process (Alabri et al., 2014).

### Ethanolic extraction procedure

A 1.0 kg leaves were air-dried for 3 days at 25°C and were pulverized using Wiley mill. It was soaked in 4.0 L of 95% ethyl alcohol for 48 hours. The mixture was filtered and the filtrate obtained was concentrated using rotary evaporator at 60°C under vacuum for 3 hours. The concentrated extract was further evaporated using water bath at 60°C to obtain a semi-solid extract (Department of Science and Technology -Industrial Technology and Development Institute).

### Aqueous extraction procedure

A 1.0 kg fresh leaves were blended and soaked in 4.0 L of distilled water for 48 hours. The mixture was filtered and the filtrate was concentrated using rotary evaporator at 60°C under vacuum for 3 hours. The concentrated extract was further evaporated using water bath at 60°C to obtain a semi-solid extract (DOST-ITDI).


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**Total phenol assay**

The extract solutions (250 µL) with a concentration of 1000 mg/ml were mixed with 4 mL of distilled water and 250µL Folin–Denis reagent. Then, 500 µL of saturated sodium carbonate ($Na2CO3$) was added to the mixture. The mixtures were mixed using vortex for 30 seconds and were incubated for 20 minutes in a dark room at room temperature. These were centrifuged for 10 minutes at 3000 rpm. The absorbance was measured at 700 nm using a UV-Vis spectrophotometer. The total phenolic content was calculated according to the standard curve that was prepared with gallic acid in five different concentrations (25, 50, 200, 300 and 400 mg/mL) and the results were expressed as gallic acid equivalents (GAE) per gram of extract (DOST-Standards and Testing Division).

**DPPH radical scavenging capacity**

DPPH radical scavenging assay was adapted from Clarke et al. (2013) with some modifications based on the nature of the extract (University of Santo Tomas-Analytical Services Laboratory). Twenty microliter of the extract (1000, 500, 250, 125 and 62.50 µg/mL) was diluted appropriately in distilled $H2O$ with 180 µL of DPPH in methanol (40 µg/mL) in wells of a 96-well plate. The plate was kept in the dark for 15 minutes then the absorbance of the solution was measured at 517 nm in SH-1000 Corona microplate reader. Distilled $H2O$ served as a blank and Ascorbic Acid served as the standard. The concentration at 50% inhibition (IC50) was computed using linear interpolation.

$$\%\ \text{inhibition} = \frac{\text{Absorbance of DPPH} - \text{Absorbance of extract}}{\text{Absorbance of DPPH}} \text{x}100$$

**Phosphomolybdenum assay**

The reducing capacity of the extracts was determined using the phosphomolybdenum method based on the conventional procedure with slight modification. This method was based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and the subsequent development of a green phosphate/Mo(V) complex at acid pH. In 47 mL of distilled water, 1 mL each of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added, producing a 50 mL solution (Phatak & Hendre, 2014). A 0.3 mL extract solution (25, 50,100 mg/mL) was dispensed into test tubes. The extracts were combined with 3 mL of reagent solution (0.6 M $H2SO4$, 28 mM sodium phosphate, 4 mM


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ammonium molybdate). The tubes were incubated at 90 oC for 90 minutes. The absorbance was measured at 695 nm using UV-Vis spectrophotometer (Hitachi U-5100) after the mixture has cooled at room temperature. A blank test was conducted using ethanol and distilled water. The antioxidant activity was determined from a linear equation that was established using ascorbic acid in 8 different concentrations (1.5630, 3.1250, 6.2500, 12.5, 25, 50, 100, 200 mg/mL) as reference standard. It was expressed as ascorbic acid equivalents (AAE) (Rivera & Uy, 2012).

**Statistical Analysis**

The experimental data were expressed as means ± SD. Statistical comparisons were made using one-way analysis of variance (ANOVA) with Post Hoc Test to determine significant differences between groups. Tukey was used to determine differences between concentrations while Dunnett's was used to differentiate the test from the control group. Independent sample t-test was used to determine relationship between paired groups. The level of significance was set at $p \leq 0.0001$. All computations were made using Graph Pad Prism Version 6.0.

## RESULTS AND DISCUSSION

**Crude ethanolic extract**

In the preparation of the crude ethanolic extract, 90.0 grams of sticky, black, semi-solid extract was obtained (Figure 2). Total percent yield was 0.09%.

**Figure 2. Crude ethanolic extract**

**Crude aqueous extract**

In the preparation of crude aqueous extract, 140.0 grams of brownish green, semi-solid extract was obtained (Figure 3). Total percent yield was 0.14%.


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**Figure 3. Crude aqueous extract**

**Total Phenolic Content**

The Folin-Ciocalteau assay (Table 1) revealed that the total phenolic content of the ethanolic extract (0.474 GAE/g) was significantly higher than the content of the aqueous extract (0.285 mg GAE/g).

**Table 1. Total Phenolic Content of Plant Extracts**

| Extract | Total phenolic content GAE/g |
|---|---|
| Ethanolic | 0.474 |
| Aqueous | 0.285 |

Azlim Almey et al. (2010) explained that the Folin-Ciocalteu reagent which is a mixture of phosphotungstic (H3PW12O40) and phosphomolybdic (H3PMo12O40) acids, is converted during phenol oxidation to blue oxides of tungstene (W8O23) and molybdene (Mo8O23) . Sodium carbonate which provides the alkaline condition makes the reaction possible. The amount of phenolic compounds present is reflected by the intensity of blue color measured using a spectrophotometer.

The extracts, as expected, contained phenolics. The study of Dey et al. (2014) showed that the plant has phenolics, alkaloids, terpenoids, flavonoids, saponins, steroids and glycosides which are responsible for its various medicinal properties. Qomariyah et al. (2012) added that S. trifasciata leaves extracted in ethanol or water has diverse phytochemical compounds such as tannins, proteins, carbohydrates and polyphenols. Furthermore, Sunilson et al. (2009) stated that the phytochemical screening of the plant



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demonstrated the presence of N-butyl-4-ol-N-propylphthalate, pregnane glycosides, and steroidal sapogenins.

According to Ignat et al. (2011), extraction is truly a critical step in the sequestration, identification and usage of phenolic compounds and a sole and standard extraction method does not exist(p. 1826). Solvent to plant material ratio, time of extraction and other factors influence the absolute value of phenolic compounds. Hence, similar plant material may lead to a different phenolic content (Nur Alam et al., 2012). The extract yield and subsequent antioxidant activities of the plant materials are strongly reliant on the nature of extracting solvent and the presence of antioxidant compounds with different chemical characteristics and polarities (Abozed, El-kalyoubi, Abdelrashid & Salama, 2014). The antioxidant compound dissolved in ethanol is the reason behind the extract's higher phenolic content Stanković (2011) and Sultana, Anwar and Ashraf (2009) attested that phenolics are frequently extracted in higher amounts in more polar solvents like aqueous methanol/ethanol as well as acetone. Do et al. (2014) narrated that ethanol can be safely used for human consumption and polyphenol extraction. Lower molecular weight polyphenols are efficiently extracted using methanol whereas higher molecular weight flavanols are efficiently extracted using aqueous acetone(p. 297). The amount of the antioxidant components that can be extracted from a plant material is mainly affected by the vigor of the extraction procedure, which may probably vary from sample to sample. Greater phenolic content was found in extracts made using a shaker (Sultana, Anwar & Ashraf, 2009).

On the other hand, the total phenolic content of the extracts diminishes with increasing water content. This can be attributed to the content of more non-phenol compounds for instance, terpene and carbohydrate in water extracts than in other extracts. The possible complex formation of several phenolic compounds in the extract that are soluble in ethanol could also be the reason. These phenolic compounds may have more phenol groups or possess greater molecular weights than the phenolics in the water extract. The best extracting solvent for total phenolic content is ethanol (Do et al., 2014).

Sabir et al. (2011) stressed that plant phenolic compounds belong to one of the chief groups of substances acting as free radical scavengers or primary antioxidants, making it rational to determine their total amount in plant extracts. Plants that are rich in polyphenolic compounds, for example, flavonoids, tannins,


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phenolic acids, carotenoids and tocopherols have excellent antioxidant activities (Nur Alam, 2012). A high correlation between total phenolic content and antioxidant activity was proven by several studies (Aliyu et al., 2013; Chinsamy et al., 2014; Erel et al., 2012; Philip, Madhumitha & Mary, 2011; Stanković, 2011; Yuan et al., 2011; Zemmouri et al., 2014).

**DPPH Free Radical Scavenging Activity**

The DPPH free radical scavenging activity (Table 2) revealed that the aqueous leaf extract at 1000 µg/mL exhibited higher percentage inhibition (84.41 ± 0.49) than the ethanolic leaf extract (80.07 ± 0.65). These data showed that the percentage of free radical inhibition increased with increasing concentrations of the extract in a dose-dependent manner.

**Table 2. DPPH free radical scavenging activity of extracts**

| Concentration (µg/mL) | % Inhibition | |
|---|---|---|
| | Ethanolic extract | Aqueous extract |
| 1000 | 80.07 ± 0.65 | 84.41 ± 0.49 |
| 500 | 64.78 ± 1.31 | 80.87 ± 0.67 |
| 250 | 46.07 ± 0.81 | 63.73 ± 1.40 |
| 125 | 35.8 ± 0.93 | 53.46 ± 1.31 |
| 62.5 | 25.75 ± 3.92 | 46.29 ± 0.73 |

**results are expressed as mean ± SD (n=6)*

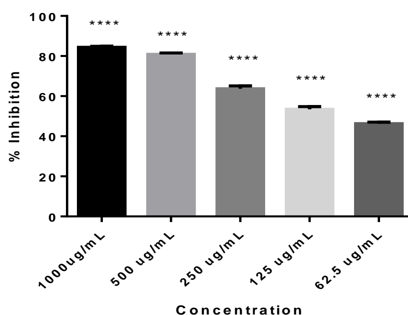


**Figure 4. Percentage inhibition of S. trifasciata aqueous extract. Data are mean ± SD of the observations. ****p<0.0001**

The aqueous extract displayed DPPH radical scavenging activity. The ability to scavenge the DPPH radical increased with increasing concentrations of the extract in a dose-dependent manner (Figure 4). The percentage of DPPH radical inhibition ranged from 21.59% at 62.5 µg/mL to 91.20% at 1000µg/mL.


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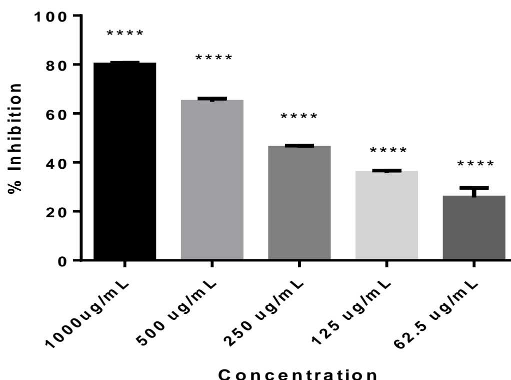


**Figure 5. Percentage inhibition of S. trifasciata ethanolic extract. Data are mean ± SD of the observations. ****p<0.0001**

The ethanolic extract displayed DPPH radical scavenging activity. The ability to scavenge the DPPH radical increased with increasing concentrations of the extract in a dose-dependent manner (Figure 5). The percentage of DPPH radical inhibition ranged from 22.76% at 62.5 µg/mL to 81.23% at 1000µg/mL.

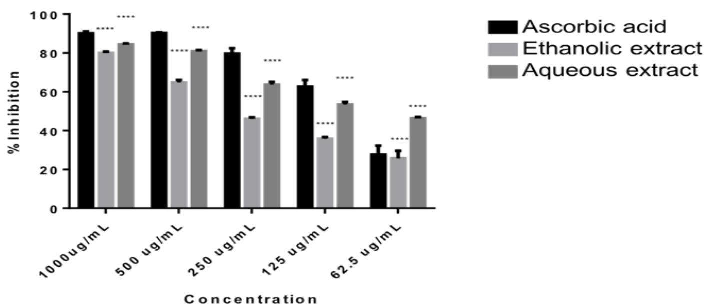


**Figure 6. Comparison of DPPH radical inhibition of the extracts compared to standard. Data are mean ± SD of the observations. ****p<0.0001**

Figure 6 shows that the percentage inhibition of the ethanolic and aqueous extracts compared to the standard is significant (p<0.0001) with dose-dependent activity in higher


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concentrations. The inhibition of the aqueous extract closely parallels that of the ascorbic acid.

**Table 3. Evaluation of $IC_{50}$ value of extracts**

| Tested Material | $IC_{50}$ |
|---|---|
| Ascorbic acid | 102.37 µg/mL |
| Ethanolic extract | 302.51 µg/mL |
| Aqueous extract | 94.84 µg/mL |

The 50% inhibitory concentration ($IC_{50}$) value (Table 3) of the aqueous extract (94.84 µg/mL) was significantly lower than the ethanolic extract (302.51 µg/mL) and the standard, ascorbic acid (102.37 µg/mL).

**Table 4. Comparison of the scavenging activity of the difference concentrations of the aqueous extract**

| | p-value | Interpretation |
|---|---|---|
| 1000 µg/mL vs. 500 µg/mL | > 0.9999 | Not significant |
| 1000 µg/mL vs. 250 µg/mL | < 0.0001 | Significant |
| 1000 µg/mL vs. 125 µg/mL | < 0.0001 | Significant |
| 1000 µg/mL vs. 62.5 µg/mL | < 0.0001 | Significant |
| 500 µg/mL vs. 250 µg/mL | < 0.0001 | Significant |
| 500 µg/mL vs. 125 µg/mL | < 0.0001 | Significant |
| 500 µg/mL vs. 62.5 µg/mL | < 0.0001 | Significant |
| 250 µg/mL vs. 125 µg/mL | < 0.0001 | Significant |
| 250 µg/mL vs. 62.5 µg/mL | < 0.0001 | Significant |
| 125 µg/mL vs. 62.5 µg/mL | < 0.0001 | Significant |

**significant at p-value <0.0001*

Statistical comparison of the scavenging activity of the different concentrations of the aqueous extract (Table 4) revealed that almost all of the concentrations differed significantly with other concentrations since the obtained p-values are less than 0.0001 level of significance. This indicates that higher aqueous extract concentration would result to a greater scavenging activity than lower concentrations. However, it was also revealed that there are no significant difference between the scavenging activity of 1000 µg/mL and 500 µg/mL aqueous extract since the obtained p-value of >0.9999 is higher than the 0.0001 level of significance. This indicates that the scavenging activity of 1000 µg/mL aqueous extract would be the same with that of the 500 µg/mL extract. This only means that better scavenging activity would be expected when either 1000 µg/mL or 500 µg/mL aqueous extract is used.



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**Table 5. Comparison of the scavenging activity of the difference concentrations of the ethanolic extract**

| | p-value | Interpretation |
|---|---|---|
| 1000 µg/mL vs. 500 µg/mL | < 0.0001 | Significant |
| 1000 µg/mL vs. 250 µg/mL | < 0.0001 | Significant |
| 1000 µg/mL vs. 125 µg/mL | < 0.0001 | Significant |
| 1000 µg/mL vs. 62.5 µg/mL | < 0.0001 | Significant |
| 500 µg/mL vs. 250 µg/mL | < 0.0001 | Significant |
| 500 µg/mL vs. 125 µg/mL | < 0.0001 | Significant |
| 500 µg/mL vs. 62.5 µg/mL | < 0.0001 | Significant |
| 250 µg/mL vs. 125 µg/mL | < 0.0001 | Significant |
| 250 µg/mL vs. 62.5 µg/mL | < 0.0001 | Significant |
| 125 µg/mL vs. 62.5 µg/mL | < 0.0001 | Significant |

**significant at p-value <0.0001*

Statistical comparison of the scavenging activity of the different concentrations of the ethanolic extract (Table 5) revealed that all of the concentrations differed significantly with other concentrations since the obtained p-values are less than 0.0001 level of significance. This indicates that higher ethanolic extract concentration would result to a greater scavenging activity than with lower concentrations. This only means that among all the concentrations, the best scavenging activity would be observed on 1000 µg/mL ethanolic extract.

DPPH assay offers the first approach for evaluating the antioxidant potential of a compound, an extract or other biological sources. It is one of the most common and relatively quick methods used for testing radical scavenging activity of various plant extracts. It provided noteworthy information on the reactivity of the compounds with stable free radicals due to the odd number of electrons. Do et al. (2014) explained that DPPH radical is a steady organic free radical with peak absorption at 517 nm. When it receives an electron or a free radical species, it turns from purple to yellow due to the loss of absorption. Various samples in a short period of time can be accommodated by this assay. It also displays high sensitivity(p. 300). The bleaching of DPPH absorption is representative of the capacity of the ethanolic and aqueous extracts to scavenge free radicals independently. Plants serve as electron donors due to their phenolic compounds. This explains the DPPH free radical scavenging power exhibited by the extracts tested. The results also agree with previous studies which showed the direct relationship between the concentration and the scavenging activity of the extracts (Moukette et al., 2015).


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Phenolic compounds and flavonoids are said to be correlated with anti oxidative action in living organisms since they act as scavengers of singlet oxygen and free radicals (Banerjee et al., 2011). Antioxidant compounds that interrupt the free radical chain reaction are regarded the most effective (Brewer,2011). The results of the Folin-Ciocalteau assay showed the presence of phenolic compounds. The difference in radical scavenging of these two extracts may be attributed to the differential solubility of S. trifasciata polyphenolic compounds in the solvents.

IC50 is the amount of the antioxidant needed to decrease DPPH concentration by 50%. There is an inverse relationship between IC50 and antioxidant activity. The lower the IC50, the greater the antioxidant activity (Do et al., 2014). The results of this study showed that the aqueous extract exhibited lower IC50 value than the ethanolic extract. This means that the aqueous extract has greater antioxidant activity in terms of scavenging free radicals.

The ethanolic extract, having higher phenolic content, appeared to be less effective in scavenging the radical compared to the aqueous extract which has lower phenolic content. This could be attributed to the active polyphenols in the extracts. Moukette et al. (2015) stressed that the availability of phenolic hydrogen, molecular structure and substitution pattern of the hydroxyl groups influence the radical scavenging activity of polyphenols and the likelihood of stabilization of the resultant NO and HO radicals through delocalization of electron expansion or donation of hydrogen (p. 3). Moreover, the difference in the scavenging activity between the two extracts could probably be due to the extraction procedure, sample processing and drying. The ethanolic extraction utilized air-dried leaf samples while the aqueous extraction used fresh samples. Alabri et al. (2014) described that the volatile compounds may have evaporated or have been destroyed from the dry samples, explaining why the activity of fresh samples is higher than the dry extracts. Furthermore, Kedare and Singh (2011) revealed that the DPPH method may be used in aqueous and nonpolar organic solvents and it may examine both lipophilic and hydrophilic antioxidants. The greater scavenging activity of the aqueous extract may be attributed to the predominance of hydrophilic antioxidants which apparently dissolved better in distilled water than in ethanol.

**Phosphomolybdenum assay**

The reducing capacity of the extracts was determined using phosphomolybdenum assay (Table 6). It revealed that the


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ethanolic extract gave higher reducing capacity (2.417 mg AAE) than the aqueous extract (0.999 mg AAE) at 100 mg/mL. The activity values were determined using the following linear equation that was established with ascorbic acid as reference standard ($A = 0.0118C + 0.3876$; $R2 = 0.8759$) where A is the measured absorbance and C is the ascorbic acid equivalent (AAE).

**Table 6. Total Antioxidant Capacity of extracts using the Phosphomolybdenum method**

| Concentration (mg/mL) | Absorbance (AAE) | |
|---|---|---|
| | Ethanolic extract | Aqueous extract |
| 25 | 2.106 | 0.559 |
| 50 | 2.315 | 0.71 |
| 100 | 2.417 | 0.999 |

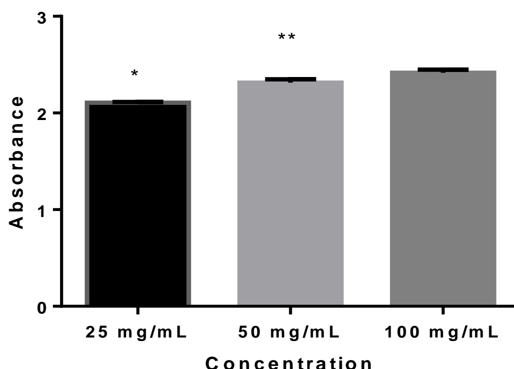


**Figure 7. Absorbance of ethanolic extract**

The ethanolic extract exhibited great reducing capacity (2.417 mg AAE). The reducing property increased with increasing concentrations of the extract in a dose-dependent manner (Figure 7).


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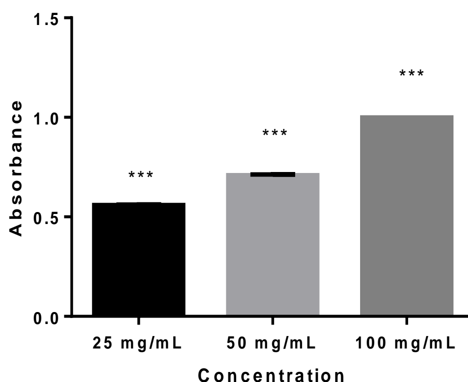


**Figure 8. Absorbance of aqueous extract**

The aqueous extract displayed significant reducing capacity (0.999 mg AAE). The reducing property increased with increasing concentrations of the extract in a dose-dependent manner (Figure 8).

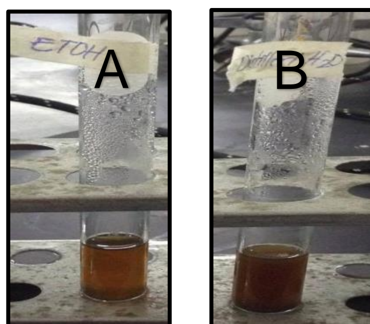


**Figure 9. Negative control of the extracts A. ethanolic extract B. aqueous extract**

Figure 9 shows the negative control of both extracts. The tubes contained the respective solvent and the ammonium molybdate solution. After incubation and heating, no green complex was formed.


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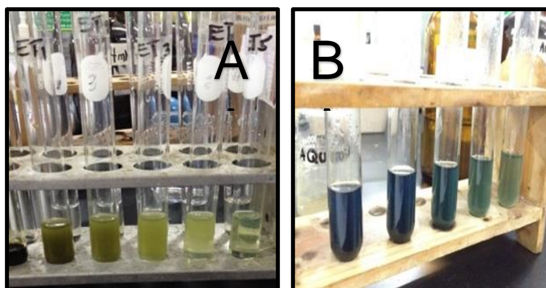


**Figure 10. Ethanolic extract treatment with molybdate reagent A. before heating B. after heating**

Figure 10 shows that the ethanolic extract was able to reduce the phosphomolybdenum complex as demonstrated by the formation of a green colored solution.

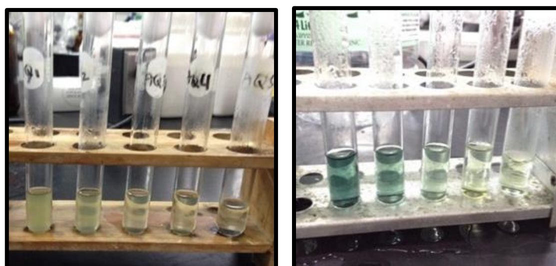

**Figure 11. Aqueous extract treatment with molybdate reagent A. before heating B. after heating**

Figure 11 shows that the aqueous extract was able to reduce the phosphomolybdenum complex evident in the formation of a green colored solution.

The reducing capacity of the ethanolic extract (Figure 12) was significantly higher (2.106 at 25 mg/mL, 2.315 at 50 mg/mL and 2.417 at 100 mg/mL) than that of the aqueous extract (0.559 at 25 mg/mL, 0.71 at 50 mg/mL and 0.999 at 100 mg/mL) at all concentrations tested.


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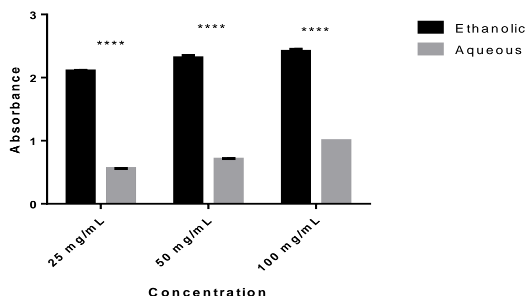


**Figure 12. Reducing capacity of the extracts in terms of GAE**

**Table 7. Comparison of the reducing capacity of the different concentrations of the ethanolic extract**

| | p-value | Interpretation |
|---|---|---|
| 25 mg/mL vs. 50 mg/mL | <0.05 | Significant |
| 25 mg/mL vs. 100 mg/mL | <0.01 | Significant |
| 50 mg/mL vs. 100 mg/mL | >0.05 | Not significant |

**significant at p-value <0.05*

Statistical comparison of the reducing capacity of the different concentrations of the ethanolic extract (Table 7) showed that there was a significant difference between the reducing capacity of 25 mg/mL extract and 50 mg/mL extract (p-value <0.05) as well as 25 mg/mL extract and 100 mg/mL extract (p-value <0.01) since the obtained p-values were less than 0.05 level of significance. This indicates that when 50 mg/mL and 100 mg/mL ethanolic extracts are used, they would produce significant reducing capacity than the use of 25 mg/mL extract. However, there was no significant difference between the reducing capacity of 50 mg/mL and 100 mg/mL extract since the obtained p-values was greater than the 0.05 level of significance. This indicates that when 50 mg/mL ethanolic extract is used, the resulting reducing capacity would be the same as that of 100 mg/mL. This only means that the use of either 50 mg/mL or 100 mg/mL ethanolic extract would result to a better reducing capacity than the use of 25 mg/mL extract.


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**Table 8. Comparison of the reducing capacity of the different concentrations of the aqueous extract**

| | p-value | Interpretation |
|---|---|---|
| 25 mg/mL vs. 50 mg/mL | < 0.0001 | Significant |
| 25 mg/mL vs. 100 mg/mL | < 0.0001 | Significant |
| 50 mg/mL vs. 100 mg/mL | < 0.0001 | Significant |

**significant at p-value < 0.0001*

Statistical comparison of the reducing capacity of the different concentrations of the aqueous extract (Table 8) showed that there are significant differences between the reducing capacity of 25 mg/mL and 50 mg/mL, 25mg/mL and 100 mg/mL and 50 mg/mL and 100 mg/mL since the obtained p-values were less than 0.0001 level of significance. This indicates that the use of 50 mg/mL and 100 mg/mL aqueous extract would result to better reducing capacities than the use of 25 mg/mL. Also, the use of 100 mg/mL aqueous extract would result to a better reducing capacity than the use of 50 mg/mL. This only means that when 100 mg/mL aqueous extract is used, the most significant reducing capacity would be observed as compared to the use of 25 mg/mL or 50 mg/mL.

Phosphomolybdenum assay is a spectroscopic method for the quantitative determination of antioxidant capacity based on the reduction of Mo (VI) to Mo (V) by the sample component and the subsequent formation of a green phosphate Mo (V) complex at acidic pH (Nur Alam et al., 2012). Phatak and Hendre (2014) added that this method examines antioxidant, oxidant and molybdenum ligand's reduction reaction rate. Thermal generation of auto-oxidation during extended incubation period at increased temperature is involved in this method. The reducing ability of an antioxidant is direct estimated(p. 34). No reducing substance was found in the negative controls indicated by the absence of green colored complex. On the other hand, appearance of a green colored complex on both extracts after heating suggested the presence of reducing compounds. The higher reducing capacity of the ethanolic extract agrees with the result of the study conducted by Philip et al., 2011).

The electron donating capacity reflects the reducing power of bioactive compounds and is related to the antioxidant activity. Antioxidants can be considered as reductants. Redox reactions can be described as inactivation of oxidants by reductants. The presence of reductants, for instance, antioxidant substances in the samples, results to the reduction of the phosphomolybdenum


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complex. The reducing activity of the extracts escalated with increasing concentration, suggesting that the electron donating capacity of both extracts is concentration dependent (Aliyu et al., 2013; Phatak & Hendre, 2014).

The reducing capacity of antioxidant components is associated with their total phenolic content. A close correlation between phenolic content and antioxidant activity was evident in the study conducted by Philip et al. (2011). The plant extracts with higher levels of such compounds demonstrated greater reducing power (Sultana, Anwar & Ashraf, 2009).

## CONCLUSION

Crude ethanolic and aqueous leaf extracts of S. trifasciata both possess significant antioxidant activities in the form of reducing power and free radical scavenging activity. This can be attributed to their phenolic content. Ethanolic extract contained greater phenolic compounds than aqueous extract. Phenolics are frequently extracted in higher amounts in more polar solvents like aqueous methanol/ethanol as well as acetone. Likewise, ethanolic extract showed higher reducing activity with increasing concentrations of the extract in a dose-dependent manner than the aqueous extract. This could be attributed to its higher phenolic content. Phenol compounds can donate electrons and inactivate oxidants through reduction. The reducing activity of the ethanolic extract has been found to be steady at increasing concentrations. However, the reducing activity of aqueous extract has been found to increase at higher concentrations. This study proves the direct relationship between phenolic content and reducing capacity. Meanwhile, the aqueous extract demonstrated higher free radical scavenging activity with increasing concentrations of the extract in a dose-dependent manner than the ethanolic extract. The active antioxidant compound in the aqueous extract may have stabilized the DPPH radical better while the active compounds in the ethanolic extract may have reduced the phosphomolybdenum molecule better.

## RECOMMENDATIONS

Other in vitro methods are recommended to be employed in assessing the free radical scavenging activity and reducing capacity of S. trifasciata. Other solvents for extraction can also be used. Highly specific and advanced analytical method for the determination of polyphenols should be employed. More detailed in vivo studies can be conducted to establish the safety and


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bioavailability of the plant. Other part of the plant, such as the rhizome can also be tested for its antioxidant properties. Also, quantification of the flavonoid content should be considered. Furthermore, separation and identification of the specific bioactive compounds responsible for the antioxidant activity can be done.

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