INTRODUCTION

Herbal plants have been widely used to treat certain diseases since the ancient era. In Malaysia, hundreds of plants are reported to exhibit medicinal properties and are yet to be discovered and commercialized. Plant contains numerous nutritional contents and produce primary and secondary metabolites or phytochemical constituents, such as tannins, phytosterols, and flavonoids that are essential for their physiological function. These metabolites are beneficial to human health and prevent disease development (Kumar et al., 2014).

Neptunia oleracea (N. oleracea) also known as water mimosa is an aquatic legume belonging to the family Fabaceae. This plant is well-adapted to its watery environment. It possesses a buoyant, spongy structure in white, aiding its flotation and stability in water. It is characterized by an astringent flavor and distinctive scent. The leaves exhibit an abrupt bipinnate arrangement with stipules that are obliquely ovate-cordate. The flowers, on the other hand, are diminutive, sessile, arranged in oblong clusters, and have smooth surfaces (Deb et al., 2013). It thrives in a variety of freshwater habitats, particularly in areas with slow-moving or stagnant water, and is usually found in rivers, swamps, ponds, lakes, and shallow streams (Atabaki et al., 2020). It is native to the tropics of both hemispheres and has been used by indigenous people as a traditional medicinal plant to treat tongue sores, epileptic convulsions, bloody diarrhea, jaundice, and uterine infections.

Neptunia oleracea also known as water mimosa is widely consumed by people worldwide including Malaysia. This plant was claimed able to treat several diseases such as epileptic convulsion, syphilis, jaundice, and bloody diarrhea. Moreover, it has been reported to possess antimicrobial, antioxidant, and anti-ulcer properties that benefit human health. Hence, this study investigates the content of macronutrients, micronutrients, phytochemical constituents, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant properties in the aqueous leaf extract of this plant. The study found that N. oleracea leaves were possessed with high fiber (52%), moisture (23.33%), fat (5.33%), protein (5.25%), carbohydrate (6.42%), ash (7.67%), and mineral content with a sequence of Potassium > Calcium > Sodium > Phosphorus. A positive result of tannins, steroids, phenols, and flavonoids were detected. High values of TPC and TFC which were 136.19 ± 0.62 mg GAE/g and 829.17 ± 19.09 mg GAE/g of dry weight respectively were obtained. Meanwhile, the hydrogen peroxide scavenging activity of the plant extract produced an IC50 of 70.09 µg/mL. The presence of a high content of polyphenolic compounds may reflect the antioxidant properties of this plant. In conclusion, the aqueous extract of N. oleracea leaves showed the presence of various phytochemical constituents and has a high content of TPC and TFC which may be derived from its nutritional content. Hence, consumption of N. oleracea leaves is useful for human health and disease prevention.

Key words: Antioxidant, macronutrient, micronutrient, phytochemical constituents
Neptunia oleracea is widely consumed as a vegetable dish such as in the form of soup in Malaysia and Thailand. Several studies have been reported on the benefits of N. oleracea extract. A study conducted by Lee et al. (2016) claimed that this plant extract exhibits strong antioxidant properties and the ability to inhibit α-glucosidase, potentially offering benefits for individuals with diabetes. Furthermore, the methanolic extract of N. oleracea possesses anticancer effects by inducing cell death in cancer cells via several pathways such as increasing the cleavage of poly ADP ribose polymerase (PARP) for cell apoptosis, reducing cellular Myc proto-oncogene levels and downregulating the abnormal extracellular signal-regulated kinase (ERK) signaling pathway in tumors (Bhumireddy et al., 2020). The ethanolic extract of N. oleracea demonstrates a notable decrease in gastric juice production and free- and total acidity. This ulcer-inhibiting effect may be attributed to the presence of flavonoids, the active constituents responsible for their anti-ulcer properties (Bhoomannavar et al., 2011). Additionally, its leaf extract using petroleum ether, chloroform, and methanol was found to possess antimicrobial activity by showing a few zones of inhibition in diameter against H. pylori (Uyub et al., 2010). Although many studies have been conducted on this plant, there is limited knowledge on the benefits of this plant when boiled in water and eaten as soup to human health such as in aqueous extract. Furthermore, there is still a lack of studies on the nutritional analysis and phytochemical screening of aqueous extracts of N. oleracea leaves. Hence, this study aimed to conduct a nutritional analysis, preliminary phytochemical screening, and antioxidant analysis on the aqueous extract of N. oleracea leaves. The findings of this study could benefit local people’s consumption of this plant.

MATERIALS AND METHODS

Materials

Collection and identification of plants

Eight kilogram (8) kg of fresh N. oleracea plants were purchased from Kasih Herb Nursery, Perlis, Malaysia. The plant authentication voucher number is MFI 0023/18, identified by the Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia (UPM).

Preparation of plant

Neptunia oleracea plants were washed with tap water. The separated leaves were stored in a freezer at -20 °C for two days and then freeze-dried for 49 hr at 0.38 mbar pressure. The leaves were ground roughly using a blender and freeze-dried for 72 hr until they became dried. The leaves were ground into a fine powder. The powders obtained were then weighed, recorded, and kept in airtight bottles at room temperature for future use.

Macronutrient and micronutrient analysis

All macronutrient and micronutrient analyses were performed according to the standard method of the Association of Official Agriculture Chemists 2000 (AOAC, 2016).

Moisture content

The empty petri dish was dried in the oven for 3 hr at 105 °C. Then, it was cooled down and weighed. 3 grams of sample was added to the dish and placed in an oven for 3 hours at 105 °C. The moisture content was then calculated using Equation 1.

\[
\text{Moisture content} = \frac{w_1 - w_2}{w_1} \times 100
\]

Equation 1

\(w_1\) is the weight of the samples before drying; \(w_2\) is the weight of the samples after drying.

Crude fat content

3 grams of sample was placed into a self-prepared thimble using 3 layers of filter paper. A flat bottom flask was weighed and a Soxhlet apparatus was set up. The thimble was placed into a Soxhlet flask, hexane was added and the flask was run for 6 hr. The thimble was then raised out and the remaining hexane was distilled until the hexane dried. The flask was placed in an oven for 30 min, reweighed and the crude fat was calculated using Equation 2.

\[
\text{Crude fat} = \frac{w_1 - w_2}{\text{weight of sample}} \times 100
\]

Equation 2

\(w_1\) is the weight of beaker; \(w_2\) is the weight of the beaker + fat.
Crude fiber content
3 grams of sample was boiled with 0.13 M sulphuric acid and 0.23 M potassium hydroxide solution. The residue from the boiled solution was filtered and washed before being transferred into a weighed crucible. The sample was placed into an oven for 24 hr and then into mulled furnace at 500 °C. The crucible was reweighed, and the crude fiber content was calculated using Equation 3.

\[
\text{Crude fiber} = \frac{w_1 - w_2}{\text{weight of sample}} \times 100
\]  
Equation 3

\(w_1\) is the weight of crucible + samples before ashing, \(w_2\) is the weight of crucible after ashing

Ash content
The Crucible was placed overnight in the furnace at 550°C. 3 grams of samples were placed into it before being heated over a low Bunsen flame with the lid half covered until no longer fumes were produced. The crucible with lid was placed in the furnace at 500°C for 8 hr/day for 3 days and then into the desiccator. The crucible was reweighed, and the ash content was calculated using Equation 4.

\[
\text{Ash content} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100
\]  
Equation 4

Crude protein content
The protein content was determined based on the Kjeldahl Method with slight modification in the amount of sample used (Saez-Plaza et al., 2013). Approximately 1 gram of sample was mixed with 30 mL of concentrated sulphuric acid. Then, one tablet of Kjeldahl catalyst was added and swirled. Then 20 mL of distilled water was added to the flask and swirled. Distillation started with the addition of 10 mL of diluted sample followed by 50 mL of 40% sodium hydroxide and 30 mL of 0.1 M hydrochloric acid was poured into the collection disk and placed with a distillation apparatus. The distillation process ended once the distilled solution was collected up to 100 mL. Then methyl red indicator was added to the collection flask while 0.1 M sodium hydroxide was added to the titrant in a burette. Titration ended when the pink solution turned yellow indicating that all acid was neutralized. The volume of sodium hydroxide used was recorded, and the protein content was calculated. The crude protein content was calculated using Equation 5.

\[
\text{Crude protein} = (\% \text{ of N}) = \frac{\text{g of N}}{\text{g of sample}} \times 100 = \% \text{ of N} \times 6.25
\]  
Equation 5

\(N\) is nitrogen, and 6.25 is the conversion from nitrogen to protein

Crude carbohydrate content
Determination of carbohydrate content was measured based on Pearson (1976). The carbohydrate content was calculated using Equation 6.

\[
\text{Carbohydrate content} = 100 - (\% \text{ Ash + % Moisture + Crude protein + % Crude fat + % Fiber})
\]  
Equation 6

Micronutrient analysis
50 grams of sample powder was outsourced to BP Food Environmental Testing, Gemmaries, Shah Alam, Selangor for analysis of the presence of potassium, calcium, phosphorus, and sodium. The sample was analyzed using the AOAC Official Method 985.35 atomic absorption spectrophotometric method.

Aqueous plant extract
The aqueous extraction was performed according to Al-Manhel & Kareem, (2015). 50 grams of leaf powder was boiled in 500 mL of hot water for thirty min. The mixture was then poured into a conical flask and left for 24 hr. The extract collected was then filtered twice using muslin cloth transferred into a vial and stored at 5 °C for two days. The extract was then lyophilized at a pressure of 0.33 mbar for 72 hr to remove the solvent and form a dried powder. The percentage yield of extracts was calculated using Equation 7.
Percentage yield of extract = quantity of extraction (g)/quantity of powder used × 100 — Equation 7

**Phytochemical screening**

**Tannins**
Approximately 0.5 g of *N. oleracea* extract was added to 10 mL of distilled water. Then, a few drops of 1% ferric chloride solution were added to the filtrate. The occurrence of a black or blue-green precipitate indicates a positive test for the presence of tannins (Iqbal *et al*., 2015).

**Steroids**
Approximately 0.5 g of *N. oleracea* extract was dissolved in 3 mL of chloroform. A few drops of concentrated sulphuric acid were added slowly to the filtrate to form a lower layer. The formation of a reddish-brown color ring at the interface was taken as a positive steroid test (Aiyegoro & Alkoh, 2010).

**Alkaloids**
Approximately 0.1 g of *N. oleracea* extract was dissolved in 5 mL of methanol and filtered. Then, 1% of 5 mL hydrochloric acid was mixed with 2 mL of the filtrate in the test tube. A few drops of Mayer’s reagent were added, and the appearance of a brown-colored precipitate indicated the presence of alkaloids (Gacem *et al*., 2019).

**Phenols**
Approximately 0.1 g of *N. oleracea* extract was dissolved in 1 mL of distilled water. The mixture was then added with 0.6 mL of 1% ferric chloride. The appearance of a bluish-green color confirms the presence of phenols (Al-Manhel & Kareem, 2015).

**Flavonoids**
Approximately 0.2 g of *N. oleracea* extract was dissolved in 2 mL of methanol and heated for one minute. Approximately 1 g of magnesium metal powder was added to the mixture followed by the addition of a few drops of concentrated hydrochloric acid (HCl). The formation of orange-to-red coloration indicates the presence of flavonoids (Gacem *et al*., 2019).

**Total Phenolic Content (TPC)**
A concentration of 1 mg/mL of extract was prepared in a test tube. 1 mL of Folin-Ciocalteu phenol reagent was added to the extract, mixed well, and left at room temperature for 5 min. Then, 10 mL of 7.5% sodium carbonate solution was added to the extract and incubated for 45 min at room temperature. The absorbance was measured against a blank sample of distilled water at 760 nm using a UV-visible spectrophotometer. The calibration curve was plotted using standard gallic acid. TPC of aqueous extract of *N. oleracea* leaves was expressed as mg gallic acid equivalents (GAE) per gram. The data are presented as the mean ± standard deviation (SD).

**Total Flavonoid Content (TFC)**
Standard solutions of gallic acid were prepared. A concentration of 1 mg/mL of extract and 1 mL of gallic acid solution in the test tubes were mixed with 3 mL of methanol and 0.2 mL of 10% aluminum chloride (AlCl₃). Then, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water were added. The mixture was incubated at room temperature for 30 min. The absorbance of the mixture was measured against a blank sample of distilled water at 420 nm with a UV-visible spectrophotometer. The calibration curve was plotted using standard gallic acid. The TFC of the aqueous extract of *N. oleracea* leaves was expressed as mg gallic acid equivalents (GAE) of each gram of dry material. The data are presented as the mean ± standard deviation (SD).

**Hydrogen peroxide scavenging activity**
Ascorbic acid (100-500 µg/mL) was used as a standard solution. A 40 mM hydrogen peroxide (H₂O₂) solution (0.45 mL of 30% hydrogen peroxide solution) was prepared in 100 mL of phosphate buffer at pH 7.4. Ascending concentrations of extracts (200, 400, 600, 800, & 1000 µg/mL) were prepared by mixing with distilled water. Then, 0.6 mL of 40 mM H₂O₂ solution was added to each test tube of extract and incubated for 10 min at room temperature. The absorbance of the mixture of hydrogen peroxide with the standard solution and extracts was measured against a blank solution containing phosphate buffer at 230 nm with a UV-visible spectrophotometer. The percentage scavenging of H₂O₂ was calculated by using equation 8. The graph of H₂O₂ scavenging activity was plotted using ascorbic acid as a standard.
percentage scavenging of $\text{H}_2\text{O}_2 = \frac{A_i - A_t}{A_i} \times 100$ – Equation 8

$A_i$ is the absorbance of the control, and $A_t$ is the absorbance of the test.

RESULTS

Macronutrient and micronutrient analysis

The results are shown in Table 1. *N. oleracea* contains 52% fiber, followed by moisture content (23.33%), ash (7.67%), carbohydrate (6.42%), crude fat (5.33%), and crude protein (5.25%). Meanwhile, Table 2 shows the micronutrient content of *N. oleracea* with Potassium (K) as the highest micronutrient measured followed by calcium (Ca), sodium (Na), and phosphorus (P).

Table 1. Summary of macronutrient analysis in 3 grams of *N. oleracea* leaves

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>23.33</td>
</tr>
<tr>
<td>Crude fat</td>
<td>5.33</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>52.00</td>
</tr>
<tr>
<td>Ash</td>
<td>7.67</td>
</tr>
<tr>
<td>Crude protein</td>
<td>5.25</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>6.42</td>
</tr>
</tbody>
</table>

Table 2. Summary of micronutrient analysis of *N. oleracea* leaves

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>61.11</td>
</tr>
<tr>
<td>Calcium</td>
<td>33.03</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.96</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Yield extract

The yield of dry powder *N. oleracea* aqueous extract using the decoction method was 4.17 g with a percentage yield of 8.34% as shown in Table 2.

Table 3. Percentage yield of *N. oleracea* leaf extract.

<table>
<thead>
<tr>
<th>Extraction yield (g)</th>
<th>Percentage yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.17</td>
<td>8.34</td>
</tr>
</tbody>
</table>

Phytochemical screening

Table 3 shows a summary of the phytochemical screening conducted on *N. oleracea* aqueous leaf extract. All phytochemicals were present except alkaloids.

Table 4. Summary of the phytochemical analysis of *N. oleracea* leaves

<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = present, - = not present

TPC

The amount of total phenolic content in the aqueous extract of *N. oleracea* leaves was 136.19 ± 0.62 mg GAE/g of dry material as shown in Table 5.

Table 5. Results of TPC in aqueous extract of *N. oleracea* leaves

<table>
<thead>
<tr>
<th>Concentration</th>
<th>TPC (mg GAE/g of dry material)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract, 1 mg/mL</td>
<td>136.19 ± 0.62</td>
</tr>
</tbody>
</table>
The amount of total flavonoid content in the aqueous extract of N. oleracea leaves was 829.17 ± 19.09 mg GAE/g of dry material as shown in Table 6.

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>TFC (mg GAE/g of dry material)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/mL</td>
<td>829.17 ± 19.09</td>
</tr>
</tbody>
</table>

Hydrogen peroxide scavenging activity
The percentage of H$_2$O$_2$ scavenging activity for each concentration is shown in Table 7. The comparison of the IC$_{50}$ values of the aqueous extract of N. oleracea leaves and ascorbic acid is shown in Table 7.

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Percentage of H$_2$O$_2$ scavenging activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>95.04</td>
</tr>
<tr>
<td>400</td>
<td>95.09</td>
</tr>
<tr>
<td>600</td>
<td>95.17</td>
</tr>
<tr>
<td>800</td>
<td>95.26</td>
</tr>
<tr>
<td>1000</td>
<td>95.31</td>
</tr>
</tbody>
</table>

Table 8. Comparison of IC$_{50}$ values of the standard and N. oleracea extract

<table>
<thead>
<tr>
<th>IC$_{50}$ of ascorbic acid</th>
<th>IC$_{50}$ of N. oleracea extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.07 µg/mL</td>
<td>70.09 µg/mL</td>
</tr>
</tbody>
</table>

DISCUSSION
Plants provide essential elements for human nutrition and play a crucial role in maintaining human health. Hence, the identification of macro- and micronutrients in a plant could explore their medicinal benefits to human health. This study found that N. oleracea is a reliable source of dietary fiber. From the macronutrient analysis, it contains more than 50% of crude fiber. Plant-derived dietary fibers are known to have many beneficial effects in improving human health by acting as antioxidants and can attenuate blood cholesterol, blood glucose, and body weight due to their physiological properties (Merenkova et al., 2020). It is also a reliable source of protein due to its nitrogen-organic compound-complex. In comparison with Saupi et al. (2015), the moisture and crude protein content of the aqueous extract of N. oleracea leaves were found higher in this study. Differences in the time of harvesting and geographical location influence the macro- and micronutrient contents of a plant (Zhou et al., 2015). Moreover, differences in method use influence the amount of macro- and micronutrients measured. A study reported that the freeze-drying method can protect against water loss and protein degradation in samples compared to the air-drying method. According to Roshanak et al. (2015), the freeze-drying method maintained the physical and nutritional characteristics of the compounds in the samples. Hence, a high yield of crude fat, protein, and carbohydrate content of N. oleracea was obtained in this study. The micronutrient content of N. oleracea leaves showed a sequence of Potassium (K)> Calcium (Ca)> Sodium (Na)> Phosphorus (P). This micronutrient is needed for plant metabolism and human health. Mills et al. (2020) stated that low Na intake and increased K in the diet are recommended to reverse blood pressure levels and improve hypertension. Hence, this finding showed that the consumption of N. oleracea can be recommended to prevent hypertension.

In this study, the extraction yield of the aqueous decoction extract was lower than that of the maceration technique conducted by Mahadi et al. (2020). Moreover, the study reported that methanolic extracts of N. oleracea produced a higher percentage yield compared to aqueous extracts. Zhang et al. (2018), claimed that maceration is an easy and simple extraction method, but it has disadvantages like low extraction efficacy. This could be the reason for the low yield of extract collected. Choosing the suitable extraction method and selecting of solvent used are crucial in measuring, determining, and identifying the phytochemical compounds in plants (Dhanani et al., 2017). The preliminary phytochemical screening of the aqueous extract of N. oleracea leaves revealed a positive result for tannins, steroids, phenols, and flavonoids. Alkaloids were not detected in the extract. The absence of alkaloids in the aqueous extract may be due to its poor solubility in aqueous solvent. However, this finding contrasted with Agidew (2022) and Tongco et al. (2015) where alkaloids were detected in aqueous instead of ethanol extracts. Tannins, phenols, and flavonoids claimed to possess antitumor and anticancer properties while steroids were reported to possess allergic, anticancer, anti-inflammatory, antimicrobial, and antinociceptive activities (Mangoale & Afolayan, 2020; Marahatba et al., 2021). Polyphenolics in plants may indicate their ability to scavenge free radicals. Further study was
then carried out to investigate the amount of phenolic and flavonoid compounds present in this plant.

In this study, the mean value of TPC of \textit{N. oleracea} leaves was higher compared to the previous finding conducted by Areekul and Phomkaivon (2015). The variations in the TPC value may be related to differences in geographical regions and the drying method used. Areekul and Phomkaivon (2015) used the oven drying method in their study. According to Azwanida (2015), freeze-drying was found to produce a higher yield of phenolic contents, which was in line with the findings by Lee \textit{et al.} (2016). Meanwhile, the TFC value obtained was higher than that of the aqueous extract of \textit{Helichrysum longifolium} leaves although a similar standard solution was used (Olayinka & Anthony, 2010). A study done by de Andrade \textit{et al.} (2014) using quercetin as a standard solution reported that an aqueous decoction of \textit{Smallanthus sonchifolius} leaves resulted in a TFC yield of 39.72 - 1.37 mg QE/g dry material. The variations in TFC values are due to the different standards used. In addition, the freeze-drying method was reported to inhibit polyphenol oxidase which subsequently prevents the oxidation of flavonoids and therefore contributes to a higher TPC yield (Chumroenphat \textit{et al.}, 2021).

Hydrogen peroxide (H$_2$O$_2$) is a toxic by-product of oxygen metabolism in plants and animals. It can easily cross the cell membrane and react with Fe$^{2+}$ and Cu$^{2+}$ ions to form hydroxyl radicals, which lead to toxic effects. Hence, antioxidant compounds discovered in the plants such as phenols, flavanols, and flavonoids were found to protect living cells from being damaged by radicals in the range of 10–100 µM (Patel, 2015). \textit{N. oleracea} leaf extract demonstrated H$_2$O$_2$ scavenging activity in a concentration-dependent manner. The study’s finding is similar to that of Mohan \textit{et al.} (2012), on aqueous extract of Psidium guajava leaves. This may be due to the high amount of TPC and TFC obtained. Furthermore, Lee \textit{et al.} (2014) claimed that \textit{N. oleracea} has good antioxidant activity as revealed by the DPPH assay. The methanolic extract of \textit{N. oleracea} leaves showed the most potent scavenging activity compared to four selected medicinal plants, \textit{Mitragyna speciosa}, \textit{Clinacanthus nutans}, \textit{Strobilanthes crispus}, and \textit{Mentha asiatica} with IC$_{50}$ values of 35.45 mg/mL. The lower IC$_{50}$ value was claimed to produce a greater free radical scavenging capability of the plant needed to produce 50% inhibition of free radicals and was correlated with the high TPC with an R-value of 78.9%. This is also in line with the study done by Hijazi \textit{et al.} (2012), in which a low total phenolic content may result in a higher IC$_{50}$ value compared to the standard.

**CONCLUSION**
This study discovered that the aqueous extract of \textit{N. oleracea} leaves produced various amounts of macro- and micronutrients, and phytochemical constituents and exhibited high contents of phenols and flavonoids. Phytochemical screening of \textit{N. oleracea} revealed the presence of phenols, flavonoids, tannins, and steroids that may contribute to its antioxidant activities. Identification of the correlation of polyphenolic compounds with antioxidant properties of \textit{N. oleracea} could be interesting to explore in the future.

**ACKNOWLEDGEMENTS**
The authors would like to acknowledge the University of Cyberjaya for financial support, laboratory staff, and all individuals who were directly or indirectly involved in this research for their kind assistance.

**ETHICAL STATEMENT**
Not applicable.

**CONFLICT OF INTEREST**
The authors declare no conflicts of interest.

**REFERENCES**
Azwanida. 2015. A review on the extraction methods uses in medicinal plants, principle, strength and


