

Research

Proximate Nutritional Analysis, Phytochemical Screening and Antioxidant Properties of Aqueous Extract of *Neptunia oleracea* Leaves

Siti Syafinaz Kamaruzzaman¹, Che Ku Afina Haida Che Ku Mazuan¹, Shamima Abdul Rahman¹, Imtiaz Khalid Mohamed¹, Zolkapli Eshak² and Mahani Mahadi^{1*}

1. Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Cyberjaya, Malaysia
 2. Department of Pharmacology, Faculty of Pharmacy, University of Technology MARA (UiTM), Malaysia
- *Corresponding author: mahani@cyberjaya.edu.my

ABSTRACT

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

Article History

Accepted: 2 November 2023

First version online: 30 December 2023

Cite This Article:

Kamaruzzaman, S.S., Che Ku Mazuan, C.K.A.H., Abdul Rahman, S. & Khalid Mohamed, I., Eshak, Z. & Mahadi, M. 2023. Proximate nutritional analysis, phytochemical screening and antioxidant properties of aqueous extract of *Neptunia oleracea* leaves. Malaysian Applied Biology, 52(6): 91-99. <https://doi.org/10.55230/mabjournal.v52i6.2696>

Copyright

© 2023 Malaysian Society of Applied Biology

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

(Sagolshemcha & Singh, 2017). *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.

$$\text{Moisture content} = \frac{w_1 - w_2}{w_1} \times 100 \quad - \text{Equation 1}$$

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.

$$\text{Crude fat} = \frac{w_1 - w_2}{\text{weight of sample}} \times 100 \quad - \text{Equation 2}$$

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 muller furnace at 500 °C. The crucible was reweighed, and the crude fiber content was calculated using Equation 3.

$$\text{Crude fiber} = w_1 - w_2 / \text{weight of sample} \times 100 \quad - \quad \text{Equation 3}$$

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.

$$\text{Ash content} = \text{weight of ash} / \text{weight of sample} \times 100 \quad - \quad \text{Equation 4}$$

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.

$$\begin{aligned} \text{Crude protein} = (\% \text{ of N}) &= \text{g of N} / \text{g of sample} \times 100 \quad - \quad \text{Equation 5} \\ &= \% \text{ of N} \times 6.25 \end{aligned}$$

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.

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

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 – Equation 7
× 100

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 standard solutions 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 $H_2O_2 = A_i - A_t/A_i \times 100$ – 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

Analysis	Percentage (%)
Moisture	23.33
Crude fat	5.33
Crude fibre	52.00
Ash	7.67
Crude protein	5.25
Carbohydrate	6.42

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

Analysis	Amount (mg)
Potassium	61.11
Calcium	33.03
Sodium	0.96
Phosphorus	0.006

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.

Extraction yield (g)	Percentage yield (%)
4.17	8.34

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

Test	Results
Steroids	+
Tannins	+
Alkaloids	-
Phenols	+
Flavonoids	+

+ = 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

Concentration	TPC (mg GAE/g of dry material)
Aqueous extract, 1 mg/mL	136.19 ± 0.62

TFC

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 6. Results of TFC in aqueous extract of *N. oleracea* leaves

Concentration	TFC (mg GAE/g of dry material)
1 mg/mL	829.17 ± 19.09

Hydrogen peroxide scavenging activity

The percentage of H₂O₂ scavenging activity for each concentration is shown in Table 7. The comparison of the IC₅₀ values of the aqueous extract of *N. oleracea* leaves and ascorbic acid is shown in Table 7.

Table 7. Percentage of H₂O₂ scavenging activity of the aqueous extract of *N. oleracea* leaves

Concentration (µg/mL)	Percentage of H ₂ O ₂ scavenging activity (%)
200	95.04
400	95.09
600	95.17
800	95.26
1000	95.31

Table 8. Comparison of IC₅₀ values of the standard and *N. oleracea* extract

IC ₅₀ of ascorbic acid	IC ₅₀ of <i>N. oleracea</i> extract
34.07 µg/mL	70.09 µg/mL

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 antiulcer and anticancer properties while steroids were reported to possess anti-allergic, anticancer, antidiabetic, antimalarial, 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 *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 *et al.* (2016). Meanwhile, the TFC value obtained was higher than that of the aqueous extract of *Helichrysum longifolium* leaves although a similar standard solution was used (Olayinka & Anthony, 2010). A study done by de Andrade *et al.* (2014) using quercetin as a standard solution reported that an aqueous decoction of *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 *et al.*, 2021).

Hydrogen peroxide (H₂O₂) is a toxic by-product of oxygen metabolism in plants and animals. It can easily cross the cell membrane and react with Fe²⁺ and Cu²⁺ 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). *N. oleracea* leaf extract demonstrated H₂O₂ scavenging activity in a concentration-dependent manner. The study's finding is similar to that of Mohan *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 *et al.* (2014) claimed that *N. oleracea* has good antioxidant activity as revealed by the DPPH assay. The methanolic extract of *N. oleracea* leaves showed the most potent scavenging activity compared to four selected medicinal plants, *Mitragyna speciosa*, *Clinacanthus nutans*, *Strobilanthes crispus*, and *Mentha asiatica* with IC₅₀ values of 35.45 mg/mL. The lower IC₅₀ 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 *et al.* (2012), in which a low total phenolic content may result in a higher IC₅₀ value compared to the standard.

CONCLUSION

This study discovered that the aqueous extract of *N. oleracea* leaves produced various amounts of macro- and micronutrients, and phytochemical constituents and exhibited high contents of phenols and flavonoids. Phytochemical screening of *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 *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

- Agidew, M.G. 2022. Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bulletin of National Research Center*, 46: 87-97. <https://doi.org/10.1186/s42269-022-00770-8>
- Al-Manhel, A.J. & Kareem N.A. 2015. Effect of aqueous and alcoholic plant extracts on inhibition of some types of microbes and causing spoilage of food. *Pakistan Journal of Food Sciences and Technologies*, 25(3): 104-109.
- Areekul, V. & Phomkaivon, N. 2015. Thai indigenous plants: Focusing on total phenolic content, antioxidant activity and their correlation on medicinal effects. *KMITL Science and Technology Journal*, 15(1): 1-14.
- Association of Official Agriculture Chemist (AOAC). 2016. *Official Method of Analysis* 925.09, 990.03, 985.35. 20th Ed. AOAC International.
- Atabaki, N., Shaharuddin, N.A., Ahmad, S.A., Nulit, R. & Abiri, R. 2020. Assessment of water mimosa (*Neptunia oleracea* Lour.) morphological, physiological, and removal efficiency for phytoremediation of arsenic-polluted water. *Plants*, 9: 1-24. <https://doi.org/10.3390/plants9111500>
- Azwanida. 2015. A review on the extraction methods uses in medicinal plants, principle, strength and

- limitation. *Medicinal & Aromatic Plants*, 4(03): 1-6.
- Bhoomannavar, V.S., Patil, V.P., Hugar, S., Nanjappaiah, H.M. & Kalyane, N. 2011. Anti-ulcer activity of *Neptunia oleracea* Lour. *Pharmacology Online*, 3: 1015-1020.
- Bhumireddy, A., Nellore, K. & Alapati, K.S. 2020. Anticancer activity of *Neptunia oleracea* methanolic extracts. *Natural Product Research*, 36(4): 1053-1057. <https://doi.org/10.1080/14786419.2020.1844693>
- Chumroenphat, T., Somboonwatthanakul, I., Saensouk, S. & Siriamornpun, S. 2021. Changes in curcuminoids and chemical components of turmeric (*Curcuma longa* L.) under freeze-drying and low-temperature drying methods. *Food Chemistry*, 339: 128121. <https://doi.org/10.1016/j.foodchem.2020.128121>
- de Andrade, E.F., de Souza Leone, R., Ellendersen, L.N. & Masson, M.L. 2014. Phenolic profile and antioxidant activity of extracts of leaves and flowers of yacon (*Smallanthus sonchifolius*). *Industrial Crops and Products*, 62: 499-506. <https://doi.org/10.1016/j.indcrop.2014.09.025>
- Deb, P.K., Das, S., Nath Bhaumik, K., Ghosh, R., Ghosh, T.K., Bhakta, T. & Bhaumik, N. 2013. Pharmacognostic & preliminary phytochemical investigations of *Neptunia prostrata* L. *Journal of Pharmacognosy and Phytochemistry*, 2(3): 5-11.
- Dhanani, T., Shah, S., Gajbhiye, N.A. & Kumar, S. 2017. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry*, 10(Supp 1): 1193-1199. <https://doi.org/10.1016/j.arabjc.2013.02.015>
- Gacem, M.A., Telli, A., Gacem, H. & Ould-El-Hadj-Khelil, A. 2019. Phytochemical screening, antifungal, and antioxidant activities of three medicinal plants from Algerian steppe and Sahara (preliminary screening studies). *SN Applied Sciences*, 1: 1-13. <https://doi.org/10.1007/s42452-019-1797-1>
- Hijazi, A., Hamad, H., Farhan, H., Rammal, H., Hijazi, A., Daher, A., Reda, M. & Badran, B. 2012. In vitro antioxidant activity of ethanolic and aqueous extracts from crude *Malva parviflora* L. grown in Lebanon. *Asian Journal of Pharmaceutical and Clinical Research*, 5(3): 234-238.
- Iqbal, E., Salim, K. A. & Lim, L.B.L. 2015. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University - Science*, 27(3): 224-232. <https://doi.org/10.1016/j.jksus.2015.02.003>
- Kumar, S.G., Gaur, K., Yogi, A., Joshi, B. & Dashora, A. 2014. Studies on phytochemical constituents of medicinal plants. *American Journal of Pharmacy and Pharmaceutical Sciences*, 1(4): 61-74.
- Lee, S.Y., Abas, F., Khatib, A., Ismail, I.S., Shaari, K. & Zawawi, N. 2016. Metabolite profiling of *Neptunia oleracea* and correlation with antioxidant and α -glucosidase inhibitory activities using ¹H NMR-based metabolomics. *Phytochemistry Letters*, 16: 23-33. <https://doi.org/10.1016/j.phytol.2016.02.014>
- Lee, S.Y., Mediani, A., Ashikin, N., Azliana, A.B.S. & Abas, F. 2014. Antioxidant and α -glucosidase inhibitory activities of the leaf and stem of selected traditional medicinal plants. *International Food Research Journal*, 21(1): 165-172.
- Mahadi, M., Izzah D.Z., Mohamad, F.Z., Rozila, I. & Kamal, N. 2020. Metabolites profiling and antimicrobial activities in roots and leaves of *Neptunia oleracea*. *International Journal of Pharmaceutical Research*, 12(3): 1998-2006. <https://doi.org/10.31838/ijpr/2020.12.03.278>
- Mangoale, R.M. & Afolayan, A.J. 2020. Comparative phytochemical constituents and antioxidant activity of wild and cultivated *Alepidea amatymbica* Eckl & Zeyh. *BioMed Research International*, 2020: 5808624. <https://doi.org/10.1155/2020/5808624>
- Marahatha, R., Gyawali, K., Sharma, K., Gyawali, N., Tandani, P., Adhikari, A., Timilsina, G., Bhattarai, S., Lamichhane, G., Acharya, A., Pathak, I., Devkota, H.P. & Parajuli, N. 2021. Pharmacologic activities of phytosteroids in inflammatory diseases: Mechanism of action and therapeutic potentials. *Phytotherapy Research*, 35(9): 5103-5124. <https://doi.org/10.1002/ptr.7138>
- Merenkova, S., Zinina, O., Stuart, M., Okuskhanova, E. & Androsova, N. 2020. Effects of Dietary Fiber On Human Health: A Review. *Human Sport Medicine*, 20(1): 106-113. <https://doi.org/10.14529/hsm200113>
- Mohan, C., Blamurugan V., Thiripura, S. & Rekha, R. 2012. Metal ion chelating activity and hydrogen peroxide scavenging activity of medicinal plant *Kalanchoe pinnata*. *Journal of Chemical and Pharmaceutical Research*, 4(1): 197-202.
- Olayinka, A. & Anthony, I.O. 2010. Preliminary phytochemical screening and In vitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complementary and Alternative Medicine*, 10: 21. <https://doi.org/10.1186/1472-6882-10-21>
- Patel, S.S. & Savjani, J.K. 2015. Systematic review of plant sterols as potential anti-inflammatory agents: Current status and future perspectives. *The Journal of Phytopharmacology*, 4(2): 121-125. <https://doi.org/10.31254/phyto.2015.4212>
- Roshan, S., Rahimmalek, M., Amir, S. & Goli, H. 2015. Evaluation of seven different drying treatments in respect to total flavonoid, phenolic, vitamin C content, chlorophyll, antioxidant activity and color of green tea (*Camellia sinensis* or *C. assamica*) leaves. *Journal of Food and Science Technology*, 53(1): 721-729. <https://doi.org/10.1007/s13197-015-2030-x>
- Sáez-Plaza, P., María, J.N., Sławomir, W., Tadeusz, M. & Agustín, G.A. 2013. An overview of the Kjeldahl method of nitrogen determination. Part II. Sample preparation, working scale, instrumental

- finish, and quality control. *Critical Reviews in Analytical Chemistry*, 43(4): 224-272. <https://doi.org/10.1080/10408347.2012.751787>
- Sagolshemcha, R. & Singh, R. 2017. Traditional and biological uses of *Neptunia oleracea* Lour: An Overview. *International Journal of Current Research*, 9(6): 51689-51694.
- Saupi, N., Zakaria, M.H., Bujang, J.S. & Arshad, A. 2015. The proximate compositions and mineral contents of *Neptunia oleracea* Loureiro, an aquatic plant from Malaysia. *Emirates Journal of Food and Agriculture*, 27(3): 266-274. <https://doi.org/10.9755/ejfa.v27i3.16876>
- Tongco, J.V.V., Añis, A.D. & Tamayo, J.P. 2015. Nutritional analysis, phytochemical screening, and total phenolic content of *Basella alba* leaves from the Philippines. *International Journal of Pharmacognosy and Phytochemical Research*, 7(5): 1031-1033.
- Uyub, M.A., Ikenna, N., Azlan, A.A. & Shaida F.S. 2010. In-vitro antibacterial activity and cytotoxicity of selected medicinal plant extracts from Penang Island Malaysia on *Helicobacter pylori* and some pathogenic bacteria. *Ethnobotany Research & Applications*, 8: 95-106. <https://doi.org/10.17348/era.8.0.95-106>
- Zhang, Q.W., Lin, L.G. & Ye, W.C. 2018. Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine*, 13(20): 106-113. <https://doi.org/10.1186/s13020-018-0177-x>
- Zhou, A.Y., Robertson, J., Hamid, N., Ma, Q. & Lu, J. 2015. Changes in total nitrogen and amino acid composition of New Zealand *Undaria pinnatifida* with growth, location and plant parts. *Food Chemistry*, 186: 319-325. <https://doi.org/10.1016/j.foodchem.2014.06.016>

