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Proximate analysis and antioxidant activities of four popular Chinese *Lamiaceae* herbs in Malaysia

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Abstract

Chinese herbal plants are known to be among the promising sources of variety of essential nutrients and antioxidant constituents. The objective of this study was to evaluate four Chinese herbs from the family of *Lamiaceae* which are popular in Malaysia; *Plectranthus amboinicus*, *Vitex negundo*, *Salvia miltiorrhiza* and *Scutellaria baicalensis*, on their proximate compositions, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities; DPPH and FRAP assays. Results showed that these herbs were relatively significant different from each other for their proximate compositions, TPC, TFC and antioxidant activities. Apparently, *P. amboinicus* has higher levels of ash (21.46±0.16%) and dietary fibre (41.24±1.25%) while *V. negundo* contained the highest crude fibre (19.41±1.98%) than the rest of the samples. *Salvia miltiorrhiza* consisted of high TPC (105.90±3.37 mg GAE/g), TFC (241.55±55.15 mg QE/g), DPPH radical scavenging activities (IC_{50}: 169.10±14.17 µg/mL) and FRAP (103.09±2.84 µg $FeSO_4$/g). Overall, the imported herb of *S. miltiorrhiza* is a potent natural antioxidant source with extremely low fat content to fight against oxidative stress and reactive oxygen species. More studies on the *Lamiaceae* family plant should be performed to determine more potential bioactivity which is beneficial to human health.

1. Introduction

Oxidative stress mediates a crucial part in the pathogenesis of various diseases for instance cardiovascular diseases, cancers, neurological diseases, respiratory diseases and many others (Farooqui *et al.*, 2016). Due to many factors such as unhealthy lifestyle, diet imbalance and ageing, our body loses its ability to fight the overwhelming free radicals which cause the occurrence of oxidative stress. Free radicals are highly reactive species which would destroy numerous molecules such as deoxyribonucleic acid (DNA), carbohydrates, proteins and lipids in the body. The impairment of the molecules might lead to cell damage and homeostatic disruption which contribute to many related diseases (Singh *et al.*, 2015). The understanding of the role of reactive oxygen species (ROS) in various diseases has raised public awareness of the importance of antioxidant intake in order to inhibit the excessive formation of free radicals and prevent oxidative stress events. Emerging research evidenced that antioxidant compounds donate their electrons to unpair, control autoxidation and reduce the detrimental effects of free radicals (Sukweenadhi *et al.*, 2020). Thus, this may help to lower the risk of getting health issues.

Herbs are known to be one of the promising sources of antioxidants as some of their phytochemicals contain natural bioactive components which potentially act as a protective agent against many diseases. Apparently, herbs with high phenolic compounds exhibit strong antioxidant properties which assist in scavenging excessive ROS thus preventing oxidative stress (Sallehuddin *et al.*, 2020; Embuscado, 2015). *Plectranthus amboinicus*, *Vitex negundo*, *Salvia miltiorrhiza* and *Scutellaria baicalensis* are common Chinese herbs that come from the family of *Lamiaceae* which are widely consumed by Malaysians. *P. amboinicus* and *V. negundo* are simply found in the climate tropical regions such as Malaysia, Indonesia and Thailand while *S. baicalensis* and *S. miltiorrhiza* can only be grown in the Northern hemisphere such as in the United States, China, and Russia, therefore they are pronounced as the imported herbs and only be bought

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from the market.

Plectranthus amboinicus which is known as 'bangun -bangun (arise)' in Malaysia is a local raw salad and has been used to treat coughs, sore throats, nasal congestion and insect bites traditionally (Ashaari *et al.*, 2020). The leaves of *P. amboinicus* were proved to have a high content of carvacrol and thymol which help in the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and hydroxyl radical formation (Arumugam *et al.*, 2016). Meanwhile, *V. negundo* or 'Legundi' was traditionally used to treat eye disease, toothache, inflammation, catarrhal fever, leukoderma, rheumatoid, arthritis, spleen enlargement, gonorrhoea and bronchitis (Tiwari and Tripathi, 2007). The *V. negundo* leaves are usually used as a natural colouring additive in a confinement healthy dish namely Nasi Lemuni in Malaysia. The leaves contain many beneficial phytochemical compounds, such as phenolic acid, flavonoid, alkaloids, terpenoids and glycosidic iridoids (Salleh *et al.*, 2014; Saklani *et al.*, 2017).

Salvia miltiorrhiza is known as Danshen in China. It is mainly used to treat cardiovascular diseases, cerebrovascular diseases, neurodegenerative diseases, blood circulation disorders, liver fibrosis and cancers (Wang, 2010). *Salvia miltiorrhiza* is composed of a high level of phenolic acid; salvianolic acid B and rosmarinic acid, the main active compounds that exhibit strong antioxidant activities and free radical scavenging effects which can be found in its roots and leaves (Zhang *et al.*, 2010). Another imported Chinese herb, *S. baicalensis* is commonly known as baical skullcap, huang qin, or huang lian. In China, it is popular to treat diarrhoea, dysentery, hypertension, wound, insomnia, inflammation and respiratory infections (Zhao *et al.*, 2019). Flavonoid compounds, for example, Baicalin, baicalein, wogonoside and wogonin which can be found in the roots of *S. baicalensis* are the main active compounds in various pharmacological activities, including anti-oxidation. The compounds are shown to have protective effects against neurodegenerative diseases which are tightly related to oxidative stress (Gaire *et al.*, 2014). The objective of the study is to evaluate and compare the proximate constituents, total flavonoid and phenolic contents and antioxidant properties of the local and imported Chinese herbs from the same family of *Lamiaceae* which are commonly used in Malaysia. Such information can be a benchmark for other species from the *Lamiaceae* family or Chinese herbs in regard to the nutritional compounds and the bioactivity of the plants.

2. Materials and methods

2.1 Collection and preparation of samples

The local herbs, *P. amboinicus* and *V. negundo* were freshly bought from a nursery in Kelantan, Malaysia while the imported herbs, *S. miltiorrhiza* and *S. baicalensis*, in the dried sample, were purchased from an online herbal store. Samples were washed using distilled water and were freeze-dried (Ilshin BioBase, Korea) at -40˚C under a vacuum for 3 days. Then, the freeze-dried samples were grounded using a blender (Waring Laboratory, US) to obtain a homogenized sample. The finely grounded samples were stored in an air-tight container to keep humidity out and kept in the freezer for future usage.

2.2 Nutritional compositions

The proximate analysis was performed to evaluate the nutritional compositions of the samples. The method was according to the standard protocols of the Association of Official Analytical Chemists (AOAC, 1984) as the following: moisture using the air-oven method, ash by dry-ashing method, crude fat by Soxhlet extraction method, protein by Kjeldahl method, crude fibre by Weende Method, dietary fibre by enzymatic gravimetric method and carbohydrate was calculated by the difference of moisture, ash, fat and protein values.

2.3 Total phenolic and total flavonoid content

Prior to TPC, TFC, DPPH and FRAP assays, the samples were freshly prepared in solution by adding 10 mg sample into 10 mL distilled water (dH_2O) and stirred for 3 hrs before being filtered. The filtrate was considered as 10 mg/mL of the respective herbs before being subjected to particular assays.

The Folin-Ciocalteau method was used to determine the TPC of the herb extracts according to Moon and Cha (2020) with some modifications. The 10 µL of sample extract was added to a 96-well plate. Then, 75 µL of 10% Folin-Ciocalteau reagent was mixed with the extract and incubated for 5 mins at room temperature. After that, 75 µL of 700 mM sodium carbonate (NA_2CO_3) was added to each well. The plate was covered and kept in the dark for 2 hrs. The absorbance was measured at 765 nm using a spectrophotometric microplate reader (Thermo Scientific, USA). Gallic acid was used as a standard to construct a calibration curve. The total phenolic concentration of the samples was expressed as milligrams of Gallic acid equivalents per gram of sample (mg GAE/g).

The TFC of samples were determined by the calorimetric assay described by Herald *et al.* (2012) with minor modifications. The extract was prepared using the

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same method as the TPC assay. First, 100 µL of dH_2O was added with 10 µL of 50 gL^{-1} sodium nitrites ($NaNO_2$) and pipetted into a 96-well plate according to the calibrator range. Next, 25 µL of sample or standard solution of quercetin was added to each well and left for 5 mins. After that, 15 µL of 100 gL^{-1} aluminium chloride ($AlCl_3$) was added. After 6 mins, 50 µL of 1 mol gL^{-1} sodium hydroxide (NaOH) was pipetted, followed by 50 µL of distilled water. The absorbance was read at 510 nm using the spectrophotometric microplate reader. The total flavonoid content was determined as quercetin equivalent (QE) and expressed as a gram of QE/100 g of sample (mg QE/g).

2.4 Antioxidant activities

2.4.1 DPPH free radical scavenging assay

The sample extracts were prepared by stirring 10 mg samples with 10 mL dH_2O for three hrs in order to run the antioxidant assays in the study. The DPPH free radical scavenging assay was adopted from the Mansour *et al.* (2016) method with slight modifications. Briefly, 80 µL of different concentrations of samples were mixed with 240 µL of 0.1 mM methanolic solution of DPPH in a 96-well plate. The plate was kept in the dark at room temperature (27°C) for 30 mins. After that, the absorbance was measured by the same spectrophotometric microplate reader at 514 nm. The mixture of 80 µL methanol and 240 µL DPPH solution was used as the control. Methanol was used as the solvent to make the mixture soluble. The free radical scavenging activity (RSA) was calculated by the equation below. The percentage of RSA was plotted against concentrations to find the median inhibition concentration (IC_{50}) of the plants.

$$RSA, \% = (A_c - A_s)/A_c \times 100$$

Where A_c = absorbance of control and A_s = absorbance of sample

2.4.2 Ferric reducing antioxidant power assay

FRAP assay was based on Saravanakumar *et al.* (2015) method with slight modifications. FRAP reagent was prepared by mixing 10 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM hydrochloric acid (HCl), 10 mL of ferric chloride solution (20 mM) and 100 mL of 300 mM acetate buffer (pH 3.6). Then, 7 µL of sample or standard was added to each well, followed by 20 µL of distilled water and 200 µL of FRAP reagent. After a 4-min incubation, the absorbance was measured at 593 nm by a similar plate reader. Different concentrations of 1 M ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) were prepared to construct a calibration curve. The value was expressed as µg $FeSO_4$ equivalent per gram (µg $FeSO_4$ E/g) of the sample

2.5 Statistical analysis

The results obtained were expressed as mean ± standard deviation (SD) values of three replicates. Analysis of variance (ANOVA) followed by Dunnett's C test, as a post hoc test in Statistical Package for the Social Sciences (SPSS) version 24.0 was used to analyse the data obtained. The significant level was set at $P < 0.05$.

3. Results and discussion

3.1 Nutritional composition

The proximate compositions of all samples are shown in Table 1. The elements of moisture and carbohydrates were excluded from the statistics calculation as the source of samples initially arrived in different conditions. The local herbs of *P. amboinicus* and *V. negundo* were obtained in fresh form while *S. miltiorrhiza* and *S. baicalensis* were received in dried form. Therefore, this may affect the result of moisture and carbohydrate in the herb plants. In the local herbs, *P. amboinicus* obtained the highest content of dietary fibre (41.24 mg/100 g sample) than other samples. A high ash content (21.46%) was found in *P. amboinicus*, indicating

Table 1. Proximate analyses of four Chinese herbs from the family of *Lamiaceae* which popular in Malaysia

Parameters	Local		Imported	
	P. amboinicus	*V. negundo*	*S. miltiorrhiza*	*S. baicalensis*
Moisture (%)	60.21±0.06	58.75±0.96	9.62±0.15^{a}	8.04±0.09^{a}
Ash (%)	21.46±0.16^{abc}	5.88±0.25^{a}	6.19±0.08^{bd}	5.07±0.12^{cd}
Crude Fat (%)	2.93±0.24^{ab}	2.70±0.37^{cd}	0.51±0.03^{ace}	0.76±0.04^{bde}
Crude Protein (%)	10.83±0.21^{abc}	15.10±0.01^{ade}	18.05±0.06^{bdf}	6.48±0.14^{cef}
Crude Fibre (%)	13.83±0.35^{a}	19.41±1.98^{b}	9.75±0.33^{abc}	15.26±0.27^{c}
Carbohydrate (%)	20.20±0.17	17.56±1.00	65.61±0.16^{a}	79.63±0.15^{a}
Total Dietary Fibre (mg/100 g sample)	41.24±1.25	36.83±1.96	30.19±0.56	32.69±0.73

Values are presented as mean±SD of three replicates. Values with different superscripts within the same row are statistically significantly different ($p<0.05$) between the plants. Statistical comparison was performed within the local and imported groups for 'moisture' and 'carbohydrate'.

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that the plant contained a high amount of inorganic nutrients compared to the other samples. *P. amboinicus* was also found to have the highest (P<0.05) fat content (2.93%) among the samples. High fat content in *P. amboinicus* would be the main contributor to gross energy as fat yields 9 kcal/g while both carbohydrate and protein only provide 4 kcal/g. Previously, Moura *et al.* (2021) reported smaller values of ash (1.24%) and fat (0.99%) in the *P. amboinicus* plant. Meanwhile, *V. negundo* has the highest crude fiber content (19.41%) among this study's samples. However, the value was lower than the previous study (28.2 %) reported by Kumar *et al.* (2013). Kumar *et al.* (2013) also reported comparable ash content (5.4%) and protein content (13.7%) of *V. negundo* with this study (ash: 5.88%; protein: 15.10%).

For the imported herb, *S. miltiorrhiza* was found to have the highest protein content (18.05%). Yang *et al.* (2017) and Wang *et al.* (2021) have reported lower protein content (11.7% and 14.3% respectively) of *S. miltiorrhiza* previously. The differences in proximate compositions of these herbs might be influenced by the cultivation region or type of sample preparation method (Yang *et al.*, 2017).

3.2 Total phenolic and total flavonoid content

TPC and TFC of two local Chinese herbs, *P. amboinicus V. negundo* and two imported, *S. miltiorrhiza* and *S. baicalensis* from *Lamiaceae* family were determined using Folin-Ciocalteau method and calometric assay accordingly. As shown in Table 2, *S. miltiorrhiza* exhibited the highest amount of TFC (241.55 mg QE/g) and second highest TPC (105.90 mg GAE/g) compared to the rest of the samples. The leaves have a significant amount of phenols and flavonoids that are able to contribute to strong antioxidant activity. The present study also demonstrated a higher amount of TPC and TFC in the plant compared to Moon and Cha (2020) and Ravipati (2012) studies. Meanwhile, *S. baicalensis* in the study obtained the highest amount of TPC (116.34 mg GAE/g) but low in TFC, 18.21 mg QE/g. A study reported by Liau *et al.* (2019) showed *S. baicalensis* has a greater amount of TPC up to three times in ethanol extract form. On the other hand, the value of TFC in Vergun *et al.* (2019) study was comparable with the present finding. *S. baicalensis* was commonly known for its baicalin and baicalein which contributed to its strong antioxidant properties. Grzegorczyk-Karolak *et al.* (2015) reported that the *Salvia* sp. Contained a high amount of phenolic acid in the roots and the aerial part. Therefore, it is suggested to consume both parts of *S. baicalensis* to derive more phenolic compounds for strong antioxidant activity and prevent cell damage caused by oxidative stress.

Concurrently, this study showed a similar value of TPC (74.07 mg GAE/g) of *V. negundo* to the study reported by Rana *et al.* (2019). Thombre *et al.* (2013) revealed that the total phenolic content in *V. negundo* could be higher by preparing the sample using methanolic as the solvent. *Plectranthus amboinicus* obtained the lowest value in both TPC (33.39 mg GAE/g) and TFC (13.74 mg QE/g). However, the herb showed a higher value of TPC than the study reported by Muhamad and Ali (2018). *Plectranthus amboinicus* usually is used as a food additive in Malaysian dishes, such as in curry to enhance the taste and aroma. *P. amboinicus* might not have high phenolic or flavonoid compounds that play a major role in antioxidant activity, yet it brings different value to local people in Malaysia.

3.3 DPPH and ferric reducing antioxidant power assays

The antioxidant activities of *P. amboinicus, V. negundo, S. miltiorrhiza* and *S. baicalensis* were tested by DPPH and FRAP assays. The DPPH in *V. negundo* obtained the highest value while *S. miltiorrhiza* had the highest value in FRAP activity among the four samples. Uniquely, *V. negundo* showed the lowest FRAP activity among all samples. A previous study suggested that *V. negundo* consisted of several bioactive compounds with different polarities which led to specific antioxidant properties (Vijayalakshmi and Rao, 2020). Nevertheless, polar fractions of *V. negundo* exhibited higher antioxidant activity in the study by Vijayalakshmi and Rao (2020). The researchers demonstrated that *V.*

Table 2. TPC, TFC and antioxidant activities of four Chinese herbs from the family of *Lamiaceae* which popular in Malaysia

Activity	Local		Imported	
	P. amboinicus	*V. negundo*	*S. miltiorrhiza*	*S. baicalensis*
TPC (mg GAE/g)	33.39 ± 7.37^{ab}	74.07 ± 7.72	105.90 ± 3.37^{a}	116.34 ± 7.55^{b}
TFC (mg QE/g)	13.74 ± 2.60^{a}	142.77 ± 47.63	241.55 ± 55.15^{b}	18.21 ± 2.80^{ab}
DPPH IC_{50} (µg/mL)	271.82 ± 29.23^{ab}	407.68 ± 17.87^{acd}	169.10 ± 14.17^{bce}	303.35 ± 23.3^{de}
FRAP (µg $FeSO_4$ E/g)	44.11 ± 0.85^{ab}	42.73 ± 1.35^{cd}	103.09 ± 2.84^{ace}	69.46 ± 0.81^{bde}

Values are presented as mean±SD of three replicates. Values with different superscripts within the same row are statistically significantly different (p<0.05) between the plants. Gallic acid was used as a standard to construct a calibration curve for TPC: y = 0.0016x + 0.0445 (R^2 = 0.998). Quercetin was used as a standard to construct a calibration curve for TFC: y = 0.0003x + 0.0476 (R^2 = 0.994). FRAP calibration curve: y = 0.0004x + 0.1158 (R^2 = 0.999).

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negundo water extract such as in this study showed the presence of flavonoids, saponins, tannins, anthraquinones and cardiac glycosides. The presence of alkaloids, triterpenoids and triple sugars could only be found in methanol and hexane but not in the water extract of the plant (Vijayalakshmi and Rao, 2020). Meanwhile, *S. miltiorrhiza* consisted of several bioactive compounds such as tanshinones, salvianolic acid A and salvianolic acid B (Wang, 2010). These bioactive compounds would contribute to the strong antioxidant properties in the plant. Moreover, the present study reported a higher value of DPPH IC_{50} of *S. miltiorrhiza* (169.10 µg/mL) than the study of Chen *et al.* (2013).

Plectranthus amboinicus showed considerably good performance in DPPH and FRAP but consisted of a low amount of phenolic and flavonoid compounds. Therefore, it can be assumed that polyphenol or flavonoid compounds are not major players in high FRAP antioxidant activity in the *P. amboinicus* plant according to Navodani *et al.* (2019). A study has displayed a lower value of DPPH IC_{50} of *P. amboinicus* when using ethanol and suggested that *P. amboinicus* ethanol extract would have better performance in free radical scavenging activity as some of the compounds were less extracted in water but highly extracted in ethanol (Muhamad and Ali, 2018). Other common bioactive compounds which contribute to the antioxidant activity in plants include caffeic acid, rosmarinic acid, coumaric acid, quercetin, rutin and gallic acid. On the other hand, the value of DPPH IC_{50} of *S. baicalensis* was found similar to the study of Lee *et al.* (2014). For *S. baicalensis* plant, Liau *et al.* (2019) proved that the antioxidant activity of the plant could be higher in ethyl acetate extract than in other extracts for instance water-, ethanol- and acetone-.

Freeze-drying was subjected to the study as the sample preparation method of the tested herbs. Higher antioxidant activities of the herbs which are noticeable in this study might be due to the way of samples were prepared. Previously, Mustafa *et al.* (2019) showed that the freeze-dried sample obtained higher ($P<0.05$) antioxidant activities in FRAP, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid scavenging (ABTS) and DPPH, TPC, TFC content compared to the fresh samples. The finding was aligned with this study where all the Chinese herbs tested demonstrated higher antioxidant values compared to the same species in other studies. Freeze-dried samples also consisted of higher phenolic content than oven-dried samples. It was said that freeze-drying can help to maintain the quality of the samples, such as nutrients, colour and flavour (Mustafa *et al.*, 2019). These findings indicated that freeze drying is a favourable drying sample method which is able to minimise the loss of compositions and bioactive compounds in the plant. Other sample preparation methods that use high temperatures such as oven-drying might affect or cause the loss of bioactive compounds due to the chemical degradation during the process (Chang *et al.*, 2016).

4. Conclusion

Chinese herbal plants consist of various essential nutrients and antioxidant compounds which bring many benefits to humans. In the study, two local (*P. amboinicus*, *V. negundo*) and two imported (*S. miltiorrhiza*, *S. baicalensis*) Chinese herbs from *Lamiaceae* family which are popular in Malaysia were investigated for their proximate compositions, TPC, TFC, DPPH and FRAP activities. It was found that Danshen or *S. miltiorrhiza* showed a potent performance in TPC, TFC, DPPH radical scavenging and FRAP activities. For local herbs, 'Legundi' or *V. negundo* derived higher antioxidant compounds and activities compared to 'Bangun-bangun' (*P. amboinicus*). Further studies are recommended to isolate the bioactive compound of antioxidants from particular plants.

Conflict of interest

The authors declare no conflict of interest.

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References

Arumugam, G., Swamy, M.K. and Sinniah, U.R. (2016). *Plectranthus amboinicus* (Lour.) Spreng: Botanical, phytochemical, pharmacological and nutritional significance. *Molecules*, 21(4), 369. https://doi.org/10.3390/molecules21040369

Ashaari, N.S., Ab. Rahim, M.H., Sabri, S., Lai, K.S., Song, A.A.L., Abdul Rahim, R., Wan Abdullah, W.M.A.N. and Ong Abdullah, J. (2020). Functional characterization of a new terpene synthase from *Plectranthus amboinicus*. *PloS ONE*, 15(7), e0235416. https://doi.org/10.1371/journal.pone.0235416

Association of Official Analytical Chemists (1984). Official Methods of Analysis. 14th ed. In Williams, S. (Ed.). Virginia: AOAC.

Mansour, R.B., Ksouri, W.M., Cluzet, S., Krisa, S.,

Richard, T. and Ksouri, R. (2016). Assessment of antioxidant activity and neuroprotective capacity on PC12 cell line of *Frankenia thymifolia* and related phenolic LC-MS/MS identification. *Evidence-Based Complementary and Alternative Medicine*, 2016, 2843463. https://doi.org/10.1155/2016/2843463

Chang, C.H., Lin, H.Y., Chang, C.Y. and Liu, Y.C. (2006). Comparisons on the antioxidant properties of fresh, freeze-dried and hot-air-dried tomatoes. *Journal of Food Engineering*, 77(3), 478-485. https://doi.org/10.1016/j.jfoodeng.2005.06.061

Chen, C.Y., Li, H., Yuan, Y.N., Dai, H.Q. and Yang, B. (2013). Antioxidant activity and components of a traditional Chinese medicine formula consisting of *Crataegus pinnatifida* and *Salvia miltiorrhiza*. *BMC Complementary and Alternative Medicine*, 13, 99. https://doi.org/10.1186/1472-6882-13-99

Embuscado, M.E. (2015). Spices and herbs: Natural sources of antioxidants–A mini review. *Journal of Functional Foods*, 18, 811-819. https://doi.org/10.1016/j.jff.2015.03.005

Farooqui, M., Hassali, M.A., Shatar, A.K.A., Farooqui, M.A., Saleem, F., Ul Haq, N. and Othman, C.N. (2016). Use of complementary and alternative medicines among Malaysian cancer patients: A descriptive study. *Journal of Traditional and Complementary Medicine*, 6(4), 321-326. https://doi.org/10.1016/j.jtcme.2014.12.008

Gaire, B.P., Moon, S.K. and Kim, H. (2014). *Scutellaria baicalensis* in stroke management: Nature's blessing in traditional Eastern medicine. *Chinese Journal of Integrative Medicine*, 20(9), 712-720. https://doi.org/10.1007/s11655-014-1347-9

Grzegorczyk-Karolak, I., Wysokińska, H. and Olas, B. (2015). Studies on the antioxidant properties of extracts from the roots and shoots of two *Scutellaria* species in human blood plasma. *Acta Biochimica Polonica*, 62(2), 253-258. https://doi.org/10.18388/abp.2014_944

Herald, T.J., Gadgil, P. and Tilley, M. (2012). High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *Journal of the Science of Food and Agriculture*, 92(11), 2326-2331. https://doi.org/10.1002/jsfa.5633

Kumar, M., Dandapat, S., Kumar, A. and Sinha, M.P. (2013). Determination of nutritive value and mineral elements of five-leaf chaste tree (*Vitex negundo* L.) and Malabar nut (*Adhatoda vasica* Nees). *Academic Journal of Plant Sciences*, 6(3), 103-108.

Lee, K. J., Jung, P.M., Oh, Y.C., Song, N.Y., Kim, T. and Ma, J.Y. (2014). Extraction and bioactivity analysis of major flavones compounds from *Scutellaria baicalensis* using in vitro assay and online screening HPLC-ABTS system. *Journal of Analytical Methods in Chemistry*, 2014, 563702. https://doi.org/10.1155/2014/563702

Liau, P.R., Wu, M.S. and Lee, C.K. (2019). Inhibitory effects of *Scutellaria baicalensis* root extract on linoleic acid hydroperoxide-induced lung mitochondrial lipid peroxidation and antioxidant activities. *Molecules*, 24(11), 2143. https://doi.org/10.3390/molecules24112143

Moon, K. and Cha, J. (2020). Enhancement of antioxidant and antibacterial activities of *Salvia Miltiorrhiza* roots fermented with *Aspergillus oryzae*. *Foods*, 9(1), 34. https://doi.org/10.3390/foods9010034

Moura, H.F.S., Souza, D.F.D., e Souza, L.B.S., Magalhães, B.E.A.D., Aragão Tannus, C.D.D., Carvalho, W.C.D., Brandão, G.C., Dos Santos, W.N.L., Korn, M.G.A., Dos Santos, D.C.M.B. and Júnior, A.D.F.S. (2021). Evaluation of multielement/proximate composition and bioactive phenolics contents of unconventional edible plants from Brazil using multivariate analysis techniques. *Food Chemistry*, 363, 129995. https://doi.org/10.1016/j.foodchem.2021.129995

Muhamad, S. and Ali, S.F.Z.M. (2018). Effect of heating at 95°C on antioxidant activity and total phenolic content in extracts of *Plectranthus amboinicus* leaves. *International Journal of Recent Scientific Research*, 9(7), 28096-28099.

Mustafa, I., Chin, N.L., Fakurazi, S. and Palanisamy, A. (2019). Comparison of phytochemicals, antioxidant and anti-inflammatory properties of sun-, oven-and freeze-dried ginger extracts. *Foods*, 8(10), 456. https://doi.org/10.3390/foods8100456

Navodani, A., Jayasekara, G.A.U., Soysa, S.S.S.B.D.P., Gooneratne, L.V. and Ranathunge, I. (2019). *In-vitro* antioxidant activity and the total phenolic content of herbal plants used in cancer treatment of Sri Lankan Ayurvedic medicine. *Proceedings of 12th International Research Conference,* 2019*,* 573-579.

Rana, Z.H., Alam, M.K. and Akhtaruzzaman, M. (2019). Nutritional composition, total phenolic content, antioxidant and α-amylase inhibitory activities of different fractions of selected wild edible plants. *Antioxidants*, 8(7), 203. https://doi.org/10.3390/antiox8070203

Ravipati, A.S., Zhang, L., Koyyalamudi, S.R., Jeong, S.C., Reddy, N., Bartlett, J., Shanmugam, K., Münch, G., Wu, M.J., Satyanarayanan, M. and Vysetti, B. (2012). Antioxidant and anti-inflammatory activities of selected Chinese

medicinal plants and their relationship with antioxidant content. *BMC Complementary and Alternative Medicine*, 12, 173. https://doi.org/10.1186/1472-6882-12-173

Saklani, S., Mishra, A.P., Chandra, H., Atanassova, M.S., Stankovic, M., Sati, B., Shariati, M.A., Nigam, M., Khan, M.U., Plygun, S. and Elmsellem, H. (2017). Comparative evaluation of polyphenol contents and antioxidant activities between ethanol extracts of *Vitex Negundo* and *Vitex trifolia L.* leaves by different methods. *Plants*, 6(4), 45. https://doi.org/10.3390/plants6040045

Salleh, R.M., Dashti, N.G. and Thong, O.M. (2014). Proximate analysis and anti-proliferative properties of *Vitex Negundo* L. *Sains Malaysiana*, 43(10), 1543 -1547.

Sallehuddin, N.A., Abdul-Hamid, A., Salleh, S.Z., Abdul -Majid, N., Halim, H.H., Ramli, N.S. and Pak-Dek, M.S. (2020). Ergogenic, anti-diabetic and antioxidant attributes of selected Malaysian herbs: characterisation of flavonoids and correlation of functional activities. *International Food Research Journal*, 27(1), 197-207.

Saravanakumar, A., Ganesh, M., Peng, M.M., Aziz, A.S. and Jang, H.T. (2015). Comparative antioxidant and antimycobacterial activities of *Opuntia ficus*-indica fruit extracts from summer and rainy seasons. *Frontiers in Life Science*, 8(2), 182-191. https://doi.org/10.1080/21553769.2015.1028655

Singh R, Devi S. and Gollen R. (2015). Role of free radical in atherosclerosis, diabetes and dyslipidaemia: larger-than-life. *Diabetes or metabolism Research and Reviews,* 31(2), 113-126. https://doi.org/10.1002/dmrr.2558

Sukweenadhi, J., Yunita, O., Setiawan, F., Siagian, M.T. and Avanti, C. (2020). Antioxidant activity screening of seven Indonesian herbal extract. *Biodiversitas Journal of Biological Diversity*, 21(5), 2062-2067. https://doi.org/10.13057/biodiv/d210532

Thombre, R., Jagtap, R. and Patil, N. (2013). Evaluation of phytoconstituents, antibacterial, antioxidant and cytotoxic activity of *Vitex Negundo* L. and *Tabernaemontana divaricata* L. *International Journal of Pharma and Bio Sciences,* 4(1), 389-396.

Tiwari, O.P. and Tripathi, Y.B. (2007). Antioxidant properties of different fractions of *Vitex Negundo* Linn. *Food Chemistry*, 100(3), 1170-1176. https://doi.org/10.1016/j.foodchem.2005.10.069

Vergun, O., Svydenko, L., Grygorieva, O., Shymanska, O., Rakhmetov, D., Brindza, J. and Ivanišová, E. (2019). Antioxidant capacity of plant raw material of *Scutellaria baicalensis* Georgi. *Potravinarstvo*, 13 (1), 1090. https://doi.org/10.5219/1090

Vijayalakshmi, N. and Rao, M.R.K. (2020). Preliminary phytochemical and antioxidant studies of leaf extracts of one medicinal plant, *Vitex Negundo*. *Research Journal of Pharmacy and Technology*, 13 (5), 2167-2173. https://doi.org/10.5958/0974-360X.2020.00390.X

Wang, B.Q. (2010). *Salvia miltiorrhiza*: Chemical and pharmacological review of a medicinal plant. *Journal of Medicinal Plants Research*, 4(25), 2813-2820.

Wang, J., Luo, Y., Li, P., Zhang, F. and Liu, N. (2021). Effect of *Salvia miltiorrhiza* aerial parts on growth performance, nutrient digestibility, and digestive enzymes in rabbits. *Animal Bioscience*, 34(12), 1981. https://doi.org/10.5713/ab.21.0070

Yang, E.J., Seon, Y.K., Seo, Y.S. and Shin, B.Y. (2017). Component analysis and comparison of biological activities of *Salvia miltiorrhiza* Bunge from different cultivation regions. *Journal of the Korean Society of Food Science and Nutrition*, 46(8), 929-936.

Zhang, Y., Li, X. and Wang, Z. (2010). Antioxidant activities of leaf extract of *Salvia miltiorrhiza* Bunge and related phenolic constituents. *Food and Chemical Toxicology*, 48(10), 2656-2662. https://doi.org/10.1016/j.fct.2010.06.036

Zhao, T., Tang, H., Xie, L., Zheng, Y., Ma, Z., Sun, Q. and Li, X. (2019). *Scutellaria baicalensis* Georgi. (Lamiaceae): A review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. *Journal of Pharmacy and Pharmacology*, 71(9), 1353-1369. https://doi.org/10.1111/jphp.13129

