

Total Phenolic Content in *Piper sarmentosum* using Different Extraction Techniques and Solvents: A Short Review

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Abstract

Piper sarmentosum is an herbaceous plant that belongs to Piperaceae family which possesses an antioxidant activity due to the presence of phenolic compounds. The extraction of phenolic compounds from this Piper species could be carried out by employing various conventional and nonconventional extraction techniques and solvents. Among the extraction techniques commonly applied are maceration, infusion, reflux, and water bath extraction which are classified as conventional methods. Meanwhile, among nonconventional approaches employed are microwaved-assisted extraction, ultrasonic-assisted extraction (UAE), microwave-assisted infusion, and agitation extraction. The recovery of phenolic content could be performed using different extracting solvents such as water, ethanol, methanol, butanol, hexane, n-hexane, acetone, chloroform, and ethyl acetate. In this manuscript, the effect of different extraction techniques and solvents on the total phenolic content (TPC) in *P. sarmentosum* was reviewed. Previous research recorded that the TPC which was expressed as gallic acid equivalent in *P. sarmentosum* ranges from 0.57 mg GAE/g for UAE to 430 mg GAE/g where the maceration technique produced the highest TPC. While for the extracting solvents, methanol gave the most significant amount of TPC with obtained value at 379 mg GAE/100 g. This research provides an overall review of different techniques of solvents which could be used as references on the optimization of extraction processes to enhance the recovery of phenolic content from *P. sarmentosum*.

Keywords: Chromatography, *Piper sarmentosum*, plant extraction, total phenolic content

INTRODUCTION

Piper sarmentosum is a creeping herbaceous plant that comes from *Piperaceae* family, which is distributed in the South-East Asia region, including Malaysia.^[1] This plant is characterized by the long creeping stem, heart-shaped, waxy surfaces leave with alternate leaves arrangement.^[2] *P. sarmentosum*, which is known as wild betel and locally known as “kaduk” has been widely used in culinary and traditional medicine to treat various diseases and ailments. Conventionally, this plant was treated as a natural antioxidant by eating it raw. Meanwhile, the decoction of the plant leaves was consumed to treat diabetes. Recent studies confirmed that the plant exhibits anticancer,^[3] antioxidant,^[4] antimicrobial,^[5] and antiinflammatory properties.^[6] Pharmacological effects exhibited by *P. sarmentosum* are due to their phytochemical constituents. For the past decades, researchers and scientists are interested in the exploration of phenolic compounds derived from this plant. Various studies have been conducted on the subject, which

encompasses the molecular aspect of the compound to their extraction techniques.

Plant bioactive compound identification and profiling can be carried out by extracting the materials using conventional and nonconventional techniques assisted by different types of solvents. The analysis could be achieved by selected chromatography techniques such as high-performance liquid chromatography and gas chromatography-mass spectrometry. The purpose of this study is to review different extraction techniques and solvents and to find the best technique to provide the highest total phenolic content (TPC) from *P. sarmentosum*.

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DISCUSSIONS

Different extraction techniques and solvents of *Piper sarmentosum* extracts

Extraction and isolation of phenolic compounds from *P. sarmentosum* can be conducted by utilizing different extraction techniques and solvents. The choice of extraction techniques and solvents is a crucial step to achieve the optimum yield of phenolic compounds from plant sarmentosum extracts. Various techniques and solvents have been applied in previous research on *P. sarmentosum* to extract its phenolic compounds, which are summarized in Table 1 which includes the detailed method and the obtained TPC.

The commonly used technique for the extraction of phenolics and determination of TPC in *P. sarmentosum* is maceration as most of the studies presented in Table 1 used this particular technique as it is simple, less expensive in terms of its apparatus plus resulted in adequately high phenolic extraction rates.^[23] Other conventional techniques that have also been used are infusion, reflux, and water bath extraction (WBE). Apart from that, nonconventional techniques, namely ultrasonic-assisted extraction (UAE), microwave-assisted infusion (MAI), and agitation extraction, have also been utilized by a number of researchers to extract phenolic compounds from this species. Overall, the TPC expressed as gallic acid equivalents ranged from 0.57 mg GAE/g to 430 mg GAE/g. However, the most significant value of TPC for *P. sarmentosum* was found when using maceration, infusion, and MAI techniques with values of 430 mg GAE/g, 245.64 mg GAE/L, and 141.48 mg GAE/L, respectively.

A study conducted a study on the antioxidant activity of several edible plants including *P. sarmentosum* and the technique was employed for the recovery of phenolic compounds is maceration and it resulted in the high value of TPC which is 430 mg GAE/g. Meanwhile, identified a low amount of TPC value which was 0.57 mg GAE/g DW when using UAE. Nevertheless, performed a comprehensive study on the effects of different extraction techniques on TPC in *Piper betle* using different extraction techniques.^[24] The result showed that higher TPC at 57.60 ± 1.06 mg GAE/10 mg extract was obtained when using UAE instead of maceration.

(Suwal and Marciniak, 2018) stated that the use of UAE has been found to increase the efficiency of phenolic compound extraction up to 35% from various plant sources.^[25] This can be explained by the irradiation between the plant tissue and the ultrasound that increases the number of solvents being exposed to the molecules as the cell wall has been broken down. Thus, the swelling and hydration degree is increased, and the diffusion and mass transfer process are more efficient. Plus, UAE took a shorter time as compared to maceration which took an average of 1–4 h to complete. Despite these drawbacks, the conventional technique the maceration still remains in use today for the extraction of phenolic compounds because the extraction apparatus is widely available and less cost is needed.

Moreover, the yield of phenolic compounds can be enhanced by combining two extraction techniques. A recent study, combined both conventional; infusion and nonconventional techniques; microwaved-assisted extraction (MAE) to extract phenolic compounds. MAI technique was also applied and a high value of TPC was achieved which is 141.48 ± 3.78 mg GAE/L. Besides, in this study, *P. sarmentosum* was extracted by using the infusion method alone and resulted in a significant value of TPC; 245.64 mg GAE/L.

There is less evidence on the uses of MAI for any *Piper* species. However, highlighted some positive aspects of MAE such as short duration, less solvent, higher extraction rate, energy saving, better-quality product (higher antioxidant activity), and less cost. Hence, the combination of these two techniques appears to be a promising option to enhance the recovery of phenolic compounds.

Different extraction solvents

Various types of solvents can be used depending on the polarity of the compound that needs to be recovered. As for *P. sarmentosum*, the choice of extracting solvents highly influences its yield of phenolic compounds and TPC. The summary of previous studies is shown in Table 2.

There are various types of solvents that could be used for the extraction of *P. sarmentosum*, and TPC in this species can be affected by this factor. A literature search revealed that water, ethanol, methanol, acetone, hexane, n-hexane, chloroform, ethyl acetate, and butanol has been used as the extracting solvents and the TPC value of *P. sarmentosum* extracted with different solvents, is presented in Table 2. Overall TPC values ranged from 1.19 mg GAE/g to 379 mg GAE/100 g. High TPC values of 379 mg GAE/100 g were recorded when using methanol. Consequently, a significant amount of TPC was also observed when using ethanol and water with TPC of 167 mg GAE/g and 129 mg GAE/g, respectively. Based on the results, it may be deduced that the recovery of the phenolic compounds is significantly influenced by the polarity of the solvent. Water, ethanol, and methanol which are polar solvents can solubilize polar compounds like phenolics with more ease in comparison to nonpolar ones.^[36]

Nevertheless, in the case of *P. sarmentosum*, it was noted that methanol would be the most suitable choice for the extraction of the phenolic compound from this species and this is in accordance with the principle of like-dissolve-like. This principle explained on the ability of solvents to solubilize solute with properties that is similar to them. Hence, it can be assumed that the polarity of the phenolics in *P. sarmentosum* closely resembles more of methanol than water even though water has a higher polarity. The second-best solvent that gave a significant amount of TPC is ethanol and followed by water. It may be deduced that ethanol is a better option than water. This finding is in line with a previous study,^[37] which reported the highest TPC value for ethanol extract and lowest TPC value for water extract with a value of 45.08 ± 2.21 mg GAE/g and 1.09 ± 0.10 mg GAE/g, respectively, in *Piper nigrum*.

Table 1: Total phenolic content values of *Piper sarmentosum* obtained using different extraction techniques

Serial number	Study	Method	Temp (°C)	Time	Solvent	Total phenolic content	Reference
1	A high antioxidant level in edible plants is associated with genotoxic properties	Maceration	-	4 h	Ultra-pure water	430±3.1 mg GAE/g	[7]
2	Antioxidant activities, flavonoids, ascorbic acid and phenolic contents of Malaysian vegetables	Maceration	RT	1 h	Distilled water	7.66 mg/GAE/g DW	[8]
3	Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand	Maceration	RT	10 min	Acetone – methanol – water (7:7:6 v/v/v)	123±0.12 mg%	[9]
4	Total phenolic content and free radical scavenging activity of representative medicinal plants of Thailand	Maceration	-	-	Ethanol: water (7:3 ratio)	100 mg GAE/g	[10]
5	Ergogenic, anti-diabetic, and antioxidant attributes of selected Malaysian herbs: characterization of flavonoids and correlation of functional activities	Maceration	-	-	Ethanol (60%)	51.27±1.91 mg GAE/g	[11]
6	Ethanol extract of Betel (<i>P. betle</i> L.) and Chaphlu (<i>P. sarmentosum</i> Roxb.) dechlorophyllized using sedimentation process: Production, characteristics, and antioxidant activities	Maceration	4	1 h	Ethanol (70%)	62.6 mg GAE/g DW	[12]
7	Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables	Maceration	RT	1 h	Ethanol (70%)	5.1±0.2 mg GAE/g DW	[13]
8	<i>Piper sarmentosum</i> as an antioxidant on oxidative stress in human umbilical vein endothelial cells induced by hydrogen peroxide	Maceration	80	3 h	Aqueous	90.8 mg GAE/g DW	[14]
9	Antioxidant and cytotoxicity activity of phenolic compounds from <i>P. sarmentosum</i> Roxb. against T47D human breast cancer cell	Maceration	35	5 min	Methanol	89.33±1.764 mg GAE/g	[15]
10	Antioxidant capacity and antimutagenicity of thermal processed Thai foods	Maceration	RT	-	Methanol (100%)	0.74 g GAE/100 g	[16]
11	Total phenolic contents and free radical scavenging capacity of <i>P. sarmentosum</i> (Pucuk kaduk) extract	UAE	-	1 h	Methanol (80%)	0.57 mg GAE/g DW	[17]
12	Lipophilic and hydrophilic antioxidant capacities of vegetable, herbs and spices in eighteen traditional Thai Dishes	UAE	37	5 min	Hexane/dichloromethane (1:1 ratio)	3.30 mg GAE.g-1 FW	[18]
13	In vitro screening of phenolic compounds, potential inhibition against α -amylase and α -glucosidase of culinary herbs in Thailand	Reflux	95	30 min	Distilled water	10.98±0.25 mg GAE/g dw	[19]
14	Determinations of five kinds of secondary metabolites from different parts of <i>P. sarmentosum</i> Roxb.	WBE	80	1 h	Ethanol (60%)	2.42% (root), 0.68% (leaf), 1.08% (stem)	[20]
15	Characterization and optimization of bioactive compounds extracted from herbal plants using microwave assisted infusion	Infusion MAI	- -	- -	- -	245.64 mg GAE/L 141.48±3.78 mg GAE/L	[21]
16	Antioxidant activities and total phenolic content of Malaysian herbs as components of active packaging film in beef patties	Agitation extraction	RT	24 h	Aqueous: ethanol (75%) (1:20 ratio)	1.47±0.01 mg GAE/L	[22]

RT: Room temperature, UAE: Ultrasonic-assisted extraction, MAI: Microwave-assisted infusion, WBE: Water bath extraction, TPC: Total phenolic content, *P. sarmentosum*: *Piper sarmentosum*, GAE: Gallic acid equivalent, FW: Fresh weight, DW: Dry extract, *P. betle*: *Piper betle*

Alara et al., 2018, noted that the use of ethanol solvent gives the most significant effect on the TPC value because the presence of a small amount of water in an organic solvent can stimulate the mass transfer process by increasing the relative polarity of the solvent hence increased the solubility of the solvent.^[38] By that, the swelling of the plant matrix will be more efficient. Another similar study also confirmed that ethanol is the best solvent for extracting phenolic compounds in two *Piper* species; *Piper cubeba* and *P. nigrum*, as high TPC value have been recorded when using ethanol solvents.^[39] Meanwhile, water seems to be less suitable for

the extraction of phenolic compounds as they are more nonphenol compounds such as carbohydrates and terpene in water extracts compared to other extracts. On the other hand, it was discovered that TPC values decreased as water content increased in the aqueous solvent, as discussed.^[40]

CONCLUSION

Extraction of phenolic compounds from *P. sarmentosum* involves the application of either conventional or

Table 2: Total phenolic content values of *Piper sarmentosum* obtained using different extraction solvents

Serial number	Experiment	Solvent	TPC	Reference
1	Antioxidant, anti-TB activities, phenolic and amide contents of standardized extracts of <i>P. sarmentosum</i> Roxb.	Water	1.19±0.01 mg GAE/g	[26]
2	Flavonoids of <i>P. sarmentosum</i> and its cytoprotective effects against oxidative stress	Water	91.02±0.02 mg GAE/g DM	[27]
3	Inhibitory effect of Thai vegetable extracts on activity of lipase, alpha-amylase, trypsin and alcohol dehydrogenase	Water	129.86±0.21 mg GAE/g crude extract	[28]
4	Evaluation of the antioxidant activities of Ya-hom Intajak, a Thai herbal formulation, and its component plants	Ethanol	167.87±2.62 mg GAE/g crude extract	[29]
5	High phenolics and antioxidants of some tropical vegetables related to antibacterial and anticancer activities	Ethanol (70%)	22.01±0.78 mg GAE/g	[30]
6	Antioxidant activity of <i>P. sarmentosum</i> Roxb. and its effect on the degradation of frying oils	Ethanol (80%)	18.42±0.02 mg GAE/g dry weight	[31]
7	Nutritional value and potential of Malaysian underutilized fruits and traditional vegetables	Methanol (70%)	17.93±0.33 mg GAE/g	[32]
8	Evaluation of anti-inflammatory, antioxidant, and antinociceptive activities of six Malaysian medicinal plants	Methanol (100%)	8.48±0.1 g GAE/100 g	[33]
9	Antioxidant properties of selected fresh and processed herbs and vegetables	Methanol	50.01 mg GAE/g DW	[34]
10	Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables	Methanol	379±24 mg GAE/100 g	[13]
11	Inhibitory effect of Thai vegetable extracts on activity of lipase, alpha- amylase, trypsin, and alcohol dehydrogenase	Acetone	2.8±0.9 mg GAE/g	[28]
12	Investigation on antibacterial and antioxidant activities, phenolic, and flavonoid contents of some Thai edible plants as an alternative for antibiotics	Hexane	127.79±0.59 mg GAE/g crude extract	[35]
		n-hexane	17.6±0.009 µg/mL	
		Chloroform	23.3±0.010 µg/mL	
		Ethyl acetate	13.5±0.013 µg/mL	
		Butanol	23.9±0.007 µg/mL	

P. sarmentosum: *Piper sarmentosum*, GAE: Gallic acid equivalent, TPC: Total phenolic content, DW: Dry extract, DM: Dry matter

nonconventional techniques. Among all of the studied techniques, maceration exhibited the highest recovery of TPC as compared to the other investigated methods, namely infusion, reflux, WBE, MAE, UAE, MAI, and agitation extraction. In terms of cost and availability of extraction apparatus, maceration surpassed the other techniques, however, the nonconventional method has major benefits as it is more efficient in terms of shorter extraction time and less usage of solvent.

Other than that, the choice of solvent is also crucial to ensure high efficiency of the recovery of phenolic contents. Polar solvents such as water, methanol, and ethanol are the most appropriate solvents to extract phenolic compounds and are commonly used in most of the studies for *P. sarmentosum*. However, the most significant value of TPC was achieved by using methanol as the extracting solvent. There are insufficient experimental data on the combination and integration of extraction techniques for the extraction of phenolic compounds from this species. Other parameters involved in the extraction process such as time and temperature could also influence the yield of phenolic compounds in *P. sarmentosum* and must be taken into consideration. Future research and work should be directed toward the application of other available extraction methods together with the optimization of several parameters involved in the recovery of phenolic compounds from *P. sarmentosum*.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Seyyedani A, Yahya F, Kamarolzaman MF, Suhaili Z, Mohd Desa MN, Mohd Khairi H, et al. Review on the ethnomedicinal, phytochemical and pharmacological properties of *Piper sarmentosum*: Scientific justification of its traditional use. TANG (Humanitas Tradit Med) 2013;3:1-32.
- Rahman SF, Sijam K, Omar D. *Piper sarmentosum* Roxb.: A mini review of ethnobotany, phytochemistry and pharmacology. J Anal Pharm Res 2016;2:00031.
- Zainal Ariffin SH, Wan Omar WH, Zainal Ariffin Z, Safian MF, Senafi S, Megat Abdul Wahab R. Intrinsic anticarcinogenic effects of *Piper sarmentosum* ethanolic extract on a human hepatoma cell line. Cancer Cell Int 2009;9:6.
- Subramaniam V, Adenan MI, Ahmad AR, Sahdan R. Natural antioxidants: *Piper sarmentosum* (Kadok) and *Morinda elliptica* (Mengkudu). Malays J Nutr 2003;9:41-51.
- Fernandez L, Daruliza K, Sudhakaran S, Jegathambigai R. Antimicrobial activity of the crude extract of *Piper sarmentosum* against methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Vibrio cholera* and *Streptococcus pneumoniae*. Eur Rev Med Pharmacol Sci 2012;16 Suppl 3:105-11.
- Zakaria ZA, Patahuddin H, Mohamad AS, Israfa DA, Sulaiman MR. *In vivo* anti-nociceptive and anti-inflammatory activities of the aqueous extract of the leaves of *Piper sarmentosum*. J Ethnopharmacol 2010;128:42-8.

7. Wan-Ibrahim WI, Sidik K, Kuppusamy UR. A high antioxidant level in edible plants is associated with genotoxic properties. *Food Chem* 2010;122:1139-44.
8. Sumazian Y, Syahida A, Hakim M, Maziah M. Antioxidant activities, flavonoids, ascorbic acid and phenolic content of Malaysian vegetables. *J Med Plants Res* 2010;4:881-90.
9. Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chem* 2005;92:491-7.
10. Chaiyasut C, Kesika P, Chaiyasut K, Sittiyuno P, Peerajan S, Sivamaruthi BS. Total phenolic content and free radical scavenging activity of representative medicinal plants of Thailand. *Asian J Pharm Clin Res* 2017;10:137-41.
11. Sallehuddin NA, Abdul-Hamid A, Salleh SZ, Abdul-Majid N, Halim HH, Ramli NS, et al. Ergogenic, anti-diabetic and antioxidant attributes of selected Malaysian herbs: Characterisation of flavonoids and correlation of functional activities. *Int Food Res J* 2020;27:197-207.
12. Tagrida M, Benjakul S. Ethanolic extract of Betel (*Piper betle* L.) and Chapflu (*Piper sarmentosum* Roxb.) dechlorophyllized using sedimentation process: Production, characteristics, and antioxidant activities. *J Food Biochem* 2020;44:e13508.
13. Sulaiman SF, Sajak AAB, Ooi KL, Seow EM. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. *J Food Compos Anal* 2011;24:506-15.
14. Hafizah AH, Zaiton Z, Zulkhairi A, Mohd Ilham A, Nor Anita MM, Zaleha AM. *Piper sarmentosum* as an antioxidant on oxidative stress in human umbilical vein endothelial cells induced by hydrogen peroxide. *J Zhejiang Univ Sci B* 2010;11:357-65.
15. Ghazali NF, Mustafa FF, Tengku Muhammad TS, Ibrahim MA. Antioxidant and cytotoxicity activity of phenolic compounds from *Piper sarmentosum* Roxb. against T47D human breast cancer cell. *Nat Prod J* 2020;9:1-8.
16. Tangkanakul P, Trakoontivakorn G, Saengprakai J, Auttaviboonkul P, Niyomwit B, Lowvitoon N, et al. Antioxidant capacity and antimutagenicity of thermal processed Thai foods. *Jpn Agric Res Q* 2011;45:211-8.
17. Jusoh HM, Haron N, Zohari SH. Total phenolics contents and free radical scavenging capacity of *Piper sarmentosum* (Pucuk kaduk) extract. *Int J Allied Health Sci* 2019;3:876-83.
18. Tangkanakul P, Trakoontivakorn G. Lipophilic and hydrophilic antioxidant capacities of vegetables, herbs and spices in eighteen traditional Thai dishes. *Agric Nat Resour* 2014;48:214-26.
19. Wongsap P, Chaiwarit J, Zamaludien A. *In vitro* screening of phenolic compounds, potential inhibition against α -amylase and α -glucosidase of culinary herbs in Thailand. *Food Chem* 2012;131:964-71.
20. Zhang Y, Zhang H. Determinations of five kinds of secondary metabolites from different parts of *Piper sarmentosum* Roxb. *Med Plant* 2016;7:1-6.
21. Mohd Shafie NS. Characterization and Optimization of Bioactive Compounds from Herbal Plants Using Microwave Assisted Infusion (Doctoral Dissertation, Universiti Tun Hussein Onn Malaysia); 2020. Available from: <http://eprints.uthm.edu.my/id/eprint/13840/1/24p%20NOR%20SHAFAWATI%20MOHD%20SHAFIE.pdf>. [Last retrieved on 2020 Dec 23].
22. Wan Yahaya WA, Abu Yazid N, Mohd Azman NA, Almajano MP. Antioxidant activities and total phenolic content of Malaysian herbs as components of active packaging film in beef patties. *Antioxidants* 2019;8:204.
23. Khoddami A, Wilkes MA, Roberts TH. Techniques for analysis of plant phenolic compounds. *Molecules* 2013;18:2328-75.
24. Das S, Ray A, Nasim N, Nayak S, Mohanty S. Effect of different extraction techniques on total phenolic and flavonoid contents, and antioxidant activity of betelvine and quantification of its phenolic constituents by validated HPTLC method. *3 Biotech* 2019;9:37.
25. Suwal S, Marciniak A. Technologies for the extraction, separation and purification of polyphenols – A review. *Nepal J Biotechnol* 2018;6:74-91.
26. Hussain K, Ismail Z, Sadikun A, Ibrahim P. Antioxidant, anti-TB activities, phenolic and amide contents of standardised extracts of *Piper sarmentosum* Roxb. *Nat Prod Res* 2009;23:238-49.
27. Ugusman A, Zakaria Z, Hui CK, Nordin NA, Mahdy ZA. Flavonoids of *Piper sarmentosum* and its cytoprotective effects against oxidative stress. *EXCLI J* 2012;11:705-14.
28. Sangchan A. Inhibitory Effect of Thai Vegetable Extracts on Activity of Lipase, Alpha-Amylase, Trypsin and Alcohol Dehydrogenase (Doctoral Dissertation, Burapha University); 2018. Available from: http://digital_collect.lib.buu.ac.th/dcms/files/56910052.pdf. [Last retrieved on 2020 Dec 23].
29. Tuekaew J, Siriwatanametanon N, Wongkrajang Y, Tamsiririrkkul R, Jantan I. Evaluation of the antioxidant activities of Ya-hom Intajak, a Thai herbal formulation, and its component plants. *Trop J Pharm Res* 2014;13:1477-85.
30. Daduang J, Daduang S, Hongsprabhas P, Boonsiri P. High phenolics and antioxidants of some tropical vegetables related to antibacterial and anticancer activities. *Afr J Pharm Pharmacol* 2011;5:608-15.
31. Kasemweerasan P. Antioxidant Activity of *Piper sarmentosum* Roxb. and Its Effect on the Degradation of Frying Oils (Doctoral Dissertation, University of Leeds); 2015. Available from: <https://etheses.whiterose.ac.uk/12042/1/Pattanan%20final%20thesis%20hardbound%20version%20approved%2022%20Jan%202016.pdf>. [Last retrieved on 2020 Dec 16].
32. Mohd Shukri MA, Mirfat AH, Erny Sabrina MN, Razali M, Salma I. Nutritional value and potential of Malaysian under utilised fruits and traditional vegetables. *Acta Hort* 2013;979:173-85.
33. Lee KH, Padzil AM, Syahida A, Abdullah N, Zuhainis SW, Maziah M, et al. Evaluation of anti-inflammatory, antioxidant and anti-nociceptive activities of six Malaysian medicinal plants. *J Med Plants Res* 2011;5:5555-63.
34. Chan EW, Tan YP, Chin SJ, Gan LY, Kang KX, Fong CH, et al. Antioxidant properties of selected fresh and processed herbs and vegetables. *Free Radic Antioxid* 2014;4:39-46.
35. Lee JH, Cho S, Paik HD, Choi CW, Nam KT, Hwang SG, et al. Investigation on antibacterial and antioxidant activities, phenolic and flavonoid contents of some Thai edible plants as an alternative for antibiotics. *Asian Australas J Anim Sci* 2014;27:1461-8.
36. Ali A, Lim XY, Chong CH, Mah SH, Chua BL. Ultrasound-assisted extraction of natural antioxidants from betel leaves (*Piper betle*): Extraction kinetics and modeling. *Sep Sci Technol* 2018;53:2192-205.
37. Zarai Z, Boujelbene E, Salem NB, Gargouri Y, Sayari A. Antioxidant and antimicrobial activities of various solvent extracts, piperine and piperic acid from *Piper nigrum*. *LWT Food Sci Technol* 2013;50:634-41.
38. Alara OR, Abdurahman NH, Mudalip SK, Olalere OA. Characterization and effect of extraction solvents on the yield and total phenolic content from *Vernonia amygdalina* leaves. *J Food Meas Charact* 2018;12:311-6.
39. Nahak G, Sahu RK. Phytochemical evaluation and antioxidant activity of *Piper cubeba* and *Piper nigrum*. *J Appl Pharm Sci* 2011;1:153.
40. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Linnophila aromatica*. *J Food Drug Anal* 2014;22:296-302.