**Food Research 2 (1) : 119 - 123 (February 2018)**
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

# Antioxidant activities of Rambutan (*Nephelium lappaceum* L) peel *in vitro*

[1]Mistriyani, [1]Riyanto, S. and [1,2]*Rohman, A.

[1]*Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*
[2]*Research Center of Halal Products, Universitas Gadjah Mada, Yogyakarta 55281 Indonesia*

***Article history:***
Received: 9 August 2017
Received in revised form: 1 September 2017
Accepted: 2 September 2017
Available Online: 28 September 2017

***Keywords:***
Rambutan peel,
ABTS,
FRAP,
Antioxidant activity

***DOI:***
https://doi.org/10.26656/fr.2017.2(1).150

**Abstract**

Rambutan, *Nephelium lappaceum* L, peel due to consumption of fresh rambutan fruit is taken into account as waste, therefore the exploration of rambutan peel as a natural antioxidant is highly needed. The aim of this study is to investigate the antioxidant activity of rambutan peel from two cultivars (Aceh and Binjai) using ABTS radical assay of and ferric reducing activity power (FRAP) and to correlate with total phenolics and flavonoids. The powdered rambutan peel is extracted using maceration technique using methanol as extracting solvent. The methanolic extract is added with warm water and fractionated using petroleum ether, chloroform, and ethyl acetate to get corresponding fractions. Rambutan cultivar Binjai revealed the higher ABTS antiradical activity than that of cultivar Aceh. Furthermore, among methanolic extract and its fraction, ethyl acetate fraction exhibited the highest antiradical activity using ABTS radical with IC50 values of 3.10 µg/mL and 0.77 µg/mL for Aceh and Binjai, respectively. The ethyl acetate fraction also revealed the highest FRAP values of 1424.897 ± 28.56 µg/mg fraction sample (Aceh) and 968.57± 7.48 µg/mg fraction sample (Binjai). These activities were correlated with phenolics and flavonoid contents. Rambutan peel exhibited strong antioxidant activities, contained high amounts of phenolics and flavonoid and is potential to be developed as a functional food.

## 1. Introduction

Rambutan (*Nephelium lappaceum* L.), family of *Sapindaceae* is important tropical fruit, widely cultivated in south East Asian such as Malaysia, Thailand, and Indonesia. In Indonesia, some cultivars of Rambutan existed with different characteristics, namely Narmada, Rapiah, Sinyonya, garuda binjai, Kapulasan, lebak bulus, Si Batuk Ganal, Tangkue, Antalagi, Lebak, Bahrang Simacan, and Sibongkok. Two cultivars commonly available are Aceh and Binjai. In most Indonesian communities, Rambutan is consumed freshly and as consequence, it produces a lot of wastes from peel and seed, therefore some scientist develops rambutan peel and seed as functional food via assessing it with several activities, including antioxidant (Thitilertdecha *et al*., 2008; Saeed *et al*., 2012).

The community awareness toward the safety of synthetic antioxidant has attracted a scientist of antioxidant coming from plants (Thitilertdecha *et al.,* 2010). The antioxidant can be defined as any substances in low concentrations, derived from natural or synthetic, capable of delaying or inhibiting oxidation reaction significantly (Antolovich *et al*., 2002). Based on reaction mechanism, antioxidant assays can be group into five categories, namely radical scavenging assay, reducing power, chelating agent, lipid peroxidation reaction and synergist. These mechanisms are commonly exploited for assessment of antioxidant from natural sources.

Several biological activities of rambutan fruit and its part (peel and seed) along with chemical composition are reported such as antioxidant, antibacterial and anti-inflammation activities due to ellagic acid, corilagin and geraniin contained (Thitilertdecha *et al*., 2008; Palanisamy *et al*., 2011; Fidrianny *et al*., 2015a), hypoglycaemic (Soeng *et al*., 2015), antidiabetic (Soeng *et al*., 2015), and anticancer (Khonkarn *et al*., 2010). Therefore, the objective of this research is to evaluate the antioxidant activities of rambutan peel from two cultivars (Aceh and Binjai) using ABTS radical assay of

*Corresponding author.
Email: *abdul_kimfar@ugm.ac.id*

eISSN: 2550-2166 

and FRAP and to correlate with total phenolics and flavonoids.

## 2. Materials and methods

### *2.1 Materials*

Rambutan fruit cultivar Aceh and Binjaiwere were obtained from district Bantul, Yogyakarta, Indonesia. The authenticity of rambutan cultivars is carried out in the Laboratory of Pharmacognosy, Department of Pharmaceutical Biology, Universitas Gadjah Mada, Indonesia. 2,2-diphenyl-picrylhydrazyl (DPPH), rutin, quercetin, and gallic acid were obtained from Sigma (Aldrich, USA). The other solvents and reagents used were of pro-analytical grade obtained from E. Merck (Darmstat, Germany).2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4- diene chloride (TPTZ) are purchased from Sigma (Alldrich, USA). The solvents and other reagents are obtained from E. Merck (Darmstat, Germany).

### *2.2 Preparation of methanolic extract and its fraction of Rambutan peel*

Preparation of extract and fraction was done according to Permatasari and Rohman (2016). The peel of Rambutan (cultivars Aceh and Binjai) was washed, cut into small and dried under sun drying. The dried peel was powdered using powdering machine and subjected to extraction with methanol using maceration process. The macerate was filtered and evaporated using vacuum rotary evaporator. The methanolic extract was added with warm distilled water and is then fractionated using petroleum ether. The residue of methanol extract was then fractionated again using chloroform and ethyl acetate to get the fractions of PE, chloroform, ethyl acetate, and water. The initial methanolic extract and the fractions were used for antioxidant assay as well as for determination of phenolics and flavonoid.

### *2.3 Antiradical assay using ABTS radical*

The ABTS radical assay of extract and fractions of rambutan peel was carried out using the decolourization assay with $ABTS^+$ radical cation according to Arnao *et al*. (2001). The solution of ABTS 7 mM and potassium persulphate 2.45 mM were mixed in ratio 1:1 and allowed to stand in the dark for 12–16 h to produce stock solution of ABTS radical cation ($ABTS^+$). This solution was further diluted with methanol to attain absorbance of 0.600-0.800 at 734 nm. The $ABTS^+$ working solution (3 mL) and 30 µL of blank, standard or sample were mixed and the absorbance was measured at 734 nm after 6 min using a spectrophotometer. The blank was run with methanol.

### *2.4 Determination of reducing power*

Determination of ferric reducing activity power was determined according to Benzie and Strain (1996). FRAP reagent was prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4- diene chloride (TPTZ)(in 40 mmol/L HCl), and 20 mmol/L ferric chloride (10:1:1, v:v:v). To 4.5 mL reagent, 150 µL ethanol plant extract was added. The absorbance readings were started after 5 min and they were performed at 593 nm. The blank consisted of FRAP reagent. The final absorbance of each sample was compared with those obtained from the standard curve made from ferric sulphate ($FeSO_4 \times 7H_2O$) (200–1000 µmol/L). Results were expressed in nmol $Fe^{2+}$/mg dried extract.

### *2.5 Determination of total phenolics content*

The levels of total phenolics in samples was analysed using Colorimetric Method (Folin-Ciocalteau method) according to Chun *et al.* (2003), while flavonoid contents of extract and fractions were determined using aluminium chloride colorimetric method according to Zou *et al.* (2004).

### *2.6 Data analysis*

All data were analysed in triplicate and expressed as mean ± standard deviation using Excel (Microsoft Inc., USA).

## 3. Results and discussion

Among antioxidant mechanisms radical scavengers and ferric reducing activity power (FRAP) are the most reported ones for the evaluation of antioxidant capacity derived from plants. The radical of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt cation ($ABTS^+$) is widely used to screen antioxidant activities of plants (Zheng *et al*., 2016). In this assay, ABTS radical is generated by oxidation of ABTS with potassium persulfate to form the blue/green colour. When anti-radical is present, the ABTS radical is reduced, and the extent of decolourization is measured at a fixed operating time at a wavelength of 734 nm (Re *et al*., 1999).

Table 1 listed the $IC_{50}$ values of methanolic extract and its fraction using ABTS radical assay. In general, Rambutan cultivar Binjai exhibited the higher ABTS antiradical activity than that of cultivar Aceh due to high

 

phenolics contents present in cultivar Binjai. This can be seen from the lower values of $IC_{50}$ in cultivar Binjai than those in Aceh from the same extract and fractions. The lower value of $IC_{50}$, the higher antiradical activity is. Furthermore, among methanolic extract and its fraction, ethyl acetate fraction exhibited the highest antiradical activity using ABTS radical. These results are in agreement with those using DPPH as radical sources (Rohman *et al*., 2016). Therefore, it is suggested that ethyl acetate fraction is sub-fractionated further to obtain active isolate as anti-radical. Figure 1 revealed the relationship between the concentration of ethyl acetate fraction (*x-axis*) and ABTS antiradical activity of rambutan peel cultivar Aceh and Binjai (y-axis). The equation for such correlation is also included in such figure.

Table 1. The antiradical activity of methanol extract and its fraction using ABTS radical assay.

| Samples | $IC_{50}$ |
|---|---|
| Methanol extract cultivar Aceh | 57.94 µg/mL |
| Petroleum ether fraction cultivar Aceh | 93.39 µg/mL |
| Chloroform fraction cultivar Aceh | 72.16 µg/mL |
| Ethyl acetate fraction cultivar Aceh | 3.10 µg/mL |
| Water fraction cultivar Aceh | 243.18 µg/mL |
| Methanol extract cultivar Binjai | 0.76 µg/mL |
| Petroleum ether fraction cultivar Binjai | 6.98 µg/mL |
| Chloroform fraction cultivar Binjai | 0.76 µg/mL |
| Ethyl acetate fraction cultivar Binjai | 0.77 µg/mL |
| Water fraction cultivar Binjai | 0.52 µg/mL |

The ferric reducing antioxidant power (FRAP) method of methanolic extract and its fraction of rambutan is relied on the reduction of $Fe^{3+}$-tripyridyltriazinetripyridyltriazine ($Fe(TPTZ)^{3+}$) by antioxidant in acidic medium to produce complex $Fe^{2+}$-$Fe(TPTZ)^{2+}$ having intense blue color, which can be measured at wavelength 593 nm. Results are obtained as absorbance increases at 593 nm and can be expressed as mg $Fe^{2+}$ equivalents (Ozgen *et al*., 2006). Chemically, the reaction which takes place can be described as:

$Fe^{3+}$ + 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4- diene chloride (TPTZ) ⟶
complex $Fe^{3+}$-TPTZ (yellow)
↓ Antioxidant
$Fe^{2+}$-TPTZ (Prussion blue)
(wavelength = 593 nm)

Table 2 compiled antioxidant activity of methanol extract and its fraction using FRAP method. The results showed that among evaluated samples of rambutan cultivar Aceh, ethyl acetate fraction revealed the highest FRAP values of 1424.897±28.56 µg/mg samples. In addition, ethyl acetate fraction also exhibited the highest FRAP in rambutan cultivar Binjai with a value of 968.57± 7.48 µg/mg samples.

Table 2. The antioxidant activity of methanol extract and its fraction using FRAP assay.

| Samples | FRAP (µg/mg samples) |
|---|---|
| Methanol extract cultivar Aceh | 864.53 ±18.7 |
| Petroleum ether fraction cultivar Aceh | 132.29 ±28.56 |
| Chloroform fraction cultivar Aceh | 883.76 ±18.7 |
| Ethyl acetate fraction cultivar Aceh | 1424.897±28.56 |
| Water fraction cultivar Aceh | 328.31 ±28.56 |
| Methanol extract cultivar Binjai | 79.80± 3.31 |
| Petroleum ether fraction cultivar Binjai | 218.94± 9.18 |
| Chloroform fraction cultivar Binjai | 178.84± 4.92 |
| Ethyl acetate fraction cultivar Binjai | 968.57± 7.48 |
| Water fraction cultivar Binjai | 273.20± 3.55 |

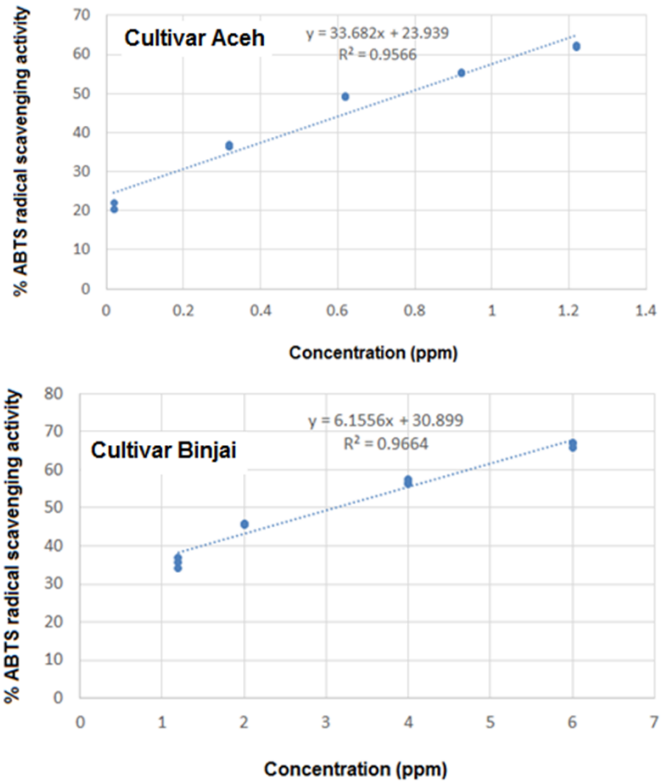


Figure 1. The relationship between concentration of ethyl acetate fraction(x-axis) with percentage of radical scavenging activity (y-axis). A = cultivar Aceh; (B) = cultivar Binjai.

A group of phenolics and flavonoids are deduced to be responsible for the antioxidant activities of plants (fruits and vegetables) due to its capability to provide radical hydrogen in ABTS assay and due to its properties as reducing agents (Prior *et al*., 1998), therefore the

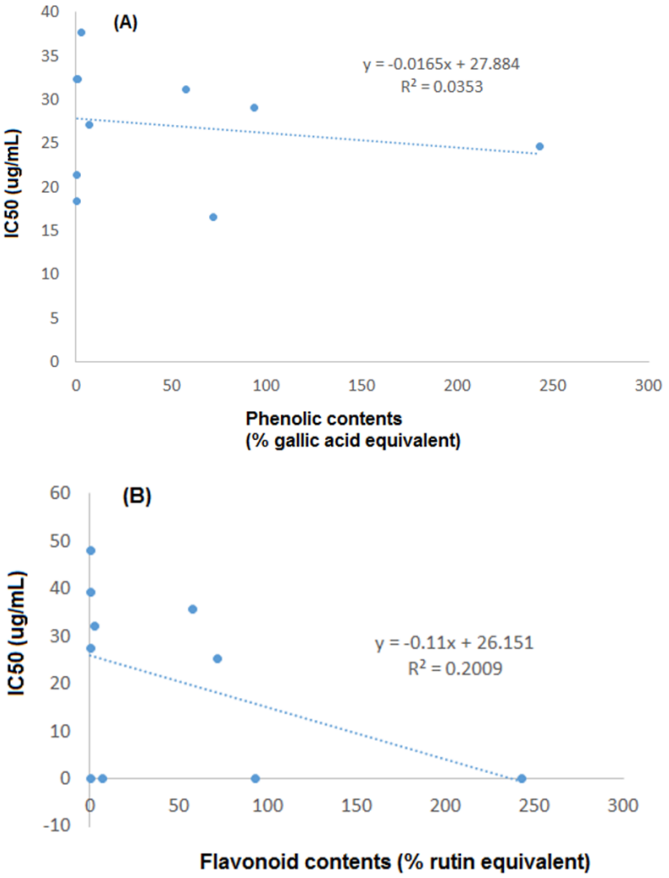


Figure 2. The correlation between (A) phenolic contents and (B) flavonoid contents (x-axis) with $IC_{50}$ values (y-axis) of extracts and fractions of Rambutan peel.

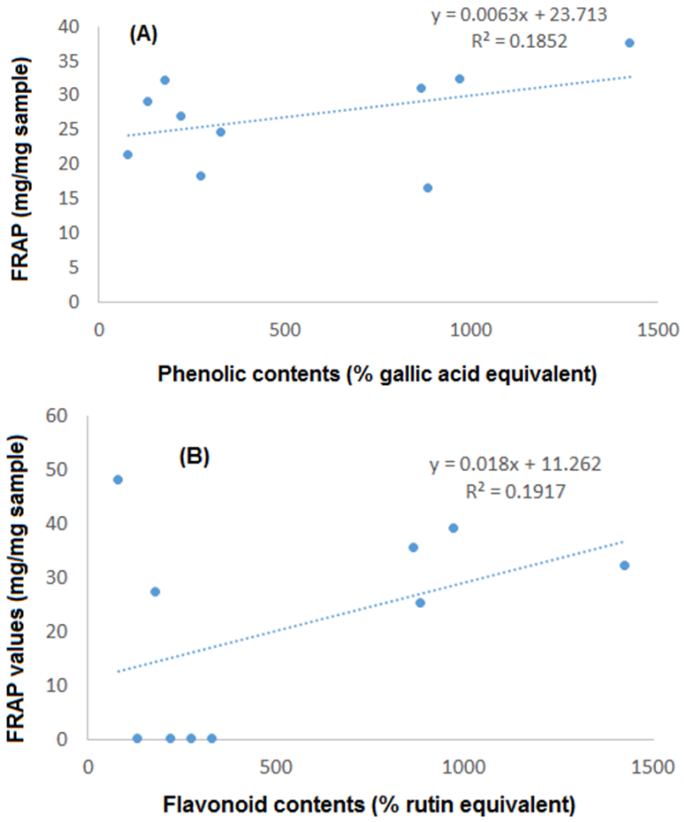


Figure 3. The correlation between (A) phenolic contents and (B) flavonoid contents (x-axis) with FRAP values (y-axis) of extracts and fractions of Rambutan peel.

antioxidants activities are frequently correlated with the contents of phenolics and flavonoid (Javanmardi *et al*., 2003; Rohman *et al*., 2006). Figure 1 and Figure 2 revealed the correlation between (A) phenolic contents and (B) flavonoid contents (x-axis) with $IC_{50}$ values (y-axis) of extracts and fractions of Rambutan peel cultivar Aceh and Binjai using ABTS assay (Figure 2) and FRAP (Figure 3). The coefficient of determination ($R^2$) is used as a means for relationship assessment. The R2 values obtained using ABTS assay are 0.0353 (phenolics) and 0.2009 (flavonoids), indicating that phenolics and flavonoids contributed to ABTS radical scavenging of 3.53% and 20.09%, respectively. In terms of FRAP, phenolics and flavonoids contributed 18.52% and 19.17%, respectively. From these results, it can be stated that ABTS radical scavenging activity and FRAP of extract and fractions of rambutan peel is not limited to phenolics and flavonoids. Antiradical activity may also come from the presence of other anti-radical components such as alkaloids, vitamins, carotenoids, and lignans.

## 4. Conclusion

Rambutan peel exhibited ABTS antiradical activity and reducing power, as determined using FRAP method. Ethyl acetate fraction showed the highest antiradical activity using ABTS radical and FRAP values. The correlation of phenolics and flavonoid contents toward antioxidant activities are relatively weak, meaning that other compounds are also responsible for those antioxidants. Rambutan peel is potential to be developed as a functional food.

## Acknowledgements

The authors thank to the Ministry of Research, Technology and Higher Education, Republic Indonesia for financial assistance during this study via English Thesis Scheme awarded to Prof. Dr. Abdul Rohman.

## References

Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S. and Robards, K. (2002). Methods for testing antioxidant activity. *Analyst*, 127, 183–198. https://doi.org/10.1039/b009171p

Arnao, M.B., Cano, A. and Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chemistry*, 73, 239–244. https://doi.org/10.1016/S0308-8146(00)00324-1

Benzie, I.F.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of

''antioxidant power'': the FRAP assay. *Analytical Biochemistry*, 239, 70–76. https://doi.org/10.1006/abio.1996.0292

Chun, O.K., Kim, D.O. and Lee, C.Y. (2003). Superoxide radical scavenging activity of the major polyphenols in fresh plums. *Journal of Agricultural and Food Chemistry*, 51, 8067-8072. https://doi.org/10.1021/jf034740d

Fidrianny, I., Sukowati, A. and Sukrasno, S. (2015). *In vitro* antioxidant activities of various leaves extracts from five varieties of rambutan (*Nephelium lappaceum*) and it's correlation with total flavonoid, phenolic, carotenoid content. *Asian Journal of Pharmaceutical and Clinical Research*, 8, 139-143.

Javanmardi, J., Stushnoff, C., Locke, E. and Vivanco, J.M. (2003). Antioxidant activity and total phenolic content of Iranian Ocimum accessions. *Food Chemistry*, 83, 547-550. https://doi.org/10.1016/S0308-8146(03)00151-1

Khonkarn, R., Okonogi, S., Ampasavate, C. and Anuchapreeda, S. (2010). Investigation of fruit peel extracts as sources for compounds with antioxidant and antiproliferative activities against human cell lines. *Food Chemical Toxicology*, 48, 2122–2129. https://doi.org/10.1016/j.fct.2010.05.014

Ozgen, M., Reese, R.N., Tulio, A.Z., Scheerens, J.C. and Miller, A.R. (2006). Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) methods. *Journal of Agricultural and Food Chemistry*, 54(4), 1151–1157. https://doi.org/10.1021/jf051960d

Palanisamy, U.D., Ling, L.T., Manaharan, T. and Appleton, D. (2011). Rapid isolation of geraniin from *Nephelium lappaceum* rind waste and its anti-hyperglycemic activity. *Food Chemistry*, 127, 21–27. https://doi.org/10.1016/j.foodchem.2010.12.070

Permatasari, L. and Rohman, A. (2016). 2,2'-diphenil-1-picrylhydrazil (DPPH) radical scavenging activity of extracts and fractions of Rambutan (*Nephelium lappaceum* L) peel. *Research Journal of Phytochemistry*, 10(2), 75-80. https://doi.org/10.3923/rjphyto.2016.75.80

Prior, R.L., Cao, G., Matin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G. and Mainland, C.M. (1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. *Journal of Agricultural and Food Chemistry*, 46, 2686–2693. https://doi.org/10.1021/jf980145d

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical in Biology and Medicine*, 26(9), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rohman, A., Riyanto, S. and Utari, D. (2006). Antioxidant activities, total phenolic and flavonoid contents of ethyl acetate extract of Mengkudu (*Morinda citrifolia*, L) fruit and its fractions. *Indonesia Journal of Pharmacy*, 17(3), 136– 142.

Rohman, A., Riyanto, S., Mistriyani, M., Shuhaera, S. and Nugroho, A.E. (2016). Antiradical Activities of Rambutan Peel: Study from Two Cultivars. *Research Journal of Phytochemistry*, 11(1), 42-47. https://doi.org/10.3923/rjphyto.2017.42.47

Saeed, N., Khan, M.R. and Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complementary and Alternative Medicine*, 12, 221-228. https://doi.org/10.1186/1472-6882-12-221

Soeng, S., Evacuasiany, E., Widowati, W. and Fauziah, N. (2015). Antioxidant and hypoglycemic activities of extract and fractions of Rambutan seeds (*Nephelium lappaceum* L.). *Biomedical Engineering*, 1, 13-18.

Thitilertdecha, N., Teerawutgulrag, A. and Rakariyatham, N. (2008). Antioxidant and antibacterial activities of Nephelium lappaceum L. extracts. *LWT Food Science and Technology*, 41, 2029–2035. https://doi.org/10.1016/j.lwt.2008.01.017

Thitilertdecha, N., Teerawutgulrag, A., Kilburnand, J.D. and Rakariyatham, N. (2010). Identification of major phenolic compounds from *Nephelium lappaceum* L. and their antioxidant activities. *Molecules*, 15, 1453-1465. https://doi.org/10.3390/molecules15031453

Zheng, L., Zhao, M., Xiao, C., Zhao, Q. and Su, G. (2016). Practical problems when using ABTS assay to assess the radical-scavenging activity of peptides: Importance of controlling reaction pH and time. *Food Chemistry*, 192, 288–294. https://doi.org/10.1016/j.foodchem.2015.07.015

Zou, Y., Lu, Y. and Wei, D. (2004). Antioxidant activity of flavonoid-rich extract of *Hypericum perforatum* L *in vitro*. *Journal of Agricultural and Food Chemistry*, 52, 5032-5039. https://doi.org/10.1021/jf049571r

eISSN: 2550-2166 