Asian Journal of Chemistry; Vol. 32, No. 7 (2020), 1741-1745

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22265

Effect of Extraction Conditions on Total Polyphenol and Flavonoid Content of Sugar Apple Seeds (*Annona squamosa* L.)

VAN THUY NGUYEN[1,2], NGOC QUY NGUYEN[1,2], NGUYEN VAN MUOI[3] and MAI HUYNH CANG[4,*]

[1]Center of Excellence for Biochemistry and Natural Products, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
[2]NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
[3]College of Agriculture, Can Tho University, Can Tho City, Vietnam
[4]Department of Chemical Engineering & Processing, Nong Lam University, Ho Chi Minh City, Vietnam

*Corresponding authors: E-mail: maihuynhcang@hcmuaf.edu.vn

Received: 6 June 2019; *Accepted*: 12 April 2020; *Published online*: 27 June 2020; AJC-19941

The objective of this study was to determine the optimal value of factors affecting the extraction of phenolic compounds from sugar apple seeds. The effects of solvent nature (ethanol, methanol and water), solvent concentration (40, 60,80 and 100%), solid-to-solvent ratio (2.5/100, 5.0/100, 7.5/100 and 10 mg/100 mL), extraction temperature (40, 50, 60 and 70 ºC) and extraction time (60, 120, 180 and 240 min) were investigated. Total phenolic content and total flavonoid content were used for the determination of phenolic compounds of sugar apple seeds extract. Experimental results showed that all the examined parameters had statistically significant ($p < 0.05$) effects on phenolic compound extraction. The optimal extraction conditions were as follows: extracting solvent 80% ethanol, sample-to-solvent ratio 5 g/100 mL, extraction contact time 120 min and extraction temperature 60 ºC with values of 356.92 ± 9.27 mgGAE/100g DW, 240.03 ± 6.16 mgQE/100g DW for the total phenolic content and the total flavonoid content, respectively.

Keywords: Extraction conditions, Total polyphenol, Total flavonoid, Sugar apple seeds (*Annona squamosa* L.).

INTRODUCTION

Nowadays, natural products become more and more popular in many topics of scientific researches due to their chemical composition, following with two main reasons: applications as natural options for food ingredients and significant influences on human health based on their antioxidant characteristics [1-8]. Sugar apple seeds (*Annona squamosa* L.) belongs to the Annonaceae family which cultivated mainly in Malaysia, Laos, Thailand and Vietnam [9]. The previous phytochemical investigations made on the plant have proved that they possess a wide variety of compounds like acetogenins, diterpenes, alkaloids, phenolics, flavonoids and lignans [10-12]. The previous study reports that various extracts of *Annona squamosa* play an essential role in against *Tribolium castaneum* (Herbst) and mosquito due to antimicrobial and larvicidal activities [13,14].

Previous studies have demonstrated the function of the aqueous leaf extract of *Annona squamosa* to ameliorate hyperthyroidism [15]. In recent day, natural antioxidant agents have receiving a great deal of scientific attention due to their ability to scavenge free radicals [16]. In natural, there are three main types of plant chemicals including alkaloids, terpenoids, and phenolic metabolites [17]. Among these three groups, phenolic compounds play a vital role in dietary applications and extensively researched [18]. Mainly, phenolic compounds include flavonoids, polyphenols and phenolic acids, which protects fruits, vegetables, plants from oxidative damage. Discovering new and safe antioxidants from natural sources become a great interest for applications in functional foods [19].

There are different techniques to obtain antioxidants from plants including microwave-assisted extraction, Soxhlet extraction, maceration and so on. Moreover, extraction yield depends not only the extraction method but also the many other extract conditions [20]. Aside from population studies, previous studies have highlighted the function of extraction on leaves and seeds of sugar apple. However, relatively little is explored about the optimization of extraction of phenolic antioxidants from sugar

apple seeds. Thus, this study was aimed to investigate the effects of solvents (distilled water, ethanol, and methanol), solvent concentration (40, 60, 80 and 100%), extraction time (60, 120, 180 and 240 min), solid-to-solvent ratio (2.5/100, 5.0/100, 7.5/100 and 10 mg/100 mL) and extraction temperature (40, 50, 60 and 70 ºC) on the extraction of phenolic compounds from sugar apple seeds using single factor experiment. The results of the study will provide scientific data on the conditions of the extraction of sugar apple seeds to obtain the highest polyphenol and flavonoid content.

EXPERIMENTAL

Plant material: *Annona squamosa* L. seeds were collected from Duyen Hai district, Tra Vinh province, Vietnam in January 2019. First, the seeds were removed. They then were washed with water and kept on absorbent paper towels at room temperature to dry to moisture contents of 10% and ground to powder.

Extraction conditions: To evaluate the effects of extraction conditions, *Annona squamosa* L. was extracted with 40 mL of solvent at the appropriate extraction conditions of temperature, time and solid to solvent ratio. Parameters such as the effects of solvent nature (ethanol, methanol and water), solvent concentration (40, 60, 80, and 100%), solid-to-solvent ratio (2.5/100, 5.0/100, 7.5/100 and 10 mg/100 mL), extraction temperature (40, 50, 60 and 70 ºC) and extraction time (60, 120, 180 and 240 min).

Total polyphenol content (TPC): Based on the method of Pham *et al*. [21], total polyphenol content was determined. First, an extract (0.5 mL) was pipetted into a test tube containing 2.5 mL Folin-Ciocalteu reagent 10% v/v. After 5 min, 2 mL Na_2CO_3 20% (w/v) was added to the sample. Next, the mixture was vigorously shaken and incubated for 30 min in the dark. Finally, the absorbance was spectrophotometrically measured at 765 nm and the results were shown in mg gallic acid equivalents per 100 gram of dried weight (mg GAE/100g DW).

Total flavonoid content (TFC): Based on the aluminum chloride colorimetric method, the total flavonoid content was determined [22]. Mixing 0.5 mL the extract with 0.1 mL 10% $AlCl_3$. Then, 0.1mL of 1M CH_3COOK and 4.3 mL distilled water was added and vigorously shaken. The absorbance was spectrophotometrically measured at 415 nm and the results were shown in mg quercetin equivalents per 100 g of dried weight (mg QE/100g DW).

Statistical analyses: The data were analyzed by one-way ANOVA followed by using Fisher's Least Significant Difference (LSD) test using Statgraphics Centurion XV Version 15.0. Differences were considered statistically significant at $p < 0.05$ for all tests.

RESULTS AND DISCUSSION

The amounts of TPC and TFC in sugar apple seeds were measured by referring to the calibration curves of gallic acid ($y = 0.017x + 0.0229$, $R^2 = 0.9990$) and quercetin ($y = 0.0094x - 0.0118$, $R^2 = 0.9991$), respectively.

Effect of solvent nature: Fig. 1 illustrates the effect of the solvent nature on total polyphenol content and total flavonoid content of sugar apple seeds. All solvents can extract phenolic compounds from sugar apple seeds. Ethanolic extract showed the highest polyphenol and flavonoid contents with values of 234.17 ± 9.25 mg GAE/100g DW and 189.15 ± 3.74 mgQE/100g DW, respectively, followed by methanol (232.01 ± 8.40 mg GAE/100g DW and 183.90 ± 6.50 mgQE/100g DW) and water extracts (113.89 ± 9.79 mg GAE/100g DW and 84.90 ± 8.99 mgQE/100g DW). There was no significant difference ($p > 0.05$) between the content of polyphenols and flavonoids obtained in ethanol and methanol solution. Therefore, ethanol was taken as the extraction solvent for the next studies.

Effect of solvent concentration: In this study, ethanol at different concentrations was used as the extracting solvent. Fig. 2 shows the effect of the solvent concentration (40, 60, 80 and 100%) on total polyphenol content and total flavonoid content of sugar apple seeds. The highest TPC value was achieved at solvent concentration of 80% (230.47 ± 4.03 mg GAE/100g DW) and 100% (216.29 ± 6.30 GAE/100g DW), respectively. The highest TFC value was achieved at solvent concentration

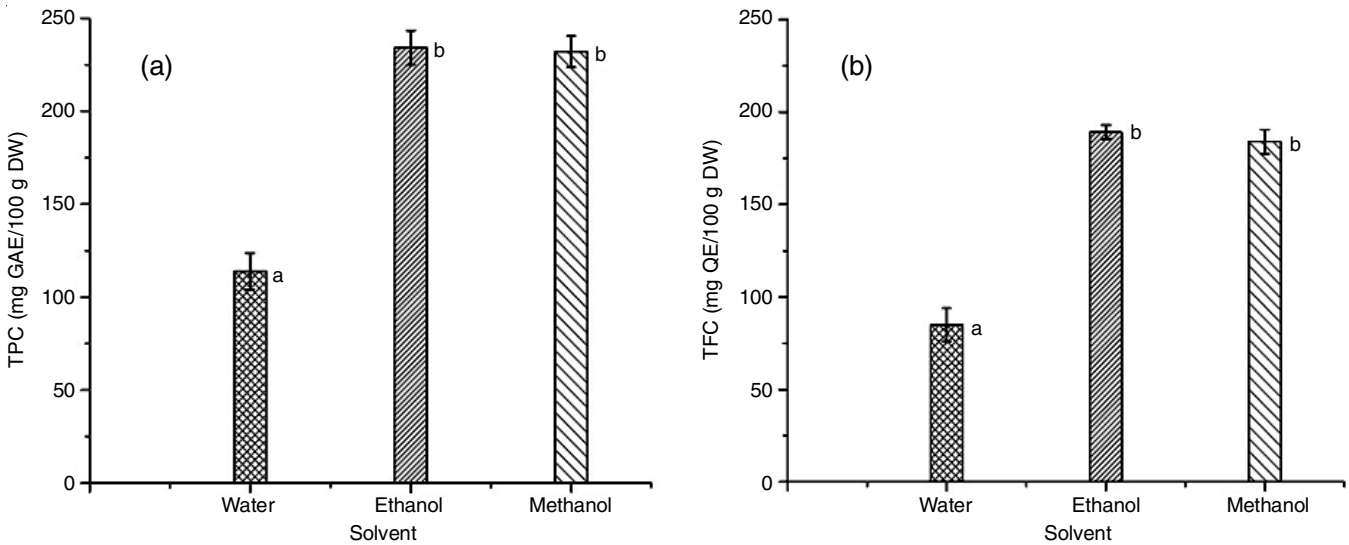

Fig. 1. Effect of the solvent nature on total polyphenol content (a) and total flavonoid content (b) of sugar apple seeds

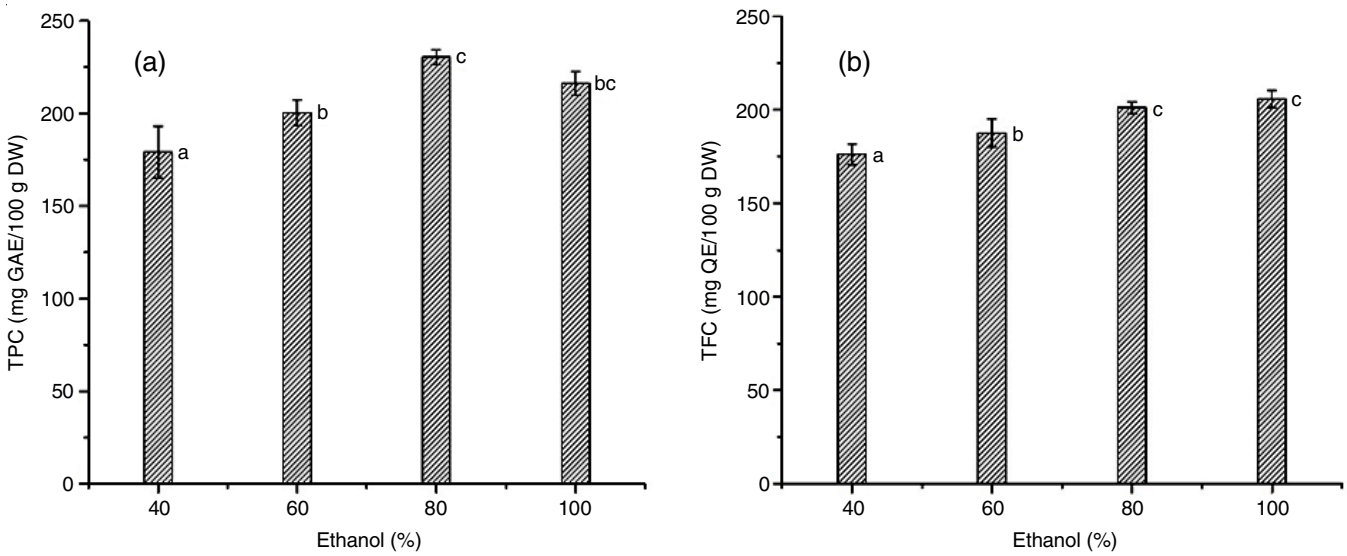

Fig. 2. Effect of the solvent concentration on total polyphenol content (a) and total flavonoid content (b) of sugar apple seeds

of 80% (201.17 ± 3.25 mg QE/100g DW) and 100% (205.80 ± 4.56 mg QE/100g DW), respectively. However, there is no significant difference ($p < 0.05$) observed in both the solvent concentration between 80% to 100%. Therefore, 80% of ethanol concentration is an optimal factor for the next studies.

Effect of sample-to-solvent ratio: In many cases, a large volume of solvent is also used for extraction and recuperation of extracts, which can be a significant problem in terms of environmental considerations. Fig. 3 demonstrated the effect of the sample-to-solvent ratio on total polyphenol content and total flavonoid content of sugar apple seeds. The optimal phenolic compound extraction was obtained with a ratio of 5 mg/100 ml with 263.16 ± 6.56 mg GAE/100g DW for TPC and 211.04 ± 6.67 mgQE/100g DW for TFC. Therefore, based on the graph we choose the liquid/solid ratio of 5 mg/100 mL.

Effect of extraction temperature: In order to determine the influence of temperature on the extraction efficiency of sugar apple seeds, the extractions were taken between temperature ranges of 40 and 70 ºC. The highest TPC and TFC value was achieved at extraction temperatures of 60 ºC (328.24 ± 4.75 mg GAE/100g DW for TPC; 232.94 mg QE/100g DW for TFC) and 70 ºC (328.85 ± 7.48 mg GAE/100g DW for TPC; 233.86 ± mg QE/100 DW for TFC), respectively (Fig. 4). Previous studies [23] demonstrates that TPC increased at elevated temperature due to increased mass transfer and diffusion rate. However, the over increasing the temperature may raising possible concurrent decomposition of phenolic compounds. Moreover, high temperature may promote solvent loss vapourization. Thus, the optimal extraction temperature for sugar apple seeds was of 60 ºC.

Effect of extraction contact time: Extraction time plays an essential role in minimizing the cost and energy of the extraction process. Fig. 5 shows effect of extraction time on TPC and TFC of sugar apple seeds and the level of TPC and TFC tends to decrease with prolonged extraction time. As shown in Fig. 5, total polyphenol and flavonoid contents increased from 60 to 120 min with the highest values of 356.92 ± 9.27 mgGAE/100g DW for TPC, 240.03 ± 6.16 mgQE/100g DW

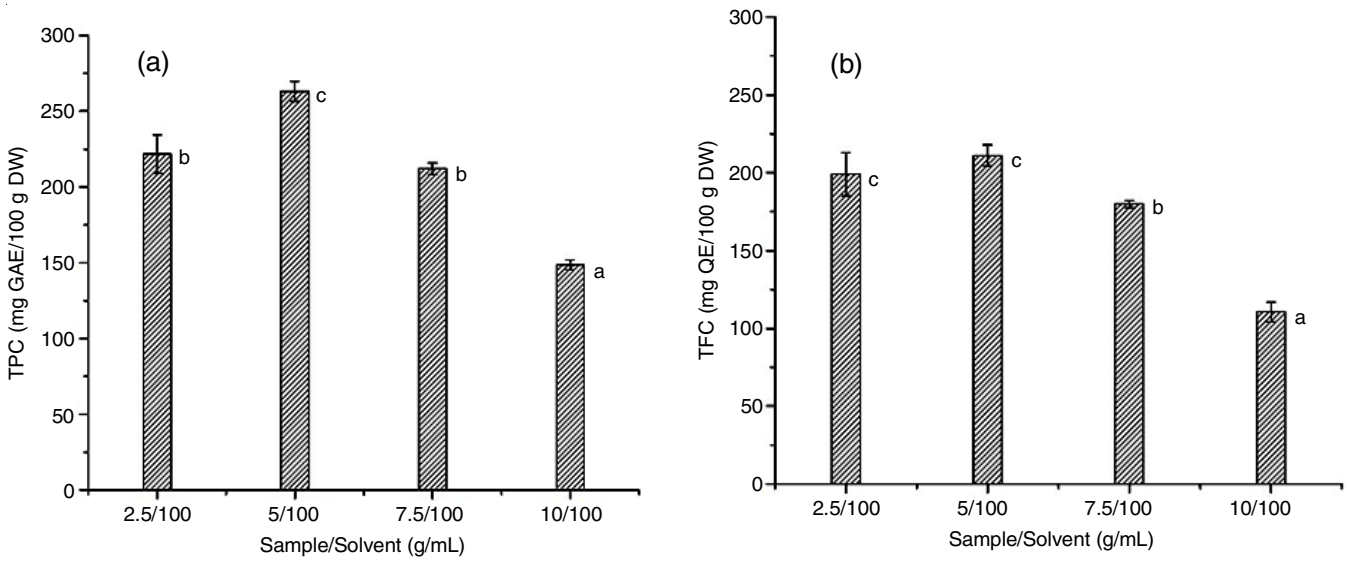

Fig. 3. Effect of the sample-to-solvent ratio on total polyphenol content (a) and total flavonoid content (b) of sugar apple seeds

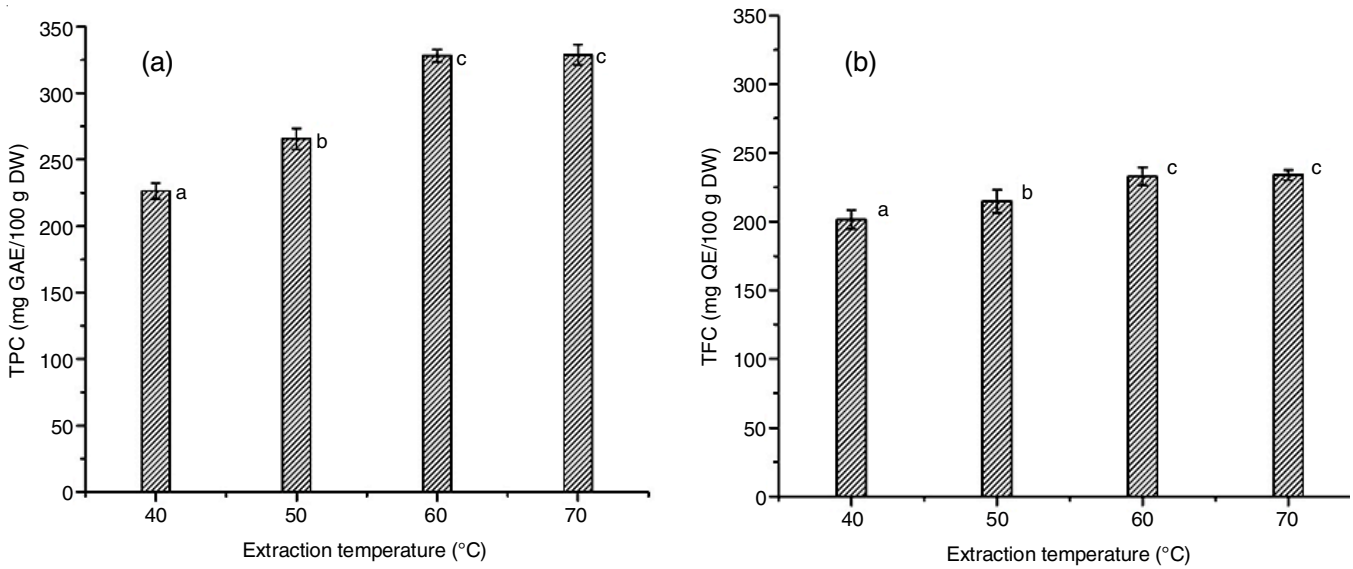

Fig. 4. Effect of the extraction temperature on TPC (a) and TFC(b) of sugar apple seeds

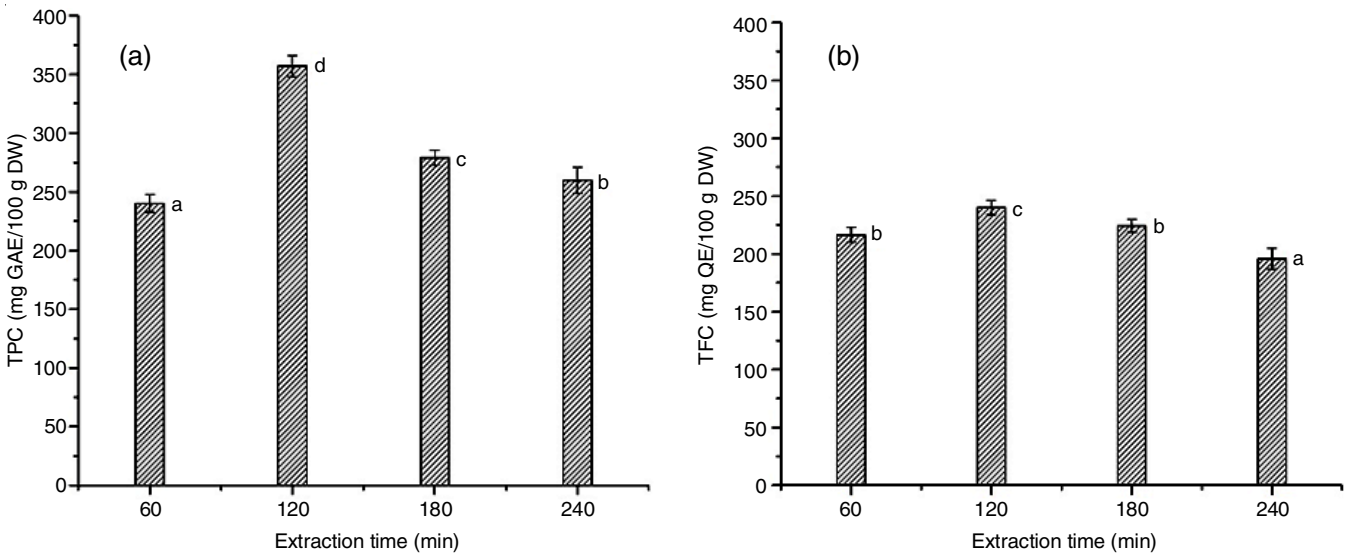

Fig. 5. Effect of the extraction time on total polyphenol content (a) and total flavonoid content (b) of sugar apple seeds

for TFC. After 120 min, the values fell significantly ($p < 0.05$). Therefore, an extraction time of 60-240 min was selected.

Conclusion

In this study, the single factor experiments determined for recognizing the optimum condition of each independent variable influencing the total phenolic content and total flavonoid content of sugar apple seeds (*Annona squamosa* L.), namely solvent nature, solid-to-solvent ratio, the solvent concentration, extraction time and temperature. Commonly, high extraction yield was achieved using 80% aqueous ethanol as the solvent, sample-to-solvent ratio 5g/100 mL, and the extraction yield could further be improved using a prolonged time of 120 min and a higher incubation temperature of 60 ºC. Under these optimized conditions, the maximum experimental yield of total phenolic content and the total flavonoid content was 356.92 ± 9.27 mgGAE/100g DW and 240.03 ± 6.16 mgQE/100g DW, respectively.

ACKNOWLEDGEMENTS

This study was supported by grants from Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. D.-P. Xu, Y. Li, X. Meng, T. Zhou, Y. Zhou, J. Zheng, J.-J. Zhang and H.-B. Li, *Int. J. Mol. Sci*., **18**, 96 (2017); https://doi.org/10.3390/ijms18010096
2. S.C. Lourenço, M. Moldão-Martins and V.D. Alves, *Molecules*, **24**, 4132 (2019); https://doi.org/10.3390/molecules24224132
3. C.M. Hasler, *J. Nutr*., **132**, 3772 (2002); https://doi.org/10.1093/jn/132.12.3772

4. M. Kebede and S. Admassu, *Adv. Food Technol. Nutr. Sci. Open J.*, **5**, 38 (2019); https://doi.org/10.17140/AFTNSOJ-5-155
5. M.S. Brewer, *Comp. Rev. Food Sci. Food Saf.*, **10**, 21 (2011); https://doi.org/10.1111/j.1541-4337.2011.00156.x
6. A. Yashin, Y. Yashin, X. Xia and B. Nemzer, *Antioxidants*, **6**, 70 (2017); https://doi.org/10.3390/antiox6030070
7. B. Olas, *Front. Pharmacol.*, **9**, 78 (2018); https://doi.org/10.3389/fphar.2018.00078
8. J. Shipp and E.S.M. Abdel-Aal, *The Open Food Sci. J.*, **4**, 7 (2010); https://doi.org/10.2174/1874256401004010007
9. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants International Book Distributors: Deharadun, India, pp 68-69 (1987).
10. D.K. Yadav, N. Singh, K. Dev, R. Sharma, M. Sahai, G. Palit and R. Maurya, *Fitoterapia*, **82**, 666 (2011); https://doi.org/10.1016/j.fitote.2011.02.005
11. R. Baskaran, D. Pullencheri and R. Somasundaram, *Food Res. Int.*, **82**, 121 (2016); https://doi.org/10.1016/j.foodres.2016.02.001
12. H.M. Kotkar, P.S. Mendki, S.V. Sadan, S.R. Jha, S.M. Upasani and V.L. Maheshwari, *Pest Manag. Sci.*, **58**, 33 (2002); https://doi.org/10.1002/ps.414
13. O.A. Adoum, N.T. Dabo and M.O. Fatope, *Int. J. Pharmacogn.*, **35**, 334 (1997).
14. M.A. Malek and R.M. Wilkins, *Bangladesh J. Zool.*, **23**, 65 (1995).
15. P. Sunanda and K. Anand, *Curr. Sci.*, **84**, 1402 (2003).
16. N. Saeed, M.R. Khan and M. Shabbir, *BMC Complement. Altern. Med.*, **12**, 1174 (2012); https://doi.org/10.1186/1472-6882-12-221
17. J.B. Harborne, eds.: J.N. Walton and D.E. Brown, Classes and Functions of Secondary Products from Plants. In: Chemicals from Plants Perspectives on Plant Secondary Products, Imperial College Press: London, UK, pp. 1-25 (1999).
18. A. King and G. Young, *J. Am. Diet Assoc.,* **99**, 213e8 (1999).
19. Q. Diem Do, A.E. Angkawijaya, P.L. Tran-Nguyen, L.H. Huynh, F.E. Soetaredjo, S. Ismadji and Y. Ju, *Journal of Food and Drug Analysis*, **22**, 296 (2014).
20. J. Dai and R.J. Mumper, *Molecules*, **15**, 7313 (2010); https://doi.org/10.3390/molecules15107313
21. H.N.T. Pham, V. Tang Nguyen, Q. Van Vuong, M.C. Bowyer and C.J. Scarlett, *J. Food Process. Preserv.*, **41**, e12879 (2017); https://doi.org/10.1111/jfpp.12879
22. M. Mahboubi, N. Kazempour and A.R. Boland Nazar, *Jundishapur J. Nat. Pharm. Prod.*, **8**, 15 (2013); https://doi.org/10.17795/jjnpp-7621
23. T. Juntachote, E. Berghofer, F. Bauer and S. Siebenhandl, *Int. J. Food Sci. Technol.*, **41**, 121 (2006); https://doi.org/10.1111/j.1365-2621.2005.00987.x