European Journal of Food Science and Technology
Vol.3, No.5, pp. 15-21, November 2015
Published by European Centre for Research Training and Development UK (www.eajournals.org)

EFFECTS OF EXTRACTION SOLVENTS ON PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITIES OF WALNUT (*JUGLANS REGIA* L.) GREEN HUSK EXTRACTS

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ABSTRACT: *The effects of extraction solvents (hexane, ethyl-acetate, acetone, ethanol, methanol, and water) on the content of phytochemicals including total polyphenols, flavonoids and condensed tannins, as well as antioxidant activities of walnut green husk were investigated. The results showed that extraction solvents significantly affected phytochemicals content and antioxidant activities of walnut green husk. The acetone, ethanol, and methanol extracts had the higher content of phytochemicals, and they exhibited stronger antioxidant activities, followed by ethyl-acetate and water extracts, and the lowest for hexane extract. These results indicated that selective extraction from walnut green husk, by an appropriate solvent, is important for obtaining fractions with high antioxidant activity, which will be useful for the developing and application of walnut green husk.*

KEYWORDS: Walnut Green Husk, Extraction Solvent, Phytochemicals, Antioxidant Activity.

INTRODUCTION

The walnut (*Juglans regia* L.) is a tree traditionally cultivated for its valuable wood and fruits. Not only dry fruit are used but also green walnuts, shells, kernels, bark, green walnut husks and leaves have been used in both foods, cosmetic and pharmaceutical industry (Stampar *et al*., 2006). All those products can be used also as a significant source of different compounds expressing antioxidant and antimicrobial potential, as well antiproliferative, antinociceptive, antiasthmatic, hepatoprotective, antidiabetic, antifertility, anti-inflammatory, lipolytic and many others properties positively affected human health (Almeida *et al*., 2008; Oliveira *et al*., 2008; Zhang *et al*., 2009; Carvalho *et al*., 2010; Tajamul *et al*., 2014).

Walnut's green husk is one of the major waste products from the walnut production that nowadays has a scarce use. Some studies have demonstrated that they are rich in natural bioactive compounds (Cosmulescu *et al*., 2010), and possess many bioactivities including antioxidant activity and antimicrobial capacity against different pathogenic Gram positive bacteria (Oliviera *et al*., 2008; *Carvalho et al*., 2010; Fernandez-Agullo *et al*., 2013). For this reason, extracting bioactive compounds in walnut green husk is basic for further analysis and its development. Extract solvents is one of the most important factors affecting the chemical composition and biological activity of plant extracts (Turkmen *et al*., 2006; Rebey *et al*., 2012; Cheok *et al*., 2012). Then, the aim of this study was to analyze the effect of the extraction solvents of varying polarity (hexane, ethyl acetate, acetone, ethanol, methanol,

ISSN 2056 - 5798(Print), ISSN 2056 - 5801(online)

water) on phytochemical contents, antioxidant activity of walnut green husk extracts to provide a reference for the comprehensive development and utilization of walnut green husk.

MATERIALS AND METHODS

Regents and Standards: Gallic acid and 2, 4, 6-Tri (2-pyridyl)-s-triazine (TPTZ) is purchased from Fluka (Switzerland). 2,2'-azino-bis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salts (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), catechin and rutin were from Sigma (USA). All other chemicals and reagent used in the experiments were of analytical grade.

Preparation of Extracts: Fresh walnut green husk were grounded before the extraction process, and then twenty-five grams of walnut green husk were extracted with 250 mL solvents and kept in a shaker at 25°C for 1 h. And then the mixture was centrifuged at 5000 rpm/min at 4°C for 10 min. The precipitation extracted with 250 mL solvent once again and mixed supernatants. The extracts were vacuum–evaporated to dryness at 40 ^{o}C, and reconstituted with methanol to a final volume of 25 mL. Extraction solvent used in our experiment contains hexane, ethyl acetate, acetone, ethanol, methanol, and water.

Determination of Total Polyphenolic Content (TPC): Polyphenolic content of extracts was determined by method (Rebey *et al*., 2012). Briefly, an aliquot (0.1 mL) extract was mixed with 2.8 mL of distilled water and 0.1 mL of Folin-Ciocalteu reagent (1.0 mol/L), and then the solution was mixed and incubated at room temperature for eight minute. Following that, 2 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added and shaken thoroughly. The mixture was incubated for 2 h in the dark at room temperature and the absorbance was determined at 765 nm. Gallic acid was used for calibration of the standard curve and total phenolic content was expressed as milligram gallic acid equivalent per gram sample.

Determination of Total Flavonoid Content (TFC): The level of total flavonoid was measured as described by Rebey et al. (2012) with some modifications. Briefly, an aliquot (1 mL) extract was mixed with 0.3 mL of 3% $NaNO_3$ solution and incubated for 6 min. After that, 0.3 mL of 10% $Al(NO_3)_3$ was added and the solution was kept at room temperature for 6 min. Finally, mix the solution with 4 mL of 4% NaOH solution and add water to 10 mL. After 20 minute of incubation, the absorbance of the mixture at 510 nm was measured. Rutin was used for calibration of the standard curve, and flavonoid content was expressed as milligram rutin equivalents per gram sample.

Determination of Condensed Tannin Content (CTC): The content of condensed tannin was measured using Vanillin-hydrochloric acid method (Rebey *et al*., 2012). Briefly, 1.0 mL of extract was mixed with 5.0 mL of vanillin-solution dissolving in methanol (0.5 g vanillin dissolves in methanol which containing 4% hydrochloric acid). The mixture was kept in the dark at room temperature for 20 min and the absorbance was measured at 500 nm. The content of condensed tannin was expressed as catechin milligram equivalents per gram sample.

ISSN 2056 - 5798(Print), ISSN 2056 - 5801(online)

DPPH Radical Scavenging Assay: DPPH radical scavenging assay was measured following the method of Xu et al. (2012) with some modifications. Extract was serially diluted to different concentrations and 0.5 mL of diluted extract mixed with 2.5 mL of 60 μmol/L DPPH solution dissolving in methanol. The mixture was shaken thoroughly and incubated in the dark at room temperature for 30 min, and the absorbance was measured at 517 nm. The scavenging rate of DPPH radical was calculated according to the formula given below: DPPH radical scavenging ability (%) = $[A_0-(A_1-A_2)]/A_0\times100$, where A_0 is the absorbance of the control in which methanol substitutes extract and A_1 is the result of the mixture of the extract and DPPH radicals while A_2 is the absorbance of the mixture of the extract and 2.5 mL methanol to eliminate the color effect of the extract. The DPPH radical scavenging activity of was measured by IC_{50} value which represents the effective concentration of the extract at which DPPH radical scavenging ability up to 50%.

ABTS Radical Scavenging Assay: ABTS radical scavenging assay was determined according to the method of Xu et al. (2012). Briefly, a certain quality of ABTS and potassium persulfate was dissolved in water to keep the final concentrations of the two substances to be 7 mmol/L and 2.45mmol/L respectively. The mixture was kept in the dark for 16~24 h to make the ABTS radical working solution and its absorbance at 734 nm was adjusted to 0.700±0.050. The ABTS radical scavenging ability was measured by adding 50 μL of diluted extract to 1.9 mL of ABTS radical working solution and the absorbance at 734 nm after 6 min was recorded. The scavenging rate and IC_{50} value were calculated using the equation described for DPPH assay.

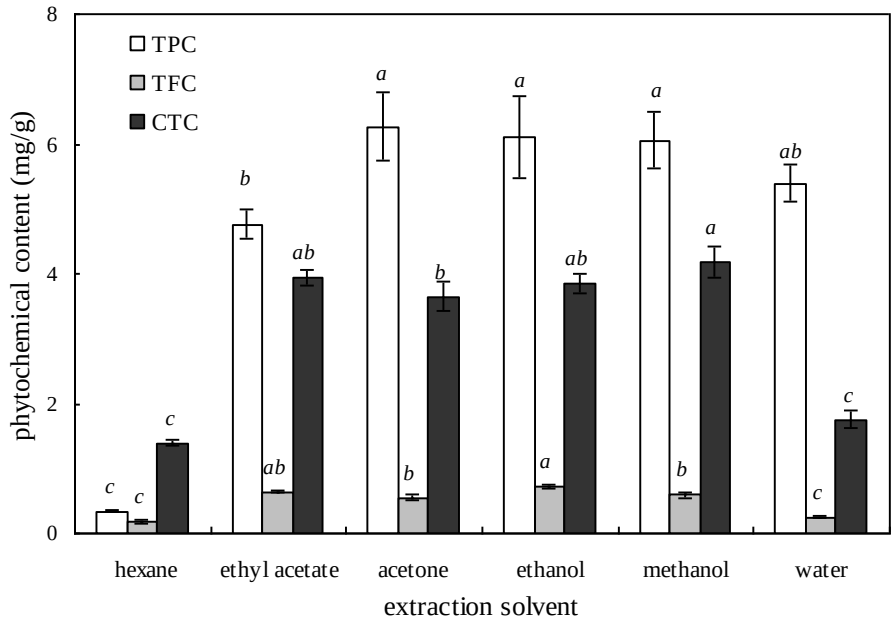
Ferric Reducing Antioxidant Power (FRAP) Assay: The reducing ability was determined by using FRAP assay described by Xu et al. (2012) with slight modifications. Briefly, the FRAP reagent was freshly prepared from 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3$ solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 ^oC in a water bath prior to use. Then 0.1 mL of samples was mixed with 1.8 mL of FRAP reagent and 3.1 mL ultrapure water. The absorption of the reaction mixture was measured at 593 nm after incubation for 30 min at 37 ^oC. Increased absorbance of the reaction mixture indicated increased reducing power.

RESULTS AND DISCUSSION

Effects of Solvents on Phytochemicals Content: The contents of phytochemicals of different solvent extracts were showed in Figure 1. Depending on the solvent used, the total polyphenol content ranged from 0.34 to 6.27 mg/g. Acetone extract had the highest polyphenol content, followed by ethanol, methanol, water, ethyl acetate and n-hexane. Among them, n-hexane extract was the lowest one and accounted for one-tenth of acetone extract. The extraction of total flavonoids was also influenced significantly by extracting solvent ($p < 0.05$), and its contents varied from 0.19 to 0.71 mg/g, respectively for hexane and ethanol. With regard to total flavonoid content, solvents could be sequenced in the

ISSN 2056 - 5798(Print), ISSN 2056 - 5801(online)

following decreasing order: ethanol>ethyl acetate>methanol>acetone>water>hexane. As regard to the condensed tannins, the content varied from 1.39 to 3.18 mg/g and their order was methanol>ethyl acetate>ethanol>acetone>water>hexane. As previously reported by



Rebey et al. (2012), these results indicates that the different solvents, as the differences in polarity, dispersibility and penetrability, could selectively extract different phytochemicals. Besides, the levels of total phenolics, flavonoids, and condensed tannins also could possibly be influenced by dielectric constant, chemical structure of organic solvents (Cheok *et al*., 2012) as well as chemical properties of plant phytochemicals (Jayaprakasha *et al*., 2003).

Figure 1. The contents of phytochemicals of different extracts

Effects of Solvents on DPPH and ABTS Scavenging Abilities: Due to the differences in the compositions and contents of the phytochemicals, the extracts showed the different antioxidant abilities. DPPH and ABTS radicals scavenging abilities of the extracts diluted to series of concentrations has been tested in our study and IC_{50} value of each sample could be calculated through regression equation. The lower IC_{50} value represents higher scavenging abilities. The Table 1 showed that there were significant differences of DPPH radical scavenging abilities of different solvent extracts. Ethanol extract of which IC_{50} value was 54.9 μg/mL owned the highest DPPH radical scavenging ability, followed by methanol and acetone. Water and hexane extracts had a poor DPPH radical scavenging ability, of which IC_{50} values were 103.0 and 143.8 μg/mL, respectively.

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Table 1. The scavenging activity of different extracts on DPPH radicals

	Extracts	Regression equation	Correlation coefficient (R^2)	IC_{50}/μg·mL^{-1}
DPPH	Methanol	y = 39.371Ln(x) + 164.04	0.9959	55.2
	Ethanol	y = 42.769Ln(x) + 174.16	0.9927	54.9
	Acetone	y = 32.829Ln(x) + 141.3	0.9905	62.0
	Ethyl acetate	y = 646.94x + 3.6	0.9992	71.7
	Hexane	y = 449.88x - 14.705	0.9893	143.8
	Water	y = 23.263Ln(x) + 102.87	0.9933	103.0

All of these extracts possessed some ABTS radical scavenging abilities in that their phytochemical components and contents might have some equivalence regarding to ABTS radical scavenging ability. Different from the results of DPPH (Table 2), IC_{50} values of acetone extract was 324.8 μg/mL, exhibiting the highest ABTS radical scavenging ability, followed by methanol, ethanol and ethyl acetate extracts, and the lowest for water extracts. However, IC_{50} values of hexane extract did not gain in it's the highest amount.

Table 2. The scavenging activity of different extracts on ABTS radicals

	Extracts	Regression equation	Correlation coefficient (R^2)	IC_{50}/μg·mL^{-1}
ABTS	Methanol	y = 126.72x + 2.1131	0.9890	377..9
	Ethanol	y = 124.64x - 0.2056	0.9869	402.8
	Acetone	y = 123.96x + 9.7354	0.9897	324.8
	Ethyl acetate	y = 126.72x + 2.1131	0.9791	377.9
	Hexane	y = 29.739x + 0.0079	0.9963	-
	Water	y = 106.18x + 3.3769	0.9988	439.1

Ferric Reducing Antioxidant Power (FRAP): Figure 2 showed that different walnut green husk extracts exhibited different ferric reducing power, but there was a significant difference ($p < 0.05$) in the reducing power among different extracts. The reducing power of extracts ranged from 0.024 to 0.509. The reducing power of acetone extracts was the highest, followed by ethanol, methanol, ethyl acetate, and water extracts, while the lowest for hexane extracts. The result suggested that extracts of walnut green husk had a remarkable potency to donate electron to reactive free radicals, converting them into more stable non-reactive species and terminating the free radical chain reaction.

ISSN 2056 - 5798(Print), ISSN 2056 - 5801(online)

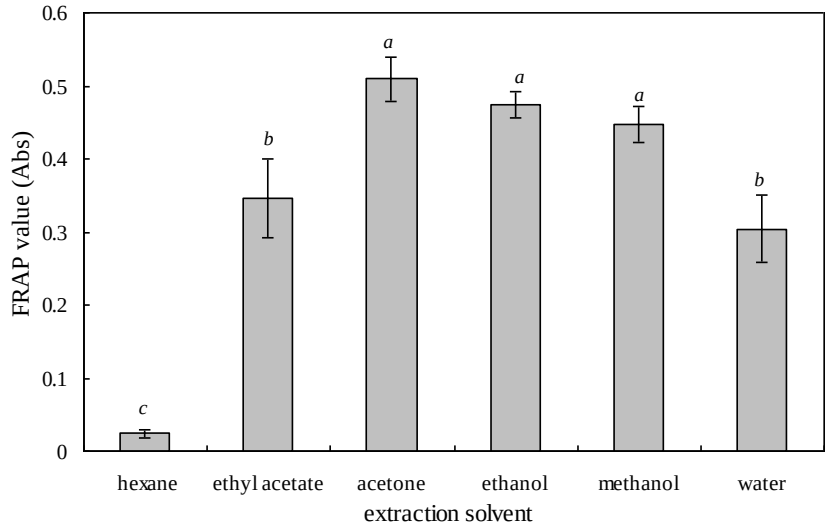

Figure 2. The reducing power of different solvent extracts

CONCLUSIONS

The extracting solvents significantly affected phytochemicals content and antioxidant activities of walnut green husk extracts. Our findings indicated that as compared to other extracts, ethanol, methanol, acetone extracts had the higher total polyphenol, flavonoid and condensed tannins content and no significant difference was found among them. Consistent with results described above, ethanol, methanol, acetone extracts showed the higher antioxidant abilities in DPPH, ABTS radicals scavenging assays and FRAP assay. In conclusion, these results indicated that selective extraction from natural sources, by an appropriate solvent, is important for obtaining fractions with high antioxidant activity and the development and utilization of walnut green husk.

REFERENCES

Almeida, I.F., Fernandes, E., Lima, J.L.F.C., Costa, P.C. and Bahia, M.F. (2008). Walnut (*Juglans regia*) leaf extracts are strong scavenger of pro-oxidant reactive species. Food Chemistry, 106: 1014–1020.

Carvalho, M., Ferreira, P.J., Mendes, V.S., Silva, R., Pereira, J.A., Jerónimo, C. and Silva, B.M. (2010). Human cancer cell antiproliferative and antioxidant activities of *Juglans regia* L. Food and Chemical Toxicology, 48: 441–447.

Cheok, C,Y., Chin, N.L., Yusof, Y.A. and Law, C.L. (2012). Extraction of total phenolic content from *Garcinia mangostana* Linn. hull. I. Effects of solvents and UV–Vis spectrophotometer absorbance method. Food and Bioprocess Technology, 5: 2928–2933.

Cosmulescu, S., Trandafir, I., Achim, G., Botu, M., Baciu, A. and Gruia, M. (2010). Phenolics of green husk in mature walnut fruits. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38(1): 53–56.

ISSN 2056 - 5798(Print), ISSN 2056 - 5801(online)

Fernandez-Agullo, A., Pereira, E., Freire, M.S., Valentao, P., Andrade, P.B., Gonzalez-Alvarez, J. and Pereira, J.A. (2013). Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts. Industrial Crops and Products, 42:126–132.

Jayaprakasha, G.K., Selvi, T. and Sakariah, K.K. (2003). Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. Food Research International, 36:117–122.

Oliveira, I., Sousa, A., Ferreira, I.C.F.R., Bento, A., Estevinho, L. and Pereira, J.A. (2008). Total phenols antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husk. Food and Chemical Toxicology, 46:2326–2331.

Rebey, I.B., Bourgou, S., Debez, I.B.S., Karoui, I.J., Sellami, I.H. and Msaada, K. (2012). Effects of extraction solvents and provenances on phenolic contents and antioxidant activities of cumin (*Cuminum cyminum* L.) seeds. Food and Bioprocess Technology, 5: 2827–2836.

Stampar, F., Solar, A., Hudina, M., Veberic, R. and Colaric, M., (2006). Traditional walnut liqueur–cocktail of phenolics. Food Chemistry, 95: 627–631.

Tajamul, I.S., Ekta, S. and Gowhar, A. (2014). *Juglans regia* Linn: a phytopharmacological review. World Journal of Pharmaceutical Sciences, 2: 364–373.

Turkmen, N., Sari, F. and Velioglu, Y.S. (2006). Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. Food Chemistry, 99: 835–841.

Xu, J.-G., Hu, Q.-P. and Liu,Y. (2012). Antioxidant and DNA-protective activities of chlorogenic acid isomers. Journal of Agricultural and Food Chemistry, 60: 11625–11630.

Zhang, Z., Liao, L., Moore, J., Wua, T., & Wang, Z. (2009). Antioxidant phenolic compounds from walnut kernels (*Juglans regia* L.). Food Chemistry, 113, 160–165.

ISSN 2056 - 5798(Print), ISSN 2056 - 5801(online)