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Full Length Research Paper

Extraction of phenolic compounds from *Temnocalyx obovatus*

C. Mahamadi*, E. Togarepi and P. Dzomba

Department of Chemistry, Faculty of Science Education, Bindura, Zimbabwe.

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Phenolic compounds yield in plant extracts depend on the method employed in the extraction process. In this study, we investigated systematically, a method of determination of extraction yield of antioxidant compounds from *Temnocalyx obovatus*. A sample treatment and preparation protocol that employs strict statistical treatment to ensure sample homogeneity was applied. As preliminary indicator of homogeneity, total nitrogen and phosphorus were determined and an iterative process using Levene test was applied to statistically test the homogeneity of the ground *T. obovatus* leaves. At p = 0.01, homogeneity of the plant material was achieved on the third ground sample. Among the six solvents used, methanol gave the best yield of extractable phytochemicals and a butylated hydroxytoluene-spike recovery of 77%, whereas diethyl ether gave the lowest yield and a butylated hydroxytoluene-spike recovery of 63%, suggesting that optimal yields tended to be favoured by more polar solvents.

Key words: Phenolic compound, antioxidant, *Temnocalyx obovatus*, homogeneity, Levene test, extraction yield.

INTRODUCTION

In recent years, the role of natural antioxidants in human health attracted increasing attention. High intake of plant products containing these compounds has been associated with a reduced risk of a number of oxidative and chronic diseases, such as cancer, coronary heart diseases and stroke (Yin et al., 2009; Gosslau and Chen, 2004). Major antioxidants found in plant parts such as fruits, leaves, seeds and oils include vitamins C and E, carotenoids, and phenolic compounds, especially flavonoids (Ismail et al., 2004). Phenolic compounds are secondary plant metabolites that possess, in common, an aromatic ring bearing one or more hydroxyl substituents. These compounds are water soluble and may occur in combination with a sugar molecule, as glycosides (Harbone, 1998). The most important natural antioxidants commercially exploited include tocopherols and ascorbic acid, which have been successfully extracted from rosemary (Tena and Valcárcel, 1997), green tea (Wang and Halliwell, 2001) and spinach (Aehle et al., 2003).

Due to the important functions of the phenolic antioxidants, systematic analytical methods have been developed for their determination. However, because phenolic compounds exist in multiple forms, the polarity of each component can vary significantly. This has lead to difficulties in developing a uniform extraction method for different phytochemical compounds from varying plant matrices and many authors therefore report differently on the methods of extracting these compounds (Pinelo et al., 2004). Various solvents have been used for the extraction of antioxidants from plant materials. Of these, water and aqueous mixtures of methanol, ethanol and acetone are commonly used in plant extractions (Sun and Ho, 2005). Wang and Helliwell (2001) reported that aqueous ethanol was superior to methanol and acetone in the extraction of flavonoids from tea. However in another study, water was found to be a better solvent than methanol or ethanol in the extraction of tea chatechins (Khokhar and Magnusdotti, 2002). Mohdaly et al. (2009) reported that extracts obtained using higher polarity solvents were more effective radical scavengers than those obtained using lower polarity solvents. Methanol showed slightly better characteristics than ethanol as a solvent for phenolic compounds, flavonols and flavonoids extraction from vegetable material waste. It can be stated that for quality of analytical data to be realized, it is essential that a control sample is

*Corresponding author. E-mail: courtiema@yahoo.com or cmahamadi@buse.ac.zw. Tel: +263777350801. Fax: +263 7536.

homogeneous so that sub-samples used in the batches are identical. A simple homogenization of a plant sample without subjecting it to strict statistical test is not a guarantee of homogeneity for all such analytical parameters (Lo'pez-Romero et al., 2005).

In Zimbabwe, despite the widespread use of wild plants as medicines, documented literature on antioxidant composition of plant extracts is still very limited and there exist a multitude of unexplored species. Muchuweti et al. (2007) reported on the phenolic composition and antioxidant properties of *Laurel noblis*, *Rosimarinus officinalis*, *Salvia officinalis*, *Origanum marjoram*, *Origanum valgare*, *Cinnamonum zeylanicum*, *Petroselium crispum*, *Ocium basilicum* and *Mentha peperita*. There are no reports on phytochemical composition of *Temnocalyx obovatus*, which grows widely in Zimbabwe, mainly on clumps on open woodland or grassland. The plant has been used to brew tea for many years by several societies in the country. In addition, the crude plant also find extensive medicinal applications on snakebite, flu, coughs (including whooping cough), muscular toning, oedema, constipation, tropical ulcers, asthma, loss of appetite and hypertension. *T. obovatus* has also been applied in the treatment of diarrhoea in chickens, stomach disorders in animals such as turkeys, goats and cows. The plant therefore presents a very interesting species for study in terms of its antioxidant composition. In this study, we explored a systematic method of extraction of phenolic compounds from *T. obovatus*. Bulky sample homogeneity was assessed by nitrogen and phosphorus analysis and extraction yields were compared for various solvent compositions.

MATERIALS AND METHODS

Butylated hydroxytoluene, methanol, hexane, acetone, ethyl acetate, petroleum ether and ethanol were purchased from Merck Co. (Germany). Hydrochloric acid, sodium hydroxide, potassium sulphate, copper sulphate and sulphuric acid were purchased from Sigma Chemical Co. (St., Louis, USA).

T. obovatus leaves were collected from Mashonaland East province of Zimbabwe (Chivhu District) and identification was validated by an herbalist at the Harare Botanical Garden. The leaves were first gently washed and lightly rubbed with gloved hands to eliminate residues of soil and media and then submerged briefly in 0.1 M HCl to eliminate any chemical residue. To remove the acid residues, the sample was washed five times successively in deionized water with total time not exceeding 1 min. The water in the first container was changed after washing three portions of fresh material. The leaves were drained of excess water before been air dried at room temperature for three weeks to obtain a constant mass.

Homogenization and testing of the material

The dried material was then ground in a plastic grinder with wooden balls, followed by sieving through a nylon sieve and the fraction passing through 250 µm was collected. The residue was ground again. Sieving and grinding was repeated until 500 g of plant material was obtained. The residue was discarded. Homogenization of the material was carried out by mixing the entire quantity of powder having particle size smaller than 250 µm in a plastic container for 60 h. Sub-samples, N_1 to N_{10} were selected from the bulky sample by random sampling (Lo'pez-Romero et al., 2005). To test sample homogeneity with respect to nitrogen, a mass of 0.5 g of powdered leaves was randomly selected from each sub-sample into a digestion flask followed by determination of total nitrogen by the the Kjeldahl method which was adopted (Willis et al., 1996). Determination of phosphorus as a second parameter for homogeneity was achieved using the method described by Reuters and Robinson (1997).

Solvent extraction

10 g of the ground material was extracted with 100 ml of organic solvents (acetone, methanol, ethanol, hexane, petroleum ether and diethyl ether) over night (12 h) (Mohdaly et al., 2009) on a shaker at room temperature followed by filtration through Whatman no. 1 filter paper. The residues were re-extracted under the same treatment and the filtrates were combined. The filtrate was then evaporated in a rotary vapour at 40°C (Ordon et al., 2006), and the extraction yield was determined. To assess the efficiency of the extraction process, 1 g of butylated hydroxytoluene was added to 10 g of the powdered leaf sample followed by extraction with 100 ml of methanol and ethanol, respectively.

RESULTS AND DISCUSSION

Homogeneity test

Samples were taken at random; 10 samples of material in the preparation (N_1 – N_2), for which total nitrogen was determined. Nitrogen was selected as the primary indicator of homogeneity because of its relative ease of determination by the Kjeldahl method. To determine the degree of homogeneity, the mean variance of the nitrogen analysis was calculated and a matrix of coefficients was set-up (Lo'pez-Romero et al, 2005) and the results are shown in Table 1.

It was considered, as suggested by Lo'pez-Romero et al. (2005), that the ground *T. obovatus* leaves complied with the primary criterion of homogeneity if $G_1 = G_2 \ldots = G_{10}$ and the value of the coefficients of the variances were close to each other. To achieve that, the grinding and statistical treatment was repeated three times until the range of the ratios was 0.17 to 1.00, which was quiet close, suggesting that the sample was homogeneous. Once the preliminary ratio was satisfied, the Levene test was applied to the data and the data for nitrogen analysis and phosphorus analysis are shown in Tables 2 and 3, respectively. One way analysis of variance (ANOVA) of one factor was performed with all residues at 5% significance level. The calculated F value of the analysis of variance was 1.01 and the critical value of $F_{(p = 0.01)}$ was 3.60, indicating that the sample satisfied the second criterion of homogeneity for the analyte. Application of ANOVA to test for homogeneity with regards to the second chemical indicator, total phosphorus, gave the value of F as 2.86 and the critical value of $F_{(p = 0.01)}$ was 3,45 suggesting that the sample was homogeneous for this second indicator. It is very crucial to note that a given sample can be homogeneous for one parameter but not

Table 1. Variance ratio matrix for preliminary estimation of homogeneity.

	S_1^2	S_2^2	S_3^2	S_4^2	S_5^2	S_6^2	S_7^2	S_8^2	S_9^2	S_{10}^2
Variances	0.004	0.004	0.014	0.023	166.41	10.43	0.014	0.014	0.040	0.040
S_1^2		1.000	0.286	0.174	-	-	0.286	0.286	1.000	1.000
S_2^2			0.286	0.174	-	-	0.286	0.286	1.000	1.000
S_3^2				0.609	-	-	1.000	1.000	1.000	1.000
S_4^2					-	-	1.000	1.000	1.000	1.000
S_5^2						-	-	-	-	-
S_6^2							1.000	1.000	0.286	0.286
S_7^2								0.286	0.286	0.286
S_8^2									1.000	1.000
S_9^2										1.000
S_{10}^2										

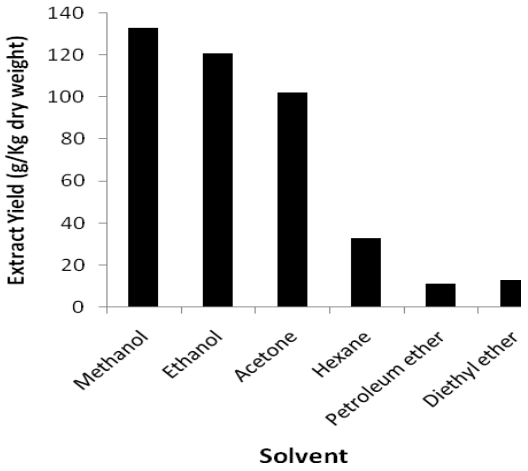

Figure 1. Extract yield (g/kg dry weight) of different solvents (shaking time of 12 h).

for the others (Lo'pez-Romero et al., 2005).

Solvent extraction

Figure 1 shows the results of extraction yield for the powdered *T. obovatus* material using methanol, ethanol, acetone, hexane, petroleum ether and diethyl ether solvents for an extraction period of 12 h. The yields varied from 13.0 to 133.2 g/kg, with methanol showing the best extraction efficiency and petroleum ether showing the least efficiency.

ANOVA computations gave $F_{calculated}$ = 13.47 and $F_{critical\ (5,12)}$ = 5.06 showing that there was significance difference between the extract yields. Variation in extract yield can be attributed to differences in polarity of compounds present in plants (Singh et al., 2002). Mohadaly et al. (2009) obtained similar findings on methanol using potatoes peels, sugar beet root pulp and sesame cake as plant samples. However, they report that hexane had the least extraction efficiency with a yield of 16.83 g/kg, whereas the present results gave an extraction efficiency of 36 g/kg. In a related study, Lin et al. (2003) showed that the best solvent for the extraction of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) from chewing gums was diethyl ether thus, differing from this study. To validate the extraction method, recovery experiments were performed by spiking with 1 g BHT and shaking for 12 h. An average recovery of 77% was achieved for methanol, while 63% was observed for ethanol, showing that methanol was a better solvent for the extraction of BHT and gave better recoveries.

Conclusion

The results of this study show that it is possible to come up with a systematic method for the extraction of phenolic compounds. Samples of *T. obovatus* leaves were successfully homogenized following an easy and low cost approach. The results illustrate the need for rigorous statistical tests in testing for homogeneity of samples and it seems plausible to speculate that the differences observed in extraction yields for different solvents may partly be due to lack of homogeneity in plant materials analysed. The results also show that methanol and ethanol had the greatest extraction yields, whereas diethyl ether had the least, which seems to suggest that polar solvents may be the most suitable for extraction of polyphenols in *T. obovatus.* Overall, the proposed method for sample treatment is suitable for the simultaneous determination of anti-oxidants in *T. obovatus*

Table 2. Levene test for nitrogen analysis.

Parameter	Sub-sample nitrogen content (mg/g)									
	N_1	N_2	N_3	N_4	N_5	N_6	N_7	N_8	N_9	N_{10}
Average nitrogen content	11.4	11.4	11.3	11.4	18.7	13.1	11.3	11.3	11.4	11.2
Residual 1	0.0179	0.0088	0.0087	0.0088	0.0089		0.0087	0.0089	0.0179	0.0182
Residual 2	0.0088	0.0088	0.0089	0.0087	0.0089	0.0089	0.0089	0.0089	0.0087	0.0090
Residual 3	0.0179	0.0088	0.0087	0.0179		0.0089	0.0087	0.0087	0.0174	0.0175

Table 3. Levene test for phosphorus analysis.

Parameter	Sub-sample nitrogen content (mg/g)									
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
Average phosphorus content	1.137	1.137	1.137	1.137	1.137	1.137	1.137	1.137	1.137	1.137
Residual 1	0	0.0009	0	0	0	0	0	0	0	0
Residual 2	0	0.0009	0	0	0	0	0	0	0	0
Residual 3	0	0.0009	0	0	0	0	0	0	0	0

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