Asian Journal of Chemistry Vol. 22, No. 5 (2010), 3851-3855

Optimization of Extraction Method for Recovery of Antioxidant Phenolics from Loranthaceae Mistletoes

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Effect of solvent composition on recovery of polyphenolics from Loranthaceae mistletoes and their antioxidant activities were examined in this work. All extracts contain higher amount of flavonoid content than proanthocyanidin content which is reflected by their higher antioxidant potential. On the basis of optimization of extraction method, the 70 % methanol extract of *D. trigona* contains higher amount of polyphenolics and flavonoids which shows IC_{50} values of 7.82 µg/mL in DPPH and 8.7 µg/mL in nitric oxide scavenging activity. Thus, it can become an important natural source of antioxidant phenolics.

Key Words: Polyphenolics, Antioxidant potential, Loranthaceae mistletoes, DPPH, Nitric oxide, IC_{50} value.

INTRODUCTION

Dendrophthoe falcata L.f. (parasitic on *Mangifera indica* L. belonging to family Anacardiaceae), *Helicanthus elastica* Desr. (parasitic on *Syzygium cumini* Linn. belonging to family Myrtaceae), *Dendrophthoe trigona* (Wt. and Arn.) Danser (parasitic on *Ficus racemosa* Linn. belonging to family Moraceae) and *Macrosolen capitellatus* (Wt. and Arn.) Danser (parasitic on *Mangifera indica* L. belonging to family Anacardiaceae) are mistletoes belonging to family Loranthaceae. They commonly found in Asian Western Ghats. The mistletoes are considered as invasive pest since they take up water, nutrients and solutes from the host plants and thus drastically reduce the growth of their hosts[1]. Traditionally, these plants were used as fodder, food, dyes and drugs[2]. High concentration of phenolics appears to be a general feature of these parasitic angiosperms[3].

Although hemiparasites develop their own secondary chemicals such as phenolics and terpenoids, the secondary chemicals are known to be transferred from host to hemiparasite[4,5]. Numerous hemiparasites do not synthesize or modify the secondary compounds taken up from their hosts[6]; thus the mistletoes constitute an important

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source of polyphenolic compounds. On this basis, the extraction method was optimized using methanol/water mixtures to recover the maximum amount of polyphenolic compounds. The extracts were further investigated for phenolic, flavonoid and proanthocyanidin content. However, antioxidant potential of such higher phenolic content in mistletoes has been rarely investigated[7]. Therefore, the present study also aimed to explore the antioxidant potential of these plants using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and nitric oxide (NO) scavenging models.

EXPERIMENTAL

The plant materials were collected from Western Ghat region of Maharashtra (16° 41' 60 N latitude and 74° 13' 0E longitude and 1000 m altitude) in November 2007. The plant specimen *D. falcata* was authenticated by Mr. Salunkhe, Botany Department, Krishna Mahavidyalaya, Rethare, India. The remaining three plant specimens were authenticated by Mr. Rao (Botanical Survey of India, Pune).

The chemicals used were Folin-Ciocalteu reagent (Merck Co.), gallic acid (Sigma Ltd., USA), sodium carbonate (Sisco Research Laboratory Pvt. Ltd., Mumbai, India), $Al(NO_3)_3$, potassium acetate, quercetin. (Sigma Ltd., USA), vanillin (Merck Co.), sulphuric acid and epicatechin (Fluka). The solvents used were of HPLC grade.

Optimization of extraction method: Accurately weighed 5 g of sample of coarsely powdered leaves was macerated with 50, 70, 90 and 100 % v/v methanol separately in a stoppered flask for 24 h with occasional shaking. After 24 h, the extracts were filtered and this procedure was repeated twice time more. The extracts were then combined in a 100 mL volumetric flask. The combined extracts were concentrated under reduced pressure on rotary vaccum evaporator and further dried in vacuum dryer and weighed. The extracts were kept in dessicator until used.

Quantification of polyphenolics

Total phenolic content: Total phenolic content of 70 % methanol extracts of all plant samples were determined using Folin-Ciocalteu reagent[8]. The blue color formed due to the polyphenol content in the extracts was measured at 760 nm using a Shimadzu UV-2400 spectrophotometer and the results were expressed as µg/mg of gallic acid equivalent.

Total flavonoid content: Total flavonoids of 70 % methanolic extracts of samples were determined using method described by Liu *et al.*[9] with some modifications. The yellow color formed due to flavonoid content was measured at 415 nm using a spectrophotometer and the total flavonoid content was calculated according to standard curve established with reference standard quercetin. The results were expressed as µg/mg of quercetin equivalent.

Total proanthocyanidin content: Total proanthocyanidin content of 70 % methanolic extracts were determined using vanillin-H_2SO_4 method[10]. The absorbance was measured at 500 nm using a Shimadzu UV-2400 spectrophotometer and the results were expressed as µg/mg of epicatechin equivalent.

Antioxidant activity

Determination of DPPH radical scavenging activity: The DPPH radical scavenging activity of the 70 % methanolic extract was performed using reported method with some modifications[11,12]. Briefly, to 100 µL of extract (10-100 µg/mL) in methanol were added 25 µL of DPPH (1 mM in ethanol) and 75 µL of ethanol. After 0.5 h maintaining at room temperature, the absorbance of reaction mixture was measured at 517 nm using Microtiter Plate Reader (Power Wave XS, Bio-tek, USA). The antioxidant activity of the extract was expressed as IC_{50}.

Determination of NO radical scavenging activity: The nitric oxide scavenging activity was determined according to method reported by Sreejayan[13]. Sodium nitroprusside (1 mL, 10 mM) was mixed with 1 mL extract (10-100 µg/mL) in phosphate buffer (pH 7.4). The mixture was incubated at 25 ºC for 2.5 h. To 1 mL of incubated solution, 1 mL of griess reagent (α-naphthyl-ethylenediamine dihydrochloride 0.1 % in water and sulfanilamide 5 % in H_3PO_4) was added and absorbance was read at 546 nm.

Statistical analysis: For total phenolics, total flavonoids, total proanthocyanidin of all samples at least three readings were considered and data were expressed as mean ± SD. Comparison was made between samples of the hemiparasite collected from different host plants by variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad Prism (Version 4) software, by considering P values = 0.05 as significant. The significance is shown in comparison to the 100 % methanol for recovery of polyphenolics and in comparison to the *M. capitellatus* for total phenolics, total flavonoids and total proanthocyanidin content of 70 % methanolic extracts.

RESULTS AND DISCUSSION

Four mistletoes of Loranthaceae family were selected and extraction method for the recovery of polyphenolics was optimized using methanol-water extract. Solvent composition significantly affects the composition as well as overall yield of extracts[14]. Consistent with these observations, methanol-water (70-30 % v/v) was found to be the best solvent in recovering maximum amount of polyphenolics (Fig. 1). The results indicate that *D. trigona* contains significant amount of polyphenolics in comparison to three other mistletoes. All the four plants of family Loranthaceae were found to contain higher amount of flavonoids than proanthocyanidins (Table-1).

In order to evaluate the contribution of the individual polyphenolic classes to the antioxidant potential of extracts, simple linear regression analysis was carried out in DPPH and NO model. For all classes determined, the correlation coefficients found were particularly high and statistically significant ($p < 0.001$), the most important link being with the total flavonoid content ($r^2 = 0.9998$).

The results indicate that almost all extracts have comparable DPPH and NO radical scavenging potential and among them *D. trigona* showed higher radical scavenging effects than reference standards (Table-2).

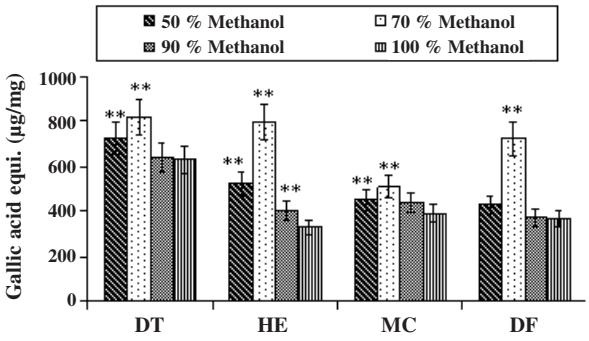

Fig. 1. Effect of solvent composition on recovery of phenolics. DT: *D. trigona*, HE: *H. elastica*, MC: *M. capitellatus*, DF: *D. falcata*. data analyzed by One-way ANOVA followed by Tukey's Multiple Comparison Test. The significance is shown in comparison to the 100 % methanol. **:Indicates p value < 0.001.

TABLE-1
TOTAL PHENOLICS, TOTAL FLAVONOIDS AND TOTAL PROANTHOCYANIDIN CONTENT OF 70 % METHANOLIC EXTRACTS OF HEMIPARASITES

Plant sample	Total phenolic content (µg/mg)	Total flavonoid content (µg/mg)	Total proanthocyanidin content (µg/mg)
D. trigona	820.2 ± 2.7220**	504.9 ± 1.4010**	312.4 ± 5.065**
H. elastica	796.7 ± 17.360**	450.5 ± 0.7550**	343.1 ± 1.858**
D. falcata	720.9 ± 10.540**	390.5 ± 0.8330**	327.1 ± 0.513**
M. capitellatus	513.0 ± 1.836	327.2 ± 0.0577	184.6 ± 0.755

Values are expressed as mean ± SEM, n = 3. Data analyzed by One-way ANOVA followed by Tukey's multiple comparison Test. The significance is shown in comparison to MC. **: Indicates p value < 0.001.

TABLE-2
DPPH AND NITRIC OXIDE (NO) RADICAL SCAVENGING ACTIVITY

S.No.	Sample	IC_{50} value in DPPH model (µg/mL)	IC_{50} value in NO model (µg/mL)
1	Ascorbic acid	9.27	8.80
2	BHT	7.89	8.48
3	*D. trigona*	7.82	8.70
4	*H. elastica*	8.09	9.10
5	*M. capitellatus*	8.29	10.11
6	*D. falcata*	8.17	9.60

REFERENCES

1. S.C. Pennings and R.M. Callaway, *Oecologia*, **131**, 479 (2002).
2. R.M. Kunwar, N. Adhikari and M.P. Devkota, *Banko Janakari*, **15**, 49 (2005).
3. S.K. Khanna, P.N. Viswanathan, C.P. Tewari P.S. Krishnan and G.G. Sanwal, *Plant Physiol.*, **21**, 949 (1968).
4. M.J. Schneider and F.R. Stermitz, *Phytochemistry*, **29**, 1811 (1990).

5. R.B. Jadhav, S.J. Anarthe, S.B. Ghokhale and S.J. Surana, *J. Plant Interac.*, **1**, 171 (2005).
6. Costs of Plant Resistance to Herbivory, Chicago: University of Chicago Press, p. 392 (1992).
7. O.U. Evren, K. Ali and A. Nazli, *Fitoterapia*, **77**, 556 (2006).
8. A. Arnous, D.P. Makris and P. Kefalas, *J. Food Compos. Anal*., **15**, 655 (2002).
9. C.T. Liu, Y.W. Ching, M.W. Yih and Y.T. Chin, *J. Ethnopharmacol*., **99**, 293 (2005).
10. Methods in Plant Biochemistry, Academic Press London, p. 389 (1989).
11. M.S. Blois, *Nature*, **26**, 1199 (1958).
12. R. Amarowicz, M. Naczk and F. Shahidi, *J. Agric. Food Chem*., **48**, 2755 (2000).
13. R. Sreejayan, *J. Pharm. Pharmacol*., **49**, 105 (1997).
14. K. Robards, P.D. Prenzler, G. Tucker, P. Swatsitang and W. Glover, *Food Chem*., **66**, 401 (1999).

(*Received*: 31 July 2009; *Accepted*: 22 January 2010) AJC-8344