Haryana Vet. **51** (December, 2012), pp 85-88

Research Article

EFFECT OF SOLVENT AND VESSEL TEMPERATURE ON EXTRACTION OF POLYPHENOL FROM LEAVES OF ANDROGRAPHIS PANICULATA AND ON THEIR ANTIOXIDANT PROPERTIES

N. MOHANTY* and P. C. BISOI
Department of Biochemistry, Orissa Veterinary College
OUAT, Bhubaneswar, Odisha

ABSTRACT

Polyphenol was extracted from the leaves of *Andrographis paniculata* using different solvents like methanol 100%, methanol 80%, acetone 100%, acetone 50% and solvent mixture of ethylacetate, methanol and water (60:30:10) through microwave assisted extraction at different vessel temperature i.e.70°C, 80°C, 90°C and 100°C at pressure of about 2.6-3.7 bar. The polyphenol content of leaves was determined by Folin-Ciocalteu method which varied between 7.58±0.46 to 36.55±1.46 mg of gallic acid equivalent/gm dry matter content. The maximum polyphenol content was found in 80% methanol and the lowest in solvent mixture. The content of polyphenol in the extract increased with an increase in extraction temperature from 70°C to 80°C and decreased at 100°C. Highest antioxidant activity was recorded in the extract obtained at 90°C (12.99±0.87 in µmole of ascorbic acid equivalent/100µg polyphenol) and the lowest activity (10.0±1.15 in µmole of ascorbic acid equivalent/100µg polyphenol) at vessel temperature of 100°C. Highest antioxidant activity was found in 50% acetone extract (26.99±0.39 in µmole of ascorbic acid equivalent/100µg polyphenol) of the leaves and the lowest activity was in the solvent mixture (4.71±0.20 in µmole of ascorbic acid equivalent/100µg polyphenol).

Key words: Polyphenol, antioxidant activity, *Andrographis paniculata*

Polyphenolic compounds constitute one of the largest and most ubiquitous group of phytochemicals. They are best known for antioxidative, antibacterial, antifungal, antimutagenic, anticancer, hypolipidemic and hepatoprotective effects. Many pharmacological activities of polyphenols are linked to their ability to act as antioxidants and free radical scavengers to chelate metal and interact with enzymes, adenosine receptor and biomarker (Middleton and Kandaswami, 1993). These antioxidants may provide protection against chronic disease, inflammation and cardiovascular diseases (Prior and Gu, 2005).

Andrographis paniculata is traditionally known as Kalmegh, Kirat, Mahatita, Bhunimba. The plant belongs to family *Acanthaceae* and is a shrub native to India, South Asia and China. It is reported to possess antihepatotoxic (Shukla *et al.,* 1992), antibacterial (Malhotra and Singh, 2005), antihepatitis (Rana and Avadhoot,1991), antithrombogenic (Koul and Kapil, 1994), antimalarial and antipyretic (Katta *et al.,* 2007) and antidiabetic and antihypertensive (Borhanuddin *et al.,* 1994) properties. Till date only alcoholic extract of leaves of *A. paniculata* obtained by cold maceration method was found to be effective in preventing liver damage (Shukla *et al.,* 1992). Therefore, the effect of different solvents system and temperature on the extraction of polyphenols from the leaves of *A. paniculata* by microwave assisted extraction (MAE) method and their antioxidant property was studied.

MATERIALS AND METHODS

Leaves of *A. paniculata* were collected from forests of Bolangir districts of Odisha. The leaves were identified as of *A. paniculata* in the Department of Botany, College of Basic Sciences and Humanities, OUAT, Bhubaneswar. The voucher specimen was kept in the herbarium in the department. The leaves of the plant were fine powdered after shade drying and were used for further extraction. Extraction from dried fine leaf powder was done by closed system of microwave assisted extraction system (MAE, Multiwave 3000

*Corresponding author: niharikavet@gmail.com

801V, Anton Par) according to standard protocol (Eskilsson and Bjorklund, 2000) with solvents like methanol (100%), methanol (80%), acetone (100%), acetone (50%) and a solvent mixture of ethylacetate, methanol and water (60:30:10). The extraction was done for 25 minutes at different temperatures i.e. at 70°C, 80°C, 90°C and 100°C with initial temperature of 27°-34°C and initial pressure between 2.6 bar to 3.7 bar. Extract was filtered using Whatman filter paper no 1. The filtered extract was divided into two parts: one part was kept as such and the other part was dried using vacuum rotary evaporator at 40°C. For each temperature in extraction method, the dried powdered leaves of *A. paniculata* were used in triplicates and data was analysed by one way ANOVA. The amount of total phenolics in the extract was determined according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965) modified by Negi and Jayaprakash (2003). A standard curve was prepared by taking different concentrations of gallic acid (1 µg-10 µg). The concentration of total phenolics was expressed as Gallic acid equivalent (GAE) per gram of dry plant material.

The total antioxidant capacity of the extract was evaluated according to method described by Prieto *et al.* (1999). An aliquot of 0.1 ml of extracted sample solution was mixed with 1 ml of reagent solution (4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M. concentrated sulphuric acid). For the blank, 0.1 ml of methanol was used in place of sample. The tubes were incubated in a water bath at 95°C for 90 min. The samples were then cooled to room temperature and the absorbance of the aqueous solution of each extract in triplicates was measured at 695 nm against a blank in a Perkin -Elmer UV-visible spectrophotometer. Standard curve was prepared by taking different concentrations of ascorbic acid (1 µmole to 10 µmole). Antioxidant activity of extracted samples was expressed as ascorbic acid equivalents (AAE) in µmol/100µg of polyphenol in the plant extract.

RESULTS AND DISCUSSION

Polyphenol Content at Different Vessel Temperature of MAE System: Table 1 represents the total polyphenol content of the leaves of *A. paniculata* and their total antioxidant activity extracted by following the technique of MAE using methanol as a solvent at different vessel temperature i.e. 70°C, 80°C, 90°C, 100°C. The polyphenol content in the extract obtained at different vessel temperature ranged from 14.04 to 16.88 mg of GAE/gm of dry matter of plant material. The polyphenol contents in leaves of *A. paniculata* are in agreement with earlier report of Kahkonen *et al*. (1999). The extraction of polyphenol from the leaves showed an increasing trend with an increase in temperature from 70°C to 90°C whereas increase in temperature from 90°C to 100°C showed a reduction in the content.

Table 1
Effect of extraction temperature on polyphenol content and antioxidant activity from *A. paniculata* leaves by MAE

Parameter	Extraction temperature			
	70°C	80°C	90°C	100°C
Total phenolics (mg of GAE/g dry matter)	14.04±0.66[a]	14.83±0.64[a]	16.88±2.26[a]	15.99±1.78[a]
Total antioxidant (µmole of AAE/100µg polyphenol)	12.79±0.99[a]	12.70±0.81[a]	12.99±0.87[a]	10.00±1.15[b]

Values with different superscripts (a, b) in a row differ significantly ($p < 0.05$).

In MAE, microwave was used as a source of heat; the heated solvent might begin the extraction process faster than the convection heating. In closed vessel system, temperature may reach well above the boiling point of the solvent (Mandal *et al*., 2007) due to pressurized condition. Thus elevated temperature resulted in improved extraction efficiencies since desorption of analyte from active sites in the matrix was increased (Eskilsson and Bjorklund, 2000). Additionally, solvents have higher capacity to solublize analytes at higher temperature while surface tension and solvent viscosity decreases with temperature which will improve sample wetting and matrix penetration, respectively. At higher temperature some of the polyphenols are unstable and gets partially or totally converted to some other compounds. Luthria (2008) also reported decrease in polyphenol content of the extract obtained at higher temperature by pressurized liquid extractor from parsley (*Petroselinum crispum*) flakes and suggested that the decrease was due to instability of polyphenol. In this

Table 2
Effect of solvent on extraction of polyphenols from *A. paniculata* leaves by MAE and their antioxidant activities

Parameter	Solvent				
	Methanol 100%	Methanol 80%	Acetone 100%	Acetone 50%	Ethylacetate, methanol and water (60:30:10)
Total phenolics (mg of GAE/g dry matter)	16.88±2.26[a]	36.95±1.46[b]	20.73±1.19[a]	26.53±1.61[c]	7.58±0.46[d]
Total antioxidant (µmole of AAE/100µg polyphenol)	12.99±0.86[a]	11.24±0.61[a]	21.67±1.29[b]	26.99±0.39[c]	4.71± 0.20[d]

Values with different superscripts (a, b, c) in a row differ significantly ($p< 0.05$)

experiment, the increase in temperature increased the efficiency of extraction of polyphenol up to 90°C and the efficiency decreased with an increase in temperature thereafter.

Antioxidant Activity at Different Vessel Temperature: The antioxidant activity of *A. paniculata* varied from 10.0 to 12.99 µmole of AAE/100µg of polyphenol (Table 1). Highest antioxidant activity was recorded at vessel temperature of 90°C and the lowest activity at vessel temperature of 100°C. Statistical analysis ($p<0.05$) indicated that the variation in the total antioxidant activity between 70°C vs 100°C, 80°C vs 100°C, 90°C vs 100°C was significant whereas variation between 70°C vs 80°C, 70°C vs 90°C, 80°C vs 90°C was not significant.

The antioxidant activity of polyphenols is mainly due to their redox properties which can play an important role in adsorbing and neutralizing free radicals, quenching oxygen or decomposing peroxides. Our results are in good agreement with those of Luthria (2008) who also reported that the phenolic compounds extracted from parsley changed significantly with the increase in temperature from 40°C to 160°C.

Polyphenol Content Using Various Solvent Systems: From the Table 2, it was evident that the polyphenol content varied between 7.58 to 36.95 mg of GAE/g of dry leaves. The efficiency of extraction decreased in the order of methanol (80%)>acetone (50%)>acetone (100%)>methanol (100%)>solvent mixture i.e. ethylacetate, methanol and water (60:30:10). The polyphenol content of different solvent extraction showed a significant variation ($p<0.05$) except methanol vs acetone 100%. Mixing of water with the solvent (methanol and acetone) increased the extraction of polyphenol from the leaves of *A. paniculata*. Maximum polyphenol content was in 80% methanol and minimum was in mixture of solvents.

In MAE method, selection of solvent played an important role. Solvent choice for MAE, is dictated by the solubility of the target analyte, the interaction between solvent and plant matrix and finally by the microwave absorbing properties of the solvent. The microwave absorbing properties of a solvent are generally characterized by its dielectric constant, its dielectric loss or by its dissipation factor. Evidently polar solvents of low molecular weight and high dielectric constant irradiated by microwave increases their temperature very rapidly like methanol, ethanol and water, which are good solvent for the MAE (Eskilsson and Bjorklund, 2000). Use of some amount of water with methanol or ethanol or even with MAE transparent solvent increases the extraction efficiency for which the polyphenol yield in aqueous methanol and aqueous acetonic were better than the pure solvent. A methanol water (90:10) mixture combination was reported to be better for extraction of paclitaxel from *Taxus bactta* by MAE than the pure solvent (Talebi *et al.,* 2004).

Antioxidant Activity with Different Solvents: There was a significant ($p<0.05$) difference in total antioxidant activity among the all groups except methanol 100% vs methanol 80% (Table 2). Maximum antioxidant activity was obtained in both acetone extract of the leave and the lowest activity was observed in the extract obtained by using the solvent mixture.

Our results are in good agreement with those of Negi and Jayaprakash (2003) who studied the antioxidant activity of pomgrenate peel extract extracted by Soxhlet extractor with ethylacetate, acetone, methanol and water. All the peel extract

exhibited marked antioxidant capacity but the aqueous extract had the lowest suggesting that selected solvent system may have different efficiency for extracting individual polyphenolic compounds. In addition to it, different polyphenols also have different antioxidant capacity (Salah *et al*., 1995). Further investigation is needed for isolation and characterization of individual polyphenolic component and their antioxidant activity from the leaves of *A. paniculata.*

REFERENCES

Borhanuddin, M., Shamsuzzoha, M. and Hussain A.H. (1994). Hypoglycaemic effects of *Andrographis paniculata* Nees on non-diabetic rabbits. *Bangladesh Med. Res. Council Bull.* **20**: 24-26.

Eskilsson, C.S. and Bjorklund, E. (2000). Analytical-scale microwave-assisted extraction. *J. Chromatogr.* A **902**: 227-250.

Kahkonen, M.P., Hopia, A.I., Vuorela, H.J, Rauha, J.P., Pihlaja, K., Kujala, T.S. and Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem*. **47**: 3954-3962.

Katta, V., Murthy, P.B.S., Kannababu, S., Syamasunder, B. and Subbaraju, G.V. (2007). Estimation of andrographolide in *Andrographis paniculata*: Herbs extracts and dosage forms. *International J. Appl. Sci. Engg.* **5**: 27-39.

Koul, I.B. and Kapil, A. (1994). Effect of diterpenes from *Andrographis paniculata* on antioxidant defense system and lipid peroxidation. *Indian J. Pharmacol.* **26**: 296-300.

Luthria, D.L. (2008). Influence of experimental conditions on the extraction of phenolic compounds from parsley (*Patroselinum crispum*) flakes using pressurized liquid extractor. *Food Chem.* **107**: 745-752.

Malhotra, S. and Singh, A. (2005). Recent advances in pharmacology of *Andrographis paniculata*: Integrative health care institute, Research studies.

Mandal, V., Mohan, Y. and Hemaltha, S. (2007). Microwave assisted extraction-An innovative and promising extraction tool for medicinal plant research. *Pharmacognosy Rev.* **1**: 7-18.

Middleton, E. and Kandaswami, C. (1993). The impact of plant flavonoids on mammalian biology: Implications for immunity, inflammation and cancer. In: The Flavonoids: Advances in Research Since 1986, edHarborne IR (Chapman and Hall, London).

Negi, P.S. and Jayaprakasha, G.K. (2003). Antioxidant and antibacterial activities of *Punica granatum* peel extracts. *J. Food Sci*. **68**: 1473-1477.

Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal. Biochem.* **269**: 337-341.

Prior, R.L. and Gu, L. (2005). Occurrence and biological significance of proanthocyanidins in the American diet. *Phytochem.* **66**: 2264-2280.

Rana, A.C. and Avadhoot, Y. (1991). Hepatoprotective effects of *Andrographis paniculata* against carbon tetrachloride-induced liver damage. *Indian Arch. Pharm. Res.* **1**: 93-95.

Salah, N., Miller, N.J., Paganga, G., Tijburg, L., Bolwell, G.P. and Rice-Evans, C. (1995). Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain breaking antioxidants. *Arch. Biochem. Biophys*. **322**: 339-346.

Shukla, B., Visen, P.K, Patnaik, G.K. and Dhawan, B.N. (1992). Choleretic effect of andrographolide in rats and guinea pigs. *Planta. Med*. **58**: 146-149.

Singleton, V.L. and Rossi, J.R. (1965). Colorimetri of total phenolics with phosphomolybedic-phosphotungstic acid reagents. *Am. J. Enolvitticult* **16**: 144-158.

Talebi, M., Ghassempour, A., Talebpour, Z., Rassoulb, A. and Dolatyarvi, L. (2004). Optimization of the extraction of palcitaxel from *Taxus baccata* L. by the use of microwave energy. *J. Sep. Sci*. **27**: 1130-1136.