e-planet **19** (2): 128-133 (December 2021)

Effect of extraction conditions on antioxidative and free radical scavenging activities of *Paederia foetida* L.

M.R. SENAPATI[1*] AND P.C. BEHERA[2]

[1*]*Agro-Polytechnic Centre (Animal Science), OUAT, Chiplima, Sambalpur, Odisha-768025, India*

[2]*Dept. of Veterinary Biochemistry, CVSc&AH, OUAT, Bhubaneswar, Odisha-753001, India*

**mitu56v@gmail.com*

Date of receipt: 10.05.2021 Date of acceptance: 13.11.2021

ABSTRACT

Extraction conditions are the major concern to enhance the efficiency in order to obtain the highest yields of phenolic compounds from natural resources which imparts antioxidative and free radical scavenging activities. The present investigation was carried out to study the effects of four extracting solvents (80% methanol, 80% acetone, 50% acetone and their mixture) and two extraction techniques (cold percolation and microwave assisted extraction) on the antioxidant activity and free radical scavenging activities of extracts of *Paederia foetida*. The tested plant materials contained appreciable amounts of total phenolic (2.22-3.98 mg GAE per gram of sample), total flavonoid (0.26-1.05 mg of rutin equnivalent per gram of sample), total antioxidant (1394.23-3143.44 µM ascorbic acid equnivalent per gram of sample), FRAP (4.60-9.53 × 103 µM of ascorbic acid equnivalent per gram of sample), reducing power (180.23-1776.80 µM ascorbic acid equnivalent per gram of sample). On the other hand, extracts of the plant under different extraction regimen was having superoxide radical scavenging activity (5.74-70.55%), scavenging of nitric oxide (34.25-73.16%), hydroxyl radical scavenging activity (30.46-41.36%) and inhibition of lipid per oxidation (23.58-41.45%). From our study it was revealed that higher content of total phenolics, total flavonoid, antioxidative and free radical scavenging activities were obtained in the extracts prepared by microwave assisted extraction (MAE) as compared to the respective cold percolation (CP) method of extraction by using four different extracting solvents.

Key words: Antioxidant, cold percolation, free radical scavenging activity, microwave assisted extraction, total flavonoids, total phenolics

INTRODUCTION

Traditional knowledge of medicinal plants, guided for searching of new cures, are often cheaper, locally available, easily consumable (raw) and has simple medicinal preparations to act a form of alternative medicine (Surveswaran et al*.,* 2007). For the prevention and treatment of human and animal diseases, natural medicines were the only option for thousands of years due to their active chemical constituents, though their efficacy and mechanisms of action have not been tested scientifically in most cases (Alasalvar et al*.,* 2006). The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, inflammation, bacterial disease, diseases associated with ageing (Kumar and Surh, 2008, Thatoi et al*.,* 2008) owing to their antioxidants and free radical scavenging activities (Veerapur et al*.,* 2009) by blocking the initiation or propagation of oxidizing chain reactions or by scavenging various types of reactive species or chelating transition metal ions (Halliwell, 2007).

Paederia foetida L. belonging to family Rubiaceae (The Ayurvedic Pharmacopeia of India, 1999) is usually found in Himalayas from Dehradun eastwards upto an altitude of 1800m and also in Assam, Bihar, Odisha, and West Bengal. It is a climbing vine with varied medicinal importance including the food value (Mishra and Bisht, 2011). Macroscopically, the fresh leaf is 10 to 15 cm long, 5 to 6 mm width and petiole 1.2 to 6 cm, surface is glabrous and mostly ovate, green in colour having a characteristic odour and indistinct bitter taste. It was reported to be used in gout, vesical calculi, piles, inflammation of the liver and emetic diarrhoea, dysentery (Atta and Mouneir, 2004) and to inhibit intestinal motility (Afroz et al., 2006). It also enters in to the preparation of Dasmularishta and used to treat enteromegaly, enterosis, flatulence, gastromegaly, rheumatism, rhinosis, sapraemia, sore, stomachache and toothache (Johnson, 1999). Ethanolic and Methanolic extract of this plant were focused on the antitussive (Nosalova et al., 2007) and antioxidant activity (Osman et al., 2009) respectively. Preliminary qualitative chemical tests showed that plant is credited with carbohydrates, proteins, amino acids, tannins, phenolics, flavonoids, steroids, mucilage and saponins (Yadav et al., 2009).

For drug discovery and development, plants are an important source of bioactive molecules. In contrast, the amounts of these bioactive molecules are always fairly low in natural medicines originated directly from plant itself. So, isolated bioactive molecules serve as starting materials for laboratory synthesis of drugs. But, today, it is very crucial to develop effective and selective methods for the extraction of those natural bioactive molecules. Several studies have revealed about the variations in the biological activities of extracts obtained by using different extraction techniques (Zhang et al., 2018; Dhanani et al., 2017). Also, incessantly growing demand for plant derived therapeutic molecules is alarming for development of innovative extraction techniques to obtain phyto-constituents in a sustainable and eco-friendly manner (Fierascu et al., 2020). Hence, the extraction conditions are the major concern to enhance the efficiency in order to obtain highest yields of antioxidative compounds from natural resources. Recovery of antioxidant compounds from plant materials is typically accomplished through different extraction techniques and the nature of extracting solvent, taking into account their varied chemical characteristics, polarities and uneven distribution in the plant matrix (Sultana et al., 2009).

Therefore, keeping in view of the above context, the present study aims to evaluate the effect of extraction conditions (types of techniques and solvents) on concentration of phenolics and their antioxidant activities.

MATERIALS AND METHODS

Plant material

Paederia foetida (Gandhali- Hindi, Shunkvine- English, Pasharuni- Odia) was identified and classified in the Department of Botany, Odisha University of Agriculture and Technology (O.U.A.T.) following the description of Saxena and Brahmam (1995). The whole shoot of the plant was collected at pre-flowering stage, cleaned, dried under shade and ground into fine structure for preparation of extracts.

Grouping

The study was classified into four groups based on the solvents such as Gr-A (80% Methanol), Gr-B (80% Acetone), Gr-C (50% Acetone), Gr-D (Solvent mixture i.e., acetone: ethanol: water: acetic acid in 40:40:19.9:0.1 v/v).

Extraction process

In trial-I, extraction of phyto-constituents was done in Microwave Assisted Extraction (MAE) system by Multiwave 3000-801V (Anton Par) digestion system following the method of Eskilsson and Bjorklund (2000) where 2 g of ground mass in 20 ml of solvent was heated at 80° C for 25 minutes followed by 15 minutes cooling. In trial-II, extraction was done by cold percolation (CP) following the method of Senapati et al., (2013), where 2 g of sample in 40 ml of solvent was kept on magnetic stirrer at 10°C temperature for 24 hrs followed by filtration through Whatman No-1 filter paper.

Phenolic estimation and antioxidant activity

Total polyphenol in the extract was determined by the method of Singh et al. (2002) and that of flavonoid was measured by the method of Mimica-Dukic (1992). The total antioxidant activity of extracts was evaluated by phosphomolybdenom and reducing power method of Prieto et al. (1999) and Oyaizu (1986) respectively. Individual antioxidant capacity of plant extract was estimated by FRAP method of Benzie and Strain (1996) where as Superoxide (SO), Nitric oxide (NO) and Hydroxyl radical (OH^-) scavenging activities were determined by method of Hyland et al. (1983), Sreejayan Rao (1997) and Halliwell et al. (1997) respectively. Besides, lipid per-oxidation inhibition assay (LPOIA) was conducted by the method of Ohkawa et al. (1979) and percentage of scavenging activities were calculated using the respective formula.

Statistical analysis

The data was subjected to analysis of variance to test the significance of difference of mean values between different groups according to Snedecor and Cochran (1994).

Table 1. Effect of extraction technique and solvents on concentration of polyphenols and flavonoids and their antioxidant activities (Mean±SE)

Groups	Gr-A		Gr-B		Gr-C		Gr-D	
Methods	MAE	CP	MAE	CP	MAE	CP	MAE	CP
TP (mg of GAE/g)	3.72 ^{a} ± 0.60	2.22 ^{b} ± 0.20	3.58 ^{a} ± 0.50	2.76 ^{a} ± 0.06	3.42 ^{a} ± 0.45	2.47 ^{a} ± 0.13	3.98 ^{a} ± 0.54	3.10 ^{a} ± 0.21
TF (mg of RE/g)	0.78 ^{a} ± 0.06	0.46 ^{b} ± 0.03	0.93 ^{a} ± 0.06	0.52 ^{b} ± 0.06	0.69 ^{a} ± 0.06	0.26 ^{b} ± 0.02	1.05 ^{a} ± 0.04	0.71 ^{b} ± 0.06
TA (µM AAE/g)	2070.55^{a} ± 11.34	1394.23^{b} ± 13.17	2844.06^{a} ± 11.83	1839.77^{b} ± 4.65	1897.53^{a} ± 12.60	1439.69^{b} ± 10.61	3143.44^{a} ± 11.80	1900.14^{b} ± 14.99
FRAP (10^3x µM AAE/g)	8.32 ^{a} ± 1.35	4.60 ^{b} ± 0.29	9.17 ^{a} ± 1.28	5.73 ^{b} ± 0.09	7.70 ^{a} ± 1.01	4.56 ^{b} ± 0.05	9.53 ^{a} ± 1.28	6.00 ^{b} ± 0.38
RP (µM AAE/g)	1333.97^{a} ± 9.50	467.11 ^{b} ± 13.17	1735.00^{a} ± 8.62	180.23 ^{b} ± 3.01	1381.07^{a} ± 13.29	194.00 ^{b} ± 3.00	1776.80^{a} ± 15.71	269.57 ^{b} ± 18.64
Scavenging of SO (%)	21.84 ^{a} ± 1.02	16.57 ^{b} ± 0.55	10.95 ± 0.21	Nil	70.55 ^{a} ± 0.57	22.46 ^{b} ± 0.61	5.74 ± 0.80	Nil
Scavenging of NO (%)	61.22 ^{a} ± 1.81	44.21 ^{b} ± 2.74	58.37 ^{a} ± 1.37	45.90 ^{b} ± 3.17	73.16 ^{a} ± 2.30	69.30 ^{b} ± 1.20	53.57 ^{a} ± 2.79	34.25 ^{b} ± 0.87
Scavenging of OH^- (%)	41.09 ^{a} ± 0.99	40.25 ^{a} ± 0.64	41.35 ^{a} ± 0.88	30.46 ^{b} ± 1.30	40.83 ^{a} ± 0.94	35.92 ^{b} ± 0.87	41.36 ^{a} ± 0.60	39.79 ^{b} ± 0.72
LPOIA	31.16 ^{a} ± 1.17	28.33 ^{a} ± 0.81	31.47 ^{a} ± 1.43	23.58 ^{b} ± 0.97	41.23 ^{a} ± 1.15	34.66 ^{b} ± 1.49	41.45 ^{a} ± 1.36	34.94 ^{b} ± 1.73

Different superscripts between columns shows significant difference ($p<0.05$) within a group. MAE-Microwave Assisted Extraction, CP- Cold Percolation, TP- Total Phenolics, GAE-Gallic Acid Equivalent, TF- Total Flavonoid, RE- Rutin Equivalent, TA- Total Antioxidant, AAE- Ascorbic Acid Equivalent, FRAP- Ferric Reducing Antioxidant Power assay, RP- Reducing Power, SO-Super Oxide, NO- Nitric Oxide, OH- Hydroxyl Radical, LPOIA- Lipid Per-Oxidation Inhibition Assay.

RESULTS AND DISCUSSION

Total phenolics in the shoot extract of *Paederia foetida* in solvent of Gr-A was significantly higher ($p<0.05$) under MAE method in comparison to that in CP. MAE also extracted significantly higher ($p<0.05$) flavonoids in all solvents. Higher amount of polyphenols in Gr-D solvents followed by Gr-A and flavonoids in Gr-D solvents followed by Gr-B were extracted in MAE method than in CP method. The result infers better efficacy of MAE method in extraction of phyto-phenols and flavonoids. The result is in good agreement with those of Kuti and Konuru (2004).

Microwave Assisted Extraction is an advanced technique where extraction of bio-active compounds is associated with solvent type and concentration (Turkmen et al., 2007). The polarity of solvents and physical and chemical properties of components play a crucial role to effect the concentration of compounds to be extracted, but non-polar solvents are not affected by microwave energy. Polyphenols and flavonoids are more soluble in organic solvent than in aqueous ones and all the solvents contain only 20% water except Gr-C which has 50% water. Less amount of water in polar solvents protects and prevents phenolic compounds from being oxidized by phenol-oxidase (Harborne and Williams, 2000). Significantly higher contents of phenolics and flavonoids in G-A, B and D and lower concentrations in Gr-C may be due to variations in the solubility and difference in properties of components.

The extracted polyphenols and flavonoids from the shoot of experimental plant under MAE method exhibited significantly higher ($p<0.05$) total anti-oxidant, FRAP, Reducing power, NO and SO scavenging activities in all groups of solvents than those extracted under CP method. On the other hand, the OH scavenging activity and LPOIA were significantly higher ($p<0.05$) in MAE method in all the groups except Gr-A than in CP method. It states that, extracted components at GR-B, C and D have better potency to exhibit anti-oxidant activities. The anti-oxidant activities of phenolics and flavonoids in different methods vary between the solvents where Gr-D solvents exhibit higher total ant-oxidant activity followed by Gr-B. Similarly, Gr-D solvents also depicts higher FRAP and reducing power activities followed by Gr-B solvents. SO and NO radical scavenging activity were estimated higher in Gr-C solvents followed by Gr-A where as OH scavenging property was higher in Gr-D solvents followed by Gr-B. Similarly, the LPOIA was higher in Gr-D followed by Gr-C. The result is in concordance with the findings of Halliwell et al. (2000).

The scavenging of superoxide, nitric oxide, hydroxyl radical and inhibition of MDA production are chemical methods to measure the antioxidative activities of bio-active compounds. Polyphenols have more than 8000 structural variants and not a single component is responsible for possessing all the anti-oxidant activities. As the components and the concentration of phenolics and flavonoids vary between the solvents and extraction methods, it contributes to their variable total and individual anti-oxidant activity which is in good agreement with Spigno et al. (2007). The literatures have reported that microwave assisted extraction is the superior technique than other conventional methods, now a day, by adopting its standard protocols within very less time, with best extraction (Dhanani et al., 2017; Zhang et al., 2018; Fierascu et al., 2020) which is equally correlates with our result. The great diversity phyto-constituents assayed by qualitative and quantitative methods is responsible to exhibit difference in antioxidant activity assayed in different chemical methods.

CONCLUSION

Microwave assisted extraction method of extraction from *Paederia foetida* recovered significantly higher ($p<0.05$) polyphenols and flavonoids than conventional method of cold percolation. Organic solvents like 80% acetone, 80% methanol and their mixture extracted significantly higher ($p<0.05$) amount and more components of polyphenols and flavonoids than 50% acetone due to increase in polarity solvents and solubility of phyto-constituents. Higher concentration of phenolics and flavonoids in these solvents exhibited significantly higher ($p<0.05$) total and individual anti-oxidant activities in different chemical methods due to variation in quality and quantity of phenolic components.

ACKNOWLEDGEMENT

The author extend thanks and gratitude to the Vice Chancellor , OUAT and the scientist and staffs of Department of Botany to facilitate the study.

REFERENCES

Afroz, S., Alamgir, M., Khan, M.T.H., Jabbar, S., Nahar, N. and Choudhuri, M.S.K. 2006. Antidiarrhoeal activity of the ethanol extracts of *Paederia foetida* Linn. (Rubiaceae). *J. Ethnopharm.* **105**: 125-130.

Alasalvar, C., Karamac, M., Amarowicz, R. and Shahidi, F. 2006. Antioxidant and antiradical activities in extracts of hazelnut kernel (*Corylus avellana* L.) and hazelnut green leafy cover. *J. Agric. Food Chem*. **54**: 4826-4832.

Atta, A.H. and Mouneir, S. M. 2004. Antidiarrhoeal activity of some Egyptian medicinal plant extracts. *J. Ethnopharm.* **92**: 303-309.

Benzie, I.E.F. and Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem*. **239**: 70-76.

Dhanani, T., Shah, S., Gajbhiye, N.A. and Kumar, S. 2017. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian J. Chem*. **10**: S1193-S1199.

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

Fierascu, R.C., Fierascu, I., Ortan, A., Georgiev, M.I. and Sieniawska, E. 2020. Innovative Approaches for Recovery of Phytoconstituents from Medicinal/ Aromatic Plants and Biotechnological Production. *Molecules* **25**(2): 309.

Halliwell, B. 2007. Dietary polyphenols: Good, bad, or indifferent for your health? *Cardiovasc. Res*. **73**: 341-347.

Halliwell, B., Gutteridge, M.C. and Aruoma, O.I. 1997. The deoxyribose method: a simple test tube assay for determination of rate constants for reactions of hydroxyl radicals. *Anal. Biochem*. **165**: 215-219.

Halliwell, B., Zhao, K. and Whiteman, M. 2000. The gastrointestinal tract: a major site of antioxidant action? *Free Radic. Res*. **33**: 819-830.

Harborne, J. and Williams, C. 2000. Advances in flavonoid research since 1992. *Phytochemistry* **55**(6): 481-504.

Hyland, K., Voisin, E., Banoun, H. and Auclair, C. 1983. Superoxide dismutase assay using alkaline dimethylsulfoxide as superoxide anion-generating system. *Anal. Biochem.* **135**(2): 280-287.

Johnson, T. 1999. *CRC Ethnobotany Desk Reference*. CRC Press, Boca Raton, Florida, pp. 580.

Kumar, J. and Surh, Y. 2008. Cancer chemopreventive and therapeutic potential of resveratrol: Mechanistic perspectives. *Cancer Lett.* **269**: 243-246.

Kuti, J. and Konuru, H. 2004. Antioxidant capacity and phenolic content in leaf extracts of tree spinach (*Cnidoscolus* spp.). *J. Agric. Food Chem.* **52**: 117-121.

Mimica- Dukic, N. 1992. *Investigation on secondary biomolecules in some Mentha species*. Ph. D. Thesis, University of Novisad.

Mishra, R. and Bisht, S.S. 2011. Medicinal values of skunk vine (*Paederia foetida* L.) - An overview. *e-planet* **9**(1): 56-59.

Nosalova, G., Mokry, J., Ather, A. and Khan, M.T.H. 2007. Antitussive activity of the ethanolic extract of *Paederia foetida* (Rubiaceae family) in Non-anaesthetized Cats. *Acta Vet.* **76**: 27-33.

Ohkawa, H., Oshishim, N. and Yagi, K. 1979. Assay for lipid peroxide in animal tissue by thiobarbituric acid reaction. *Anal. Biochem.* **95**: 351-358.

Osman, H., Rahim, H.A., Isa, N.M. and Bakhir, N.M. 2009. Antioxidant activity and phenolic content of *Paederia foetida* and *Syzygium aqueum*. *Molecules* **14**: 970-978.

Oyaizu, M. 1986. Studies on product on browning reaction prepared from glucose amine. *Jpn. J. Nutr.* **44**: 307-315.

Prieto, P., Pineda, M. and Miguel, A. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of Phosphomolybdenum Complex. *Anal. Biochem.* **269**: 337-341.

Saxena, H.O. and Brahmam, M. 1995. Rosaceae to Martyniaceae, In: *The flora of Orissa*. Vol-II. Regional Research Laboratory, Bhubaneswar and Orissa Forest Development Corporation Ltd., Bhubaneswar.

Senapati, M.R., Behera, P.C., Bisoi, P.C., Maity, A. and Parija, S.C. 2013. HPTLC finger print analysis of phytophenols of *Paederia foetida* under different extraction regimen. *Bioscan* **8**(2): 603-609.

Singh, R.P., Jayaprakasha, G.K and Murthy, K.N.C. 2002. Studies on antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *J. Agric. Food Chem.* **50**: 81-86.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods*, 8th edn, Iowa State University Press, Ames, USA.

Spigno, G., Tramelli, L. and Faveri, D.M.D. 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *J. Food Eng.* **81**: 200-208.

Sreejayan Rao, M.N.A. 1997. Nitric oxide scavenging by curcuminoids. *J. Pharm*. **49**: 105-107.

Sultana, B., Anwar, F. and Ashraf, M. 2009. Effect of extraction solvent/ technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* **14**: 2167-2180.

Surveswaran, S., Cai, Y.Z., Corke, H. and Sun, M. 2007. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *J. Food Chem.* **102**: 938-953.

Thatoi, H.N., Panda, S.K., Rath, S.K. and Dutta, S.K. 2008. Antimicrobial activity and ethnomedicinal uses of some medicinal plants from Similipal Biosphere Reserve, Orissa. *Asian J. Plant Sci.* **7**: 260-267.

The Ayurvedic Pharmacopeia of India 1999. Ministry of Health and Family Welfare Department and Indian System of Medicine and Homeopathy, New Delhi. **2** (Part I): 137-140.

Turkmen, N., Velioglu, Y.S., Sari, F. and Polat, G. 2007. Effects of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of Black Tea. *Molecules* **12**: 484-496.

Veerapur, V.P., Prabhakar, K.R., Parihar, V.P., Kandadi, M.R., Ramakrishana, S., Mishra, B., Satish Rao, B.S., Srinivasan, K.K., Priyadarsini, K.I. and Unnikrishnan, M.K. 2009. *Ficus racemosa* stem bark extract, a potent antioxidant and a probable natural radioprotector. *Evid.- Based Complement Alternat. Med*. **6**(3): 317-324.

Yadav, V.K., Kumar, S.P., Singh, U.P., Bhat, H.R. and Zaman, M.K. 2009. Pharmacognostical and phytochemical study on the leaves of *Paederia foetida* linn. *Int. J. Pharm. Tech. Res.* **1**(3): 918-920.

Zhang, Q.W., Lin, L.G. and Ye, W.C. 2018. Techniques for extraction and isolation of natural products: a comprehensive review. *Chin. Med.* **13**: 20.