Journal of King Saud University – Science (2015) 27, 204–208

King Saud University
Journal of King Saud University – Science
www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Effect of method and time of extraction on total phenolic content in comparison with antioxidant activities in different parts of *Achyranthes aspera*

Vinayak Upadhya [a], **Sandeep R. Pai** [b,*], **Harsha V. Hegde** [a,b]

[a] *Herbal Medicine Division, Regional Medical Research Centre (RMRC), Indian Council of Medical Research (ICMR), Nehru Nagar, Belgaum 590 010, Karnataka, India*
[b] *Plant Biotechnology and Tissue Culture Division, Regional Medical Research Centre (RMRC), Indian Council of Medical Research (ICMR), Nehru Nagar, Belgaum 590 010, Karnataka, India*

Received 6 March 2015; accepted 19 April 2015
Available online 25 April 2015

KEYWORDS

Achyranthes aspera;
Continuous shaking;
Ultrasonic;
Microwave;
Total phenolics;
Antioxidant

Abstract The main purpose of this study was to evaluate the effect of extraction method with respect to time of exposure on total phenolic content and antioxidant potential of methanolic extracts (95%) from *Achyranthes aspera* leaves, stem and roots. Total phenolic content (TPC) was determined using the Folin–Ciocalteu method and antioxidant potential was tested using DPPH radical scavenging and FRAP assays. Plant populations aged 60 and 120 days were considered during the study. Overall, highest TPC was evident in mature plants (120 days) compared to younger (60 days). Similarly, leaves accumulated higher phenolics followed by roots and stem. Results showed the MAE technique to be efficient over USE and CSE methods. Same was evident in both the antioxidant assays tested. A similar trend was observed in both antioxidant assays as that of TPC, indicating phenolics to be the major contributor in the antioxidant potential of the plant. In conclusion it can be said that the yield of phenolic compounds depends on parameters *viz*. age of plant, part used for extraction, method of extraction and time required for the same.

Abbreviations: TAE, tannic acid equivalent; CAE, caffeic acid equivalent; TPC, total phenolic content; AEAC, ascorbic acid equivalent antioxidant capacity; TEAE, trolox equivalent antioxidant capacity; CSE, continuous shaking extraction; MAE, microwave assisted extraction; USE, ultra sonic extraction

* Corresponding author. Tel.: +91 831 2475477/78; fax: +91 831 2475479.
E-mail address: drpaisr@gmail.com (S.R. Pai).
Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

1. Introduction

Achyranthes aspera L. commonly known as devils horse whip, is a weed belonging to family Amaranthaceae, widely distributed throughout tropical and warmer parts of the world (Hooker, 1885). It has spread in many countries of Asia, Africa, America, Europe and Australia (Prain, 1963; deLange et al., 2004). It has been listed as a noxious weed in many countries including South Africa (Henderson, 2001). Although listed as a weed in India too, it has been identified

http://dx.doi.org/10.1016/j.jksus.2015.04.004
1018-3647

among the 46 high volume traded medicinal species by Ved and Goraya (2007).

The plant individually and in combination is widely used in traditional medicine all over the world (Pai et al., 2010, 2015; Fikru et al., 2012; Shibeshi et al., 2006). It is used as an anti-inflammatory and in anti-arthritic treatment (Girach and Khan, 1992; Gokhale et al., 2002); as an abortifacient (Pakrashi and Bhattacharya, 1977). It has been tested as an antifertility agent (Prakash, 1986; Varshney et al., 1986; Wadhwa et al., 1986), and also for its anti-cancer activity (Chakraborty et al., 2002). Barua and co-workers (2012) have examined it for healing wounds and burns, while its antimicrobial potential is reported by many others (Sharma et al., 2011; Raman et al., 1996). It has also been investigated for energy production (Subramanian and Sampathrajan, 1999). The plant has also been reported to enhance the immunity of fish in aquaculture (Kaleeswaran et al., 2012).

The medicinal properties of the plants are mainly attributed to their phytochemical constituents such as polyphenols, saponins, alkaloids etc. (Dinda et al., 2007a,b; Francis et al., 2002; Podsędek, 2007). These phytochemicals are responsible for the magnitude of biological effects, including antioxidant, antimicrobial and anti-cancer activities. Extraction is an important rate limiting step to attain optimum yield of any compound. Two major parameters affecting content yield of analytes, includes method of extraction and time required for it. There are a number of methods identified for extraction of plant based activities and metabolites (Pai et al., 2011a,b, 2015; Murugan and Parimelazhagan, 2014; Nimbalkar et al., 2012; Patil et al., 2012; Pawar et al., 2011).

As there are no such studies carried out in *A. aspera* and owing to its medicinal importance the present work was undertaken. Thus the study herein evaluates total phenolic content with antioxidant potential of *A. aspera* on the basis of age of plant; parts (leaves, stem roots) and different extraction methods with various times of exposures.

2. Materials and methods

2.1. Chemicals

Trolox, caffeic acid (3-(3,4-dihydroxyphenyl)-2-propenoic acid) were procured from Sigma Aldrich, India. Folin and Ciocalteu phenol reagent, sodium acetate trihydrate, sodium hydrogen carbonate, tannic acid, ascorbic acid, 2,2-diphenyl-1-picryl hydrazyl (DPPH), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), and ferric chloride were obtained from Hi media, India. Solvents like methanol, glacial acetic acid, and hydrochloric acid were from Qualigens, India. All chemicals used in the study were of analytical grade.

2.2. Sample preparation

Leaves, stem and roots of *A. aspera* were obtained from a single population from the north central corridor of the Western Ghats from Belgaum district in Karnataka state, India (GPS: N 15.88°; E 74.52°, 801 M above MSL). Plants were collected at 60 days and 120 days interval after germination. A specimen was authenticated and deposited at the Herbarium, Regional Medical Research Centre, Belgaum (Voucher Number: RMRC 1250). The material obtained was dried separately at 40 ± 5 °C for 48 h, finely powdered and sieved through a 20 μm stainless sieve and used for extraction.

2.3. Efficiency of extraction method

The extraction methods earlier described by Pai et al. (2011a) for the extraction of betulinic acid from *Ancistrocladus heyneanus*, with minor modifications were employed during the study.

2.3.1. Continuous shaking extraction (CSE)

Continuous shaking extractions were carried out by subjecting 5 g of fresh plant material (leaves, stem and roots separately) into a 150 mL conical flask. The flask was subjected with 100 mL of 95% methanol and placed on an orbital shaker (Rivotek, Riviera, India). A constant stirring of 110 ± 2 rpm was maintained for the suspensions at a controlled temperature (25 ± 5 °C). Plant materials were extracted with 95% MeOH for 30, 180 and 360 min separately.

2.3.2. Ultrasonic extraction (USE)

Ultrasonic extractions were performed by subjecting 1 g of plant material with 20 mL 95% methanol in a 150 mL beaker on an ultrasonic bath (Soncis vibracell) at a working amplitude of 60 Hz. Samples were prepared as above and were exposed to sonication for 5, 15 and 30 min at room temperature.

2.3.3. Microwave assisted extraction (MAE)

Leaves, stem and roots (1 g) were individually put into 150 mL Erlenmeyer flasks and were added with 20 mL 95% methanol. All flasks with suspension were exposed for 1, 3 and 5 min in a microwave oven (Godrej, GMX 30GAI SIM, India) at 180 W. The suspensions were cooled at regular intervals to avoid bumping of the solvent out of the flask. The above steps were repeated in order to complete the required time of microwave irradiation. The extracts were then filtered and the volume was made up to 20 mL with the solvent.

All extracts were filtered through filter paper (Whatman No. 1) re-volumised (to obtain 5% concentration) and the filtrates were used for further analysis.

2.4. Total phenolic content (TPC)

Total phenolic content was quantified using the modified Folin–Ciocalteu method previously described by Wolfe et al. (2003). The absorbance of blue color was read at 760 nm using distilled water instead of standards in the reaction mixture as blank on a double beam spectrophotometer (Thermo Scientific, multiskan Go 1510, USA). Similarly, extracts prepared (5%) were also quantified and the results were compared to the standard curves and expressed as mg/tannic or caffeic acid equivalent per gram dry powder for the samples.

2.5. Antioxidant activities

2.5.1. DPPH radical scavenging assay

Antioxidant activities were determined for the plant extracts as a measure of radical scavenging, using the DPPH assay determined by Brand-Williams et al. (1995). Various concentrations of ascorbic acid and/or Trolox were used instead of the plant extract as reference standard during the experiment. The