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Research paper

Oxidative DNA damage protective activity and antioxidant potential of Ashtvarga species growing in the Indian Himalayan Region

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ABSTRACT

Ashtvarga is a popular Ayurvedic group comprises of eight herbs used in various rejuvenating Ayurvedic tonics like Chyvanprash. Considering the importance of Ashtvarga group in various preparations, the phenolic composition, antioxidant and antimutagenic activities of all eight species extracts were evaluated. Total phenolic content measured by Folin- Ciocalteu assay of Ashtvarga species showed the highest value in *Polygonatum cirrhifolium* and minimum was recorded in *Roscoea procera*. However, *R. procera* showed significantly higher tannin and flavonoid content as compared to other Ashtvarga species. Total fifteen phenolic compounds were quantified using HPLC analysis, and maximum 11 phenolic compounds were detected in *Malaxis acuminata* extract. Antioxidant activities using in vitro assay like 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging (ABTS), 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH), Ferric reducing antioxidant power (FRAP) and Superoxide scavenging activity were also determined. The results showed all the Ashtvarga plants possess strong ability to protect DNA and therefore, these species could be a potential source of natural antioxidant.

1. Introduction

Traditional knowledge on medicinal plants in the discovery of a new drug is gaining popularity all across the globe considering their effectiveness in curing various diseases with lesser side effects, cheaper and easy availability. Although, their efficacy and mechanism are not well investigated but the presence of several bioactive compounds make them special candidate for detail investigations.

Among others, 'Ashtvarga' a group of eight medicinal plants ('Asht' means eight and 'Varga' means group), namely, Vriddhi (*H. edgeworthii*), Ridhi (*Habenaria intermedia*), Meda (*Polygonatum verticillatum*), Mahameda (*P. cirrhifolium*), Jeevak (*Malaxis acuminata*), Rishvak (*M. muscifera*), Kshirakakoli (*Lilium polyphyllum*) and Kakoli (*Roscoea procera*), distributed in North West Himalayan region in small patches with an altitudinal range of 1600–4000 m asl. This group is well known for (i) rejuvenating health promoting activity, (ii) strengthening vital force of the body, (iii) enhancing cell regeneration capacity, and (iv) improving immunity system (Mathur, 2003; Pandey, 2005; Singh and Duggal, 2009; Balkrishna et al., 2012). Plants of this group are also considered useful in curing the healing fractures, seminal weakness, fever, diabetes, etc. This group of plants was invented by *Rishi Chyvan* in ancient time and developed a formulation called Chyvanprash for rejuvenating and restoring youthfulness. Since then, this preparation was offered mostly to the Kings as the availability of these plants is scarce in wild (Balkrishna et al., 2012). Inspite of the fact that the plants of this group are important source of vitality strengthening, systematic investigations with respect to antiageing property are poorly known. Generally, vitality strengthening and anti-ageing attributes are controlled by antioxidant properties present in the different group of chemicals i.e., phenol, flavonoid, isoflavone, flavones, anthocyanin, etc. which are known to scavenge Reactive Oxygen Species (ROS) species (Dimitrios, 2006). These ROS species have ability to oxidize cellular biomolecules, i.e., DNA, protein and carbohydrate (Ardestani and Yazdanparast, 2007; Bendich, 1996) and cause various degenerative diseases (Wiseman and Halliwell, 1996). Ashtvarga plants are considered threatened in its natural habitat. For instance, *Lilium polyphyllum* and *H. intermedia* are Critically Endangered, *Malaxis muscifera* is Vulnerable, *Habenaria edgeworthii* is rare (Saha et al., 2015a,b; Chinmay et al., 2009) and other four species i.e. *Polygonatum verticillatum P. cirrhifolium, Roscoea procera* and *Malaxis acuminata* are scarcely found in the wild.

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As such, phytochemical investigation in some species of Ashtvarga like *Habenaria edgeworthii, H. intermedia* and *Roscoea procera* and *Polygonatum verticillatum* with regards to phenol, flavonoid, thiamine, riboflavin, mineral contents, etc. has been carried out (Giri et al., 2012a,b, 2016; Rawat et al., 2014, 2016; Khan et al., 2012, 2016) but in depth studies on the antioxidant and antiageing properties of the plant of this group is lacking. Therefore, the present study was undertaken to (i) assess phytochemicals content, (ii) quantify individual phenolic compounds using HPLC, and (iii) evaluate the antioxidant and antimutagenic activity. The outcome of this study will form the baseline data on these species and will be helpful in development of some antioxidant and antiageing products from the species. This will also help in promoting the species for conservation and cultivation.

2. Materials and methods

2.1. Plant samples

Plants of Ashtvarga group were collected from different localities of Uttarakhand (India) and details of site characteristics is given (Table 1). Individuals of each species (5–10 No.) were collected in flowering stage (Fig. 1) and further used for quantification of polyphenolic content, antioxidant and antimutagenic activities. Botanical identity of each species was authenticated from Botanical Survey of India (BSI), Deharadun and the specimens (except *Lilium polyphyllum*) were deposited at G. B. Pant National Institute of Himalayan Environment and Sustainable Development (GBPNIHESD) herbarium. Tuber powder of *Lilium polyphyllum* was obtained from High Altitude Plant Physiology Research Centre (HAPPRC), Srinagar Garhwal, Uttarakhand.

2.2. Chemicals and reagents

2,2-Diphenyl-1-picryhydrazyl (DPPH), ascorbic acid and phenolic standard compounds (rutin hydrate, phloridzin dihydrate, *p*-coumaric acid, catechine hydrate, gallic acid, quercetin dihydrate, 3-hydroxybenzoic acid, 4-hydroxybezoic acid, ellagic acid, vanillic acid, caffeic acid, *m*-coumaric acid, ferulic acid, *trans*-cinnamic acid and chlorogenic acid) were procured from Sigma-Aldrich (St. Louis, Missouri, United States). Sodium carbonate, potassium persulphate, hydrogen peroxide, ferric chloride, sodium acetate, potassium acetate, aluminium chloride, acetic acid and hydrochloric acid from Qualigens (Mumbai, India), and 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), and methanol were procured from Merck *KGaA* (Darmstadt, Germany). All the chemicals were of analytical and HPLC grade.

2.3. Preparation of extract

Below ground portion (rhizome or tuber or bulb) of each species was separated and washed thoroughly with tap water followed by ultra pure water (Rions India Lab Water System, India). The plant material was dried separately in hot air oven 48 °C for 3–5 days and grounded into fine powder. Dried powder (0.1 g) was mixed with 10 mL (80% v/v) methanol and the suspension was incubated in water bath at 60°C for 1 h. Further the suspension was incubated for 14 h at 25 °C and sonicated at 22 °C for 10 min (40 Hz). Extracts of *Habenaria edgeworthii* (HEE), *H. intermedia* (HIE), *Malaxis acuminata* (MAE), *M. muscifera* (MME), *Polygonatum cirrhifolium* (PCE), *P. verticillatum* (PVE), *Lilium polyphyllum* (LPE) and *Roscoea procera* (RPE) were filtered and filtrate was centrifuged at 8000 rpm for 10 min. Supernatant were stored at 4 °C prior to use within 2 days.

2.4. Determination of total phenols

Total phenolic content in the methanolic extract was determined by Folin- Ciocalteu's colorimetric method (Singleton and Rossi, 1965). In 0.25 mL of diluted methanolic extract, 2.25 mL distilled water and 0.25 mL Folin-Ciocalteu's reagent was added and allowed to stand for reaction upto 5 min. This mixture was neutralized by 2.50 mL of 7% sodium carbonate (w/v) and kept in dark at room temperature for 90 min. The absorbance of resulting blue colour was measured at 765 nm using UV–vis spectrophotometer. Quantification was done on the basis of standard curve of gallic acid prepared in 80% methanol (v/v) and results were expressed in mg gallic acid equivalent (GAE) per g DW.

2.5. Determination of total flavonoids

Flavonoid content in the methanolic extract of each plant was determined by aluminium chloride colorimetric method (Chang et al., 2002). Briefly, 0.50 mL of methanolic extract of sample was diluted with 1.5 mL of distilled water and 0.50 mL of 10% (w/v) aluminium chloride added along with 0.10 mL of 1 M potassium acetate and 2.80 mL of distilled water. This mixture was incubated at room temperature for 30 min. The absorbance of resulting reaction mixture was measured at 415 nm UV–vis spectrophotometer. Quantification of flavonoid was done on the basis of standard curve of quercetin prepared in 80% methanol and results were expressed in mg quercetin equivalent (QE) per g DW.

2.6. Determination of total flavonols

Total flavonols in the methanolic extract was determined using the method of Kumaran and Kaunakaran (2007) with slight changes. Briefly, 2.0 mL of metahnolic extract sample, 2% (w/v) aluminium chloride ethanolic solution (2.0 mL) and 50 g/L sodium acetate solution (3.0 mL) were added. This mixture was incubated at room temperature for 2.5 h at 20 °C. The absorbance of resulting reaction mixture was measured at 440 nm UV–vis spectrophotometer. Quantification of total flavonols was done on the basis of standard curve of catechin prepared in 80% (v/v) methanol and results were expressed in mg catechin equivalent (CE) per g of DW.

2.7. Determination of total tannins

Total tannin content was estimated following Nwinuka et al. (2005) with slight modification. The methanolic extract (5.0 mL) was added to Folin–Dennis reagent (0.5 mL) and saturated with 1.0 mL of sodium carbonate solution. The solution was diluted up to 10.0 mL with distilled water and allowed to stand for 20 min at 22 ± 1 °C. The absorbance of resulting reaction mixture was measured at 700 nm and quantification of tannin was done on the basis of standard curve of tannic acid prepared in 80% (v/v) methanol and results were expressed in mg tannic acid equivalent (TAE) per g of DW.

2.8. Antioxidant activity

2.8.1. Radical scavenging activity (ABTS assay)

Total antioxidant activity was measured by improved ABTS method described by Cai et al. (2004). ABTS salt (7.0 μM) and potassium persulfate (2.45 μM) was added for the production of ABTS cation ($ABTS^{\bullet+}$) and kept in dark for 16 h at 23◦C. $ABTS^{\bullet+}$ solution was diluted with 80% (v/v) ethanol till an absorbance of 0.7 ± 0.005 at 734 nm was obtained. Diluted $ABTS^{\bullet+}$ solution (3.90 mL) was added in 0.10 mL of methanolic extract and the resulting mixture was mixed thoroughly. Reaction mixture was allowed to stand for 6 min in dark at 25 °C and absorbance was recorded at 734 nm using