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Full Length Research Paper

Evaluation of antioxidant activities of *Withania somnifera* leaves growing in natural habitats of North-west Himalaya, India

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Evaluation of antioxidant properties of medicinal plants from Indian Himalayan region has been very rarely carried out. *Withania somnifera* L. is one of the commercially available and most preferred medicinal plants in the Himalayan region due to its aphrodisiac property and potential to cure various diseases. The review of literature has indicated that the antioxidant activities of *W. somnifera* have not been carried out for so long. Therefore, in the present study an attempt was made to evaluate the antioxidant properties of *W. somnifera* collected from two different habitats that is, forest and roadside at Kullu, north-west Himalaya. The total phenolic and flavonoid contents and DPPH (1, 1-Diphenyl-2-pycrylhydrazyl) scavenging potential of leaves extract of *W. somnifera* varied significantly between the habitat (*p<0.05*). The results further showed that the DPPH scavenging potential of leaves extract at forest site was found significantly higher that is 51% over the roadside. The study indicates that antioxidant activities of leaves of *W. somnifera* L. varies habitat to habitat and antioxidant properties decrease if plants are exposed to vehicular pollution. Therefore, plantation and cultivation of this species in vehicular pollution free areas are suggested.

Key words: *Withania somnifera*, phenolics, flavonoids, 1, 1-Diphenyl-2-pycrylhydrazyl (DPPH), habitats.

INTRODUCTION

Many plant species are found rich in novel antioxidant compounds (Pourmorad et al., 2006) and the activities of antioxidants in a plant depend on phenolic compounds (Pietta, 2000). Medicinal plants have been recognized as a potential source of natural antioxidants throughout the world. Among the various medicinal plants, few endemic species are of particular interest as they are commonly being used for producing raw materials or preparations containing phytochemicals with significant antioxidant capacities (Exarchou et al., 2002). Naturally produced reactive oxygen species (ROS) can attack cell component and creates several types of biological damage (Hutadilok-Towatana et al., 2006). Free radicals (FR) or ROS capable of causing damage to deoxyribonucleic acid (DNA) have been associated with various health problems (for example, carcinogenesis, coronary heart disease, etc.) related to advancing age (Cadens and Davies, 2000). Recent studies have clearly shown potential of plant products to serve as antioxidant against various diseases induced by free radicals (Hou et al., 2003). Minimizing anitoxidative damage through scavenging free radicals may be one of the most important approaches to prevent the human being from these ageing associated disease and health problems.

Since antioxidants have the capacity to terminate the direct attack of ROS and free radicals mediated oxidative reactions and appear to be or primary importance in the prevention of these diseases and health problems. Many studies have reported the biological activities of phenolics as a potent antioxidant and free radical scavengers (Kahkonen et al., 1999; Sugihara et al., 1999). Motor vehicles have been closely related with increasing air pollution levels in urban areas responsible for more than 60% of the air pollution of an urban environment (Singh et al., 1995). Depending upon the fuel type, the main

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exhaust emissions are oxides of nitrogen (NOx), oxides of carbon, oxides of sulphur (SOx), carbon particles, heavy metals, water vapour and hydrocarbons including aldehydes, single and poly aromatic hydrocarbons, alcohols, olefins, alkylnitriles besides a number of secondary pollutants such as ozone, etc., causing serious environmental and health impacts. *W. somnifera* L. commonly known as Ashwagandha has been a commercially viable medicinal shrub in Ayurvedic and Indigenous Systems of Medicines for many centuries in India (Jaleel et al., 2008). It is also known as the Indian Ginseng due to its ability of curing a large number of diseases (Samant et al., 2008). The free radical scavenging potential of the *W. somnifera* from different habitats of the Himalaya has not been reported yet. Therefore, an attempt has been made to evaluate the antioxidant potential of the methanol extracts of the leaves of *W. somnifera* collected from two different habitats that is, forest and roadside at Kullu valley of the Himachal Pradesh, India.

MATERIALS AND METHODS

Chemicals were purchased from Sigma and Merck Chemical Company India. All the chemicals and reagents were of the analytical grade.

Sampling and analysis

Five mature plants, each of *W. somnifera* having heights of 60 cm were collected in March 2010 from the roadside habitat of NH-21 at a distance of 50 m both sides of the road and from forest habitat, 300 to 500 m away from the NH-21. The numbers of vehicles running on NH-21 were at Kullu averaged from 2,900 per day during 6 am to 6 pm (Kuniyal et al., 2007). The samples were washed with running tap water and air dried. The leaves were separated and used for chemical analysis. For extraction, 1g of leaf was weighed and crushed in 10 ml of 80% methanol using a mortar and pestle, and were kept at 0°C for 24 h. Finally, the supernatant was taken and final volume was maintained to 10 ml by adding 80% methanol and stored in refrigerator for further analyses. The amounts of total phenolics in methanol extracts of leaf samples were determined by the modified method of Wolfe et al. (2003). An aliquot (1ml) of the extract was mixed with 1ml of Folin-Ciocalteu Phenol Reagent (previously diluted with double distilled water in 1:1 v/v) and 2 ml of 2% of sodium carbonate. The final volume was maintained to 10 ml by double distilled water and whole mixture was then heated at 80°C for 30 min or till the blue color appeared.

The absorbance of blue color solution after cooling at room temperature was determined using Spectrophotometer (Ultraspec 2100 Pro) at 650 nm. The content of the total phenolics was expressed in mg tannic acid g^{-1} fresh leaf. A standard curve was also prepared using different concentrations of the tannic acid. Total flavonoids contents in the methanol extracts of the leaf samples was quantified using the modified method of Ordon-Ez et al. (2006) based on the formation of complex flavonoid-aluminum and absorbance of yellow color was determined using Spectrophotometer (Ultraspec 2100 Pro) at 420 nm. An amount of 1 ml of aliquot was mixed to 1 ml of 2% ethanolic aluminium chloride ($AlCl_3$) vigorously. The reaction mixture was left for 1 h at room temperature. Total flavonoid contents were calculated as mg quercetin g^{-1} fresh leaf using standard curve prepared from different concentrations of quercetin.

The effect of methanol extracts of leaf samples on scavenging DPPH radical was estimated using the method of Liyana-Pathirama and Shahidi (2005). A solution of 0.135 mM DPPH in methanol was prepared and 5 ml of the solution was mixed vigorously with 1ml of leaf extract in methanol. This mixture was left for 30 min in dark at room temperature. The absorbance of mixture was measured using Spectrophotometer (Ultraspec 2100 Pro) at 517 nm. The ascorbic acid or butylated hydroxyl toluene (BHT) solutions were used as reference solution. The DPPH free radical scavenging activity was calculated using following equation.

DPPH radical scavenging activity (%) = $(O.D_C – O.D_S) \times 100 / O.D_C$

Where O. D_C is the absorbance of DPPH radical + methanol; O. D_S is the absorbance of DPPH radical + leaf extract/standard.

Statistical analysis

Means and standard error for the each site were analysed. The significance differences between the sites were analysed using Student's T-test at probability levels (0.05, 0.01). All statistical analyses were performed by using SPSS software, version 12.

RESULTS AND DISCUSSION

The contents of the total phenolics and flavonoids, and DPPH scavenging potential of the methanol extracts of fresh leaves of *W. somnifera* L. are presented in Table 1 and Figure 1, respectively. The results showed that methanol extracts of leaves of *W. somnifera* L. collected from the roadsides had significantly higher contents of total phenolics and flavonoids (100.10±0.90 and 92.88 ± 1.12 mg g^{-1} fresh leaf, respectively) as compared to forest (Table 1). On the other hand, DPPH radical scavenging potential was found significantly higher ($p<0.05$) in the leaf extract of plants collected from forest as compared to roadside (Table 1). The present study suggests that plants exposed to vehicular pollution have higher contents of total phenolics and flavonoids, but have a lower DPPH radical scavenging potential. The higher contents of both total phenolics and flavonoids in the leaf extracts of plants collected from road side may be ascribe to increased levels of free radicals due to emission of pollutants from running vehicles. Under normal conditions free radicals usually do not showed any harmful action because they are unstable, and change to a non radical product.

The reduced form of phenolic compound acts as antioxidant while the oxidized one (phenoxy radical) may produced cytotoxic effects, which is toxic to a living system because of their ability to initiate free radical chain reaction in membrane and their propensity to cross-link with a variety of molecule. An increase of phenolic content was correlated to increase in activity of enzyme involved in phenolic compound metabolism under stress condition and increase in flavonoid concentration is mainly the result of conjugated hydrolysis. The earlier studies have clearly shown that the antioxidants such as peroxidase activity, phenolics, superoxide dismutase and glutathione reductase increased significantly due to

Table 1. Total phenolics, flavonoid contents and DPPH inhibition potential of methanol extracts of the fresh leaves of *W. somnifera* collected from roadside and forest of north-west Himalaya.

Parameter	Methanol extracts	
	Forest site	Roadside
Total phenolics (µg tannic acid. g^{-1})	74.27 ± 0.76	100.10** ± 0.90
Total flavonoids (µg quercetin. g^{-1})	75.12 ± 0.89	92.88* ± 1.12
DPPH inhibition (%)	81.63 ± 0.56	54.25* ± 3.44

Values are means ± S.E. of five replicates. The significant differences between polluted and non-polluted sites were analyzed using the student's t-test. Level of significance: ***p≤0.01*, **p≤0.05*.

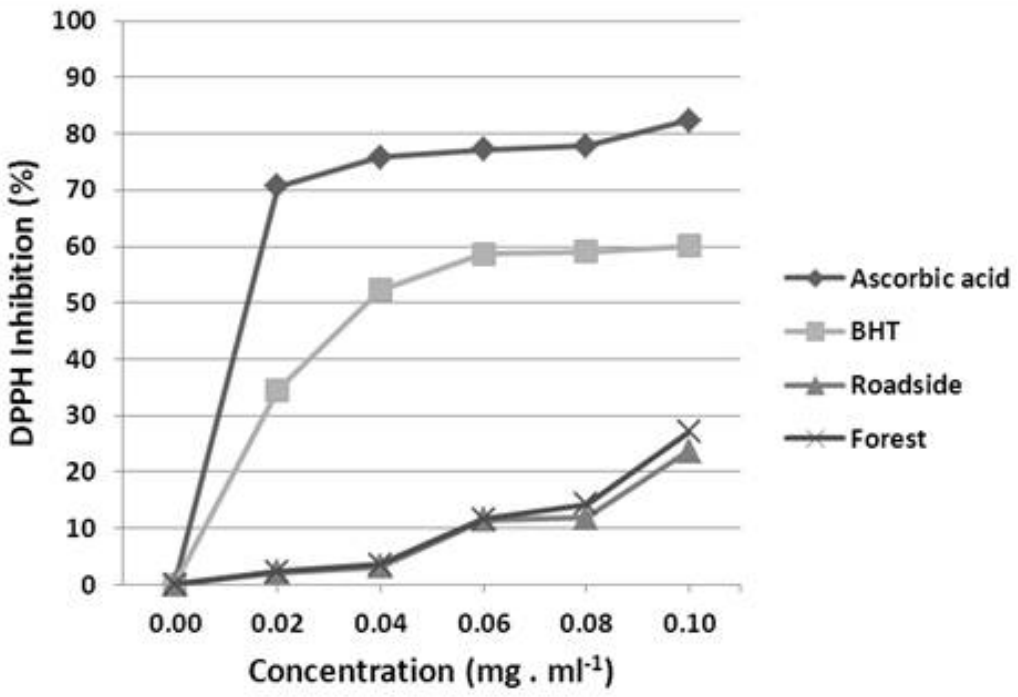

Figure 1. DPPH radical scavenging activity of methanol extracts of the leaves of *W. somnifera* collected from roadside and forest habitats of north-west Himalaya.

ozone and heavy metals in plants (Tiwari et al., 2010; Sharma et al., 2010). Mir et al. (2009) have reported increased levels of total flavonoids and phenolic content in selected medicinal plants growing on roadsides. Vehicular emissions have reduced the chlorophyll and protein content and reduced leaf areas in roadside plants (Wagh et al., 2006). Photosynthetic pigments content decreased progressively as a stress increased. Decreased in chlorophyll level under a stress condition may be due to reduction in pigment biosynthesis or enzymatic chlorophyll degradation and slight reduction in carotenoides may be due to their protective role against ROS.

Vehicular emission can lead to oxidative stress and causing significant decrease to photosynthetic system. Similarly protein content is also reduced under vehicular emission which is present in the form of enzyme in plants. These enzymes provide additional defense against oxidative stress and keep the metabolic activities. Under stress conditions, enzyme activities increase in the plants. The continued exposure of soil to vehicular pollution may get rich in toxic chemicals such Cd, Pb, etc., which may be ascribed to increased levels of total phenolics and flavonoids in roadside plants. The free radical scavenging potential of medicinal plants mainly depends on its active ingredients. The phenolic compounds have antioxidant activity due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Total phenolics are also effective free radical scavengers and have antioxidative property. The positive correlations between

Table 2. DPPH scavenging capacities of methanol extracts of the fresh leaves of *W. somnifera* L. collected from roadside and forest of north-west Himalaya.

Extract	Forest	Roadside	BHT	Ascorbic acid
IC_{50} (µg . ml^{-1})	206.77	224.96	60.87	27.47

antioxidant potential and phenolic compounds of the extracts of *Tanacetum* sp. have also been described (Tepe and Sokmen, 2007). Plants containing flavonoids have also been reported to have strong antioxidant properties. It was estimated that vehicles account for 70% of CO, 50% of HC, 30-40% of NO_X , 30% of SPM and 10% of SO_2 of the total pollution load in the major metros of India, of which two thirds are contributed by two wheelers alone (CPCB, 2010).

DPPH, a chemical compound has a proton free radical with a characteristic absorption which decreased significantly due to exposure to reagents/solutions containing proton radical scavengers (Yamaguchi et al., 1998). According to Baumann et al. (1979) and Chen and Ho (1995) DPPH radical scavenging by the natural or synthetic antioxidants is attributed to their hydrogen donating ability. The dose response curve for inhibition (%) of DPPH radicals by the tested samples at a range of 0.02 to 0.10 mg ml^{-1} are presented in Figure 1. The present study observed that a concentration of 0.1 mg ml^{-1}, the scavenging activity of the methanol extracts of the leaves of all the tested plants from the roadsides reached to 23.73%, while at the same concentration, that of leaves from forest was 27.10%. The DPPH radical scavenging abilities of the leaves extracts from of the tested plants were found further very less than those of BHT (60.07%) and ascorbic acid (82.38%). In addition, the IC_{50} values (the concentration that inhibits radical formation by 50%) of different tested samples are given in Table 2. Among the tested samples, the IC_{50} value was found maximum for the plant extracts from the polluted site followed by non-polluted site, BHT and ascorbic acid (Table 2). The present study reported for the first time that the methanol extracts of *W. somnifera* of Himalayan origin from the roadsides have higher antioxidant contents, but a lower (51%) free radical scavenging potential as compared to a forest. Thus, based on the present study it can be suggested that the medicinal plants for commercial purpose could not be grown near the national highways as their medicinal properties get reduced due to their continuous exposure to vehicular emissions.

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REFERENCES

Baumann J, Wurn G, Brushlausen FV (1979). Prostaglandin synthetase inhibiting O_2- radical scavenging properties of some flavonoids and related phenolic compounds. Deutsche Pharmakologische Gesellschaft Abstract of 20th spring meeting, Naunyn-Schmiedebergs Abstract No: R27 cited in Arch. Pharmacol., 307: R1-R7.

Cadens E, Davies KJA (2000). Mitochondrial free radical generation, oxidative stress, and aging. Free Radic. Biol. Med., 29: 222-230.

Central Pollution Control Board (CPCB) (2010). Status of the vehicular pollution control programme in India. Ministry of Environment and Forests, Govt. Of India, New Delhi – 110 032.

Chen CW, Ho CT (1995). Antioxidant properties of polyphenols extracted from green and black tea. J. Food Lipids, 2: 35-46.

Exarchou V, Nenadis N, Tsimidou M, Gerothanassis IP, Troganis A, Boskou D (2002). Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage, and summer savory. J. Agric. Food. Chem., 50: 5294-5299.

Hutadilok-Towatana N, Chaiyamutti P, Panthong K, Mahabusarakam W, Rukachaisrikul V (2006). Antioxidative and free radical scavenging activities of some plants used in Thai Folk Medicine. Pharm. Biol., 44: 221-228.

Hou WC, Lin RD, Cheng KT, Hung YT, Cho CH, Chen CH, Hwang SY, Lee MH (2003). Free radical scavenging activity of Taiwanese native plants. Phytomedicine, 10: 170-175.

Jaleel CA, Gopi R, Manivannan P, Panneerselvam R (2008). Exogenous application triadimefon affects the antioxidant defense system of *Withania somnifera* Dunal. Pesticide Biochem. Physiol., 91(3): 170-174.

Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem., 47: 3954-3962

Kuniyal JC, Rao PSP, Momin GA, Safai PD, Tiwari S, Ali K (2007). Trace gases behavior in sunshine area of northwestern Himalaya – A case study in Kullu–Manali tourist complex, India. indian J. Radio Space Phys., 36: 197-203.

Liyana-Pathirama CM, Shahidi, F (2005). Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH condition. J. Agric. Food Chem., 53: 2433-2440.

Mir AQ, Yazdami T, Ahmad S, Yunus M (2009). Total Flavonoids and Phenolics in Catharanthus roseus L. and Ocimum sanctum L. as Biomarkers of Urban Auto Pollution. Caspian J. Envon. Sci., 7 (1): 9-16.

Ordon-Ez AAL, Gomez JD, Vattuone MA, Isla MI (2006). Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. Food Chem., 97: 452-458.

Pourmorad F, Hosseinimehr SJ, Shahabimajd N (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotechnol., 11: 1142.

Pietta PG (2000). Flavonoides as antioxidant. J. Nat. Prod., 63: 1035-1042.

Samant SS, Butola JS, Manohar L (2008). Agrotechniques of Commercially Viable Medicinal Plants in the Indian Himalayan Region. GBPIHED, Himachal Unit, Mohal-Kullu, Himachal Pradesh, India.

Singh N, Yunus M, Srivastava K, Singh SN, Pandey V, Misra J, Ahmad J (1995). Monitiring of auto exhaust pollution by road side plants. Environ. Monit. Assess, 34: 13-25.

Sugihara N, Arakawa T, Ohnishi M, Furuno K (1999). Ant- and pro-oxidative effects of flavonoids on metal induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes located with a-

linolenic acid. Free Radic. Bio. Med., 27: 1313-1323.
Tepe B, Sokmen A (2007). Screening of the antioxidant properties and total phenolic contents of three endemic *Tanacetum* subspecies from Turkish flora. Bioresour. Technol., 98: 3076-3079.
Tiwari S, Agrawal M, Marshall FM (2010). Seasonal variations in adptational strategies of *Beta vulgaris* L. plants in response to ambient air pollution: Biomass allocation, yield and nutritional quality. Trop. Ecol., 51(2S): 353-363.
Wolfe K, Wu X, Liu RH (2003). Antioxidant activity of apple peels. J. Agric. Food Chem., 51: 609-614.
Wagh ND, Shukla Poonam V, Tambe Sarika B, Ingle ST (2006). Biological monitoring of roadside plants exposed to vehicular pollution in Jalgaon city. J. Environ. Biol., 27(2): 419-421.
Yamaguchi T, Takamura H, Matoba T, Terao J (1998). HPLC method for the evaluation of the free radical scavenging activity of foods by using 1, 1 diphenyl-2-picrylhydrazyl. Biosci. Biotechnol. Biochem., 62: 1201-1204.