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Research Article

Antioxidant potential and total phenolic content of *Leucas aspera* of Sonitpur district, Assam

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ABSTRACT

Antioxidants are capable of inhibiting oxidative reactions which produces free radicals in the body responsible for various diseases. In current decade, attention is being given on plants having antioxidant property to replace synthetic antioxidants. *Leucas aspera* (Willd.) Link (Lamiaceae), a wild edible vegetable was analyzed to determine antioxidant property of methanolic leaf extract by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay and IC_{50} was calculated by plotting nonlinear regression curve. The total phenolic content (TPC) was determined by Folin-Ciocalteu's method using gallic acid as standard. *L. aspera* exhibit potent antioxidant property with IC_{50} value 54.78µg/ml. Total phenolic content was found to be 45.30 ± 0.64 mg GAE/g dry extract. *L. aspera* which is consumed as common vegetable in Sonitpur district of Assam (India) are potent source of antioxidants and can be recommended as healthy vegetable.

Keywords: DPPH; Free radical scavenging activity; IC_{50}; TPC

INTRODUCTION

Antioxidants are paid much attention in the recent years due to their capability of inhibiting oxidative reaction (Seal, 2011). Oxidative reaction produces free radicals in the body which are responsible for more than 100 disorders like gastric, cancer, AIDS, arthritis, etc (Pourmorad et. al., 2006) and play an important role in ageing (Koheil et al., 2011). Synthetic antioxidant like butylhydroxyanisol (BHA), butylhydroxytoluene (BHT), propylgallate (PG) and tertbutylhydroxyltoluene (TBHQ) are largely used in food industry but due to their potential health risks and toxicity, emphasis is on to replace by natural antioxidant (Kahl and Kappus,1993).

Fruits and vegetables are major sources of dietary antioxidant (Namiki, 1999) which help in cellular defenses and prevent cellular components against oxidative damage (Evans and Halliwell, 2001). Antioxidant capacity of a plant is largely contributed by presence of phenolic compounds and flavonoids (Williams, 2004) and a large number of wild edible plants are rich in phenolic compounds (Simopoulos, 2004) and search is still going on to find a better source of natural antioxidant. *Leucas aspera* locally called Durun is widely distributed throughout the state of Assam and had been a part of ethnomedicine used in the treatment of various diseases like headache, dysentery, skin diseases, conceive failure, paralysis, piles, sour mouth, abdominal pain, gastric ulcer (Kalita and Phukan, 2010; Dutta and Nath, 1998; Das et al., 2008; Nawaz et al., 2009; Parinitha et al., 2004). Leaves are consumed as vegetable throughout the state of Assam and providing food security during times of drought and poor harvest. The present study was undertaken to determine antioxidant activity and total phenolic content of methanolic leaf extract of *L. aspera*.

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MATERIALS AND METHODS

Chemicals and solvents

Folin-Ciocalteu's phenol reagent and sodium carbonate were obtained from Merck, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and L-Ascorbic acid from Sigma and gallic acid from BDH.

Preparation of crude extract

Leaves along with tender shoots of *L. aspera* were collected from Sonitpur district, Assam (India). Samples were made dried and pulverized. 50g coarsely powdered sample were soaked in 100 ml Methanol for overnight and filtered through Whatman paper No. 41. The residue was re-extracted twice with increasing time. The total filtrate was concentrated by rotary evaporator at 45°C under reduced pressure and stored at -4°C until further use.

Figure 1: *Leucas aspera* in flowering stage

Determination of antioxidant activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method

The DPPH assay was done by the method given by Brand-Williams *et. al.* (Brand-Williams, 1995) with some modifications. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of plant extract solution of varying concentrations (5, 10, 25, 50, 100, 250 and 500 µg/ml). Corresponding blank sample were prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard. Mixer of 1ml methanol and 1ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer (SPECORD-250 Analytic Jena, AG, Germany). The inhibition % was calculated using the following formula:

$$Inhibition\,\% = \frac{[A_{(control)} - A_{(test\,sample)}]}{A_{(control)}} \times 100$$

Determination of total phenolic content (TPC)

The total phenolic content was determined by the Folin-Ciocalteu's method (Singleton and Rossi, 1965) with reduced volumes. Stock solution was prepared by mixing 10mg of gallic acid in 100ml of distilled water. Different volumes of stock solution (100µl-500µl) were mixed with 2ml water and 0.3ml Folin Ciocalteu's Phenol reagent in test tubes. After 5 minutes 0.8ml 20% $NaCO_3$ was added and final volume was made to 5ml. In a separate test tube 200 µl leaf extract was mixed with the same reagents as described above and final volume was made to 5ml. The absorbance was measured at 765 nm after 30 minutes using UV-Vis spectrophotometer (SPECORD-250 Analytic Jena, AG, Germany). Results were expressed as mg/g (Gallic acid equivalent/dry weight). Total phenolic content was calculated using the formula:

$$\mathrm{TPC} = c \times \frac{\mathrm{V}}{m}$$

Where, '*c*' is the concentration of gallic acid in mg/ml;

'V' is the volume of plant extract in ml; and

'm' is the weight of pure plant extract in g.

Statistical analysis

The analyses were performed in triplicates and the experimental results obtained were expressed as mean with 95% confidence interval for IC_{50} and mean±SD for TPC, evaluated by GraphPad Prism 5 software. TPC was expressed as of Gallic acid equivalent/dry weight.

RESULT AND DISCUSSION

DPPH is one of the most common methods used to determine the antioxidant activities of various plants extract (Wojdylo, 2007). This method is based on decrease in purple/dark violet colour of alcoholic DPPH solution (Ersoy et al., 2011; Ayoola et al., 2008) when contacted with antioxidant substances like phenolic compounds and have a strong absorption range at 517 nm (Bondet et al., 1997). The methanolic leaf extract of *L. aspera* exhibits potent antioxidant activity (Fig. 2).

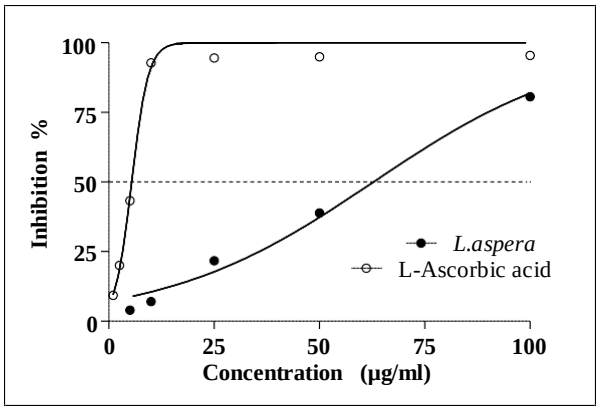

Figure 2: DPPH free radical scavenging activity *L. aspera* leave extract and standard

An IC_{50} value (50% inhibitory concentration) is the concentration of extract required to scavenge 50% of DPPH free radicals. The IC_{50} value of *L. aspera* leaf extract was found 54.78µg/ml with 95% confidence interval between 51.42µg/ml - 58.36µg/ml. The IC_{50} value of L-Ascorbic acid was 4.985µg/ml with 95% confidence interval between 4.49µg/m - 5.53µg/ml.

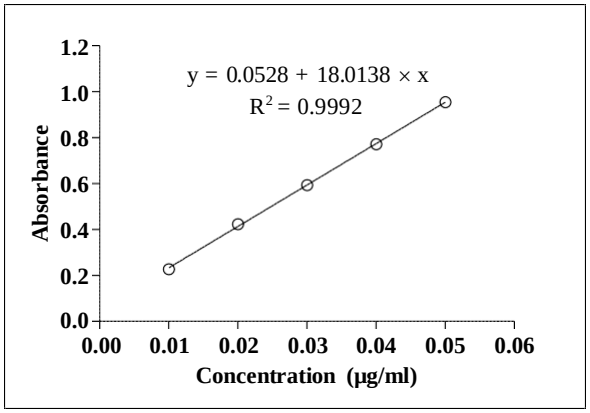

Figure 3: Gallic acid standard curve for determination of TPC

Phenolic compound of plant acts as primary antioxidants or free radical scavenger (Ayoola et al., 2008). Several comprehensive works have been done in the recent years and significant correlation was found between TPC and antioxidant activity (Olajire and Azeez, 2011). TPC was determined by plotting gallic acid standard curve (Fig. 3). TPC of *L. aspera* leaf extract was found 45.30±0.64 mg GAE/g dry extract.

CONCLUSION

The leaves of *L. aspera* are having potent antioxidant activity. However, low TPC value confirms that some other compounds are responsible for antioxidant activity. The plant is consumed as alternative and low cost vegetable by the people of Assam (India). The present finding partially validates their traditional knowledge about the goodness of consumption of this plant. Further study is going on to isolate the bioactive phytochemicals.

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REFERENCES

Ayoola AG, Ipav SS, Sofidiya MO, Adepoju-Bello AA, Coker AB, Odugbemi TO. 'Phytochemical screening and free radical scavenging activities of the fruits and leaves *of Allanblackia floribunda* Oliv (Guttiferae)', Int J Health Res, vol. 1, no. 2, June, 2008 pp. 87-93.

Bondet V, Brand-Williams W, Berset C. 'Kinetics and mechanism of antioxidant activity using the DPPH free radical method', Lebens-Wissen & Tech, vol. 30, 1997 pp. 609-615.

Brand-Williams W, Cuvelier ME, Berset C. 'Use of a free radical method to evaluate antioxidant activity', Lebens-Wissen & Tech, vol. 28, 1995 pp. 25-30.

Das AK, Dutta BK, Sharma GD. 'Medicinal plants used by different tribes of Cachar district, Assam', Ind J Trad Know, vol 7, no. 3, July, 2008 pp. 446-454.

Dutta ML, Nath SC. 'Ethno-medico botany of the *Deories* of Assam, India', Fitoterapia, vol. 69, no. 2, 1998 pp. 147-154.

Ersoy N, Bagci Y, Gok V. 'Antioxidant properties of 12 cornelian cherry fruit types (*Cornus mas* L.) selected from Turkey', Scienti Res Essays, vol. 6, no. 1, Jan, 2011 pp. 98-102.

Evans P, Halliwell B. 'Micronutrients: oxidant/antioxidant status', Br J Nutr, vol. 85, 2001 pp. S67-S74.

Kahl R, Kappus H. 'Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E', Z Lebensm-Unters Forsch, vol. 196, no. 4, 1993 pp. 329-338.

Kalita D, Phukan B. 'Some ethnomedicines used by *Tai Ahom* of Dibrugarh district, Assam, India', Indian J Nat Pro Resour, vol. 1, no. 4, Dec, 2010, pp. 507-511.

Koheil MA, Hussein MA, Othman SM, El-Hadda A. 'Anti-inflammatory and antioxidant activities of *Moringa peregrina* Seeds', Free Rad Antiox, vol. 1, no. 2, Apr-Jun, 2011 pp. 49-61.

Namiki M. 'Antioxidant/antimutagens in foods', Crit Rev Food Sci Nutr, vol. 29, 1999 pp. 273-300.

Nawaz AHMM, Hossain M, Karim M, Khan M, Jahan R, Rahmatullah M. 'An ethnobotanical survey of Jessore district in Khulna division, Bangladesh', Am-Eurasian J Sustain Agric, vol. 3, no. 2, 2009 pp. 238-243.

Olajire AA, Azeez L. 'Total antioxidant activity, phenolic, flavonoid and ascorbic acid contents of Nigerian vegetables', African J Food Sc Tech, vol. 2, no. 2, Feb, 2011 pp. 22-29.

Parinitha M, Harish GU, Vivek NC, Mahesh T, Shivanna MB. 'Ethno-botanical wealth of Bhadra wildlife sanctuary in Karnataka', Ind J Trad Know, vol. 3, no. 1, Jan, 2004 pp. 37-50.

Pourmorad F, Hosseinimehr SJ, Shahabimajd N. 'Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants', African J Biotech, vol. 5, no. 11, 2006 pp. 1142-1145.

Seal T. 'Determination of nutritive value, mineral contents and antioxidant activity of some wild edible plants from Meghalaya state, India', Asian J Applied Sciences vol. 4, 2011 pp. 238-246.

Simopoulos AP. 'Omega-3 Fatty Acids and Antioxidants in Edible Wild Plants', Biol Res, vol. 37, 2004 pp. 263-277.

Singleton VL, Rossi JA. 'Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents', American J Enol Viticult, vol. 16, 1965 pp.144-158.

Williams RJ, Spencer JP, Rice-Evans C. 'Flavonoids: Antioxidants or signaling molecules?', Free Radic Biol Med, vol. 36, 2004 pp. 838-849.

Wojdylo A, Oszmianski J, Czemerys R. 'Antioxidant activity and phenolic compounds in 32 selected herbs', Food Chem, vol. 105, 2007 pp. 940-949.