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Research Article

ANTIOXIDANT AND FREE RADICAL SCAVENGING PROPERTIES OF *TYLOPHORA INDICA* (BURM. F.) MERRILL AN ANTI-ASTHMATIC PLANT

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ABSTRACT

The present study was undertaken to evaluate the antioxidant activity of various parts of *T. indica*. The crude methanolic extracts of *T. indica* were screened for their antioxidant and free radical scavenging properties using 2, 2-diphenyl 1-picrylhydrazyl (DPPH). The overall antioxidant activity of *T.indica* was strongest in leaf explants (59.25 µg/ml) followed in descending order by callus (95.15 µg/ml), stem (112.50 µg/ml) and root (115.25 µg/ml), respectively. However, IC_{50} values of different plant parts have also been estimated to exhibit their antioxidant activities and compared with standard ascorbic acid.The tested plant extracts showed promising antioxidant and free radical scavenging activity, thus justifying their traditional and medicinal uses.

Keywords: Ascorbic acid, DPPH, Methanolic extract, *Tylophoraindica* (Burm. f.) Merrill.

NTRODUCTION

Plants are being used as a source of medicine since time immemorial. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities with no side effects and have economic viability[1]. An antioxidant is any compound, may be a vitamin, mineral, nutraceutical, or herb that protects cells against cellular damage from reactive oxygen species (ROS) including free radicals,single oxygen atoms and hydrogen peroxide etc. Some of the well known antioxidantsare ascorbic acid (Vitamin C), alpha-tocopherol (Vitamin E), beta-carotene andenzymes such as catalase, superoxide dismutase and glutathione peroxidase etc. Free radicalsplay important roles in the etiology of several major diseases, including cancer, cataract, asthma, alzheimer's and diabetes[2- 6]. However, majority of the ailments of body are mainly linked to oxidative stress due to free radicals[7]. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism[8]. The most common reactive oxygen species (ROS) include superoxide (O_2^-) anion, hydrogen peroxide (H_2O_2), peroxyl (ROO^-) radicals and reactive hydroxyl (OH^-) radicals etc. The nitrogen derived free radicals are nitric oxide (NO^-) and per-oxynitrite anion ($ONOO^-$). These antioxidants interfere with the oxidation process by reacting with free radicals, chelates, catalytic metals and also acting as oxygen scavengers[9-10]. ROS have been implicated over a hundreds of diseases which ranges from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome (AIDS). Antioxidants therapy has gained an immense importance in the treatment of these above mentioned diseases.

Consumption of a wide variety of antioxidants, vitamins, minerals, nutraceuticals and herbs may be the best way to provide the body with the most complete protection against free radical damage[11].

Keeping in view the importance of natural antioxidants from plants, we have selected an important medicinal plant *Tylophoraindica* (Burm. f.) Merrill. Itis also known as "Antmul" is a perennial, branching climber with long fleshy roots. It grows widely in plains and hilly places of India up to an altitude of 1,000 m in Bengal, Assam, Orissa and Southern India[12]. Traditionally, this plant has been used in the treatment of bronchial asthma[13-14]. However, alcoholic extract and total alkaloids such as phenanthroindolizidine, tylophorine have been used to cure CNS depression, myocardial depression, fall of blood pressure (hypotension), non specific relaxation of smooth muscles, antagonized contractile effects of histamine & acetylcholine, pentobarbital sleeping time, leukaemia and anti-inflammatory effects[15]. It inhibits systemic anaphylaxis and also the responses of adjuvant-induced arthritis. Moreover, it was found that plant is a good muscle relaxant and also helps in anti-histamine, hypotensive and antitumor activities[16], respectively. The present investigation was undertaken to estimate the antioxidant potential and free radical scavenging properties of different parts such as leaves, stem, root and callus of *Tylophoraindica*(Burm f.) Merrill methanolic extracts through DPPH *in vitro* assay.

MATERIAL AND METHODS

Collection of Plant Material

Plant of *Tylophoraindica* were collected from nursery, Department of Botany, University of Rajasthan, Jaipur. The plant was authenticated (RUBL20929) by Herbarium, Department of Botany, University of Rajasthan, Jaipur. Different plant parts (leaf, stem, root) and stock callus established from leaves of *T. indica* were used for antioxidant studies.

Preparation of the Methanolic Extracts

Different plant parts (leaf, stem and root) and calliwere oven dried at 60°C for overnight and crushed to a crude powder. Powdered material (20 gm) was soxhlet extracted with 80% methanol for 12 hours. Extracts were evaporated in vacuum under reduced pressure. All extracts were stored in sterile glass bottles at room temperature until screened.

DPPH Radical Scavenging Activity

The free radical scavenging activity of methanolic extract was measured by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) by the modified method of Hatano*et al.[* 17]. DPPH is stable nitrogen centered radical. The odd electrons in the DPPH free radical give a strong absorption maximum at 517 nm. In this assay, 1.0 ml of extract solution (concentrations ranging from 10–1000 µg/ml) was mixed with 2.0 ml of DPPH in methanol solution. The absorbance of reaction mixture at 517 nm was taken by UV spectrophotometer (Optigen 2020 plus UNICAM), which was compared with the corresponding absorbance of standard ascorbic acid concentrations (10-1000 µg/ml). The radical scavenging activities were expressed

as percentage of inhibition and calculated according to the following formula.

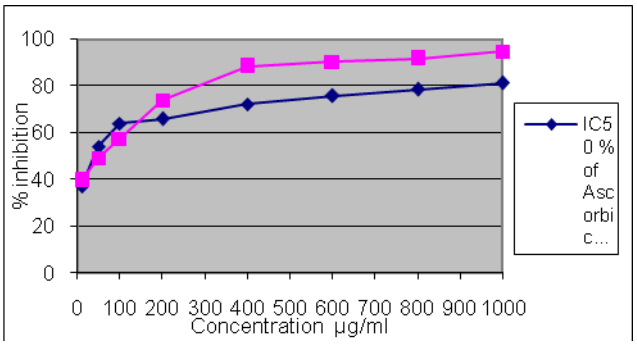
$$\text{Percentage of DPPH inhibition} = \frac{Ac - As}{Ac} \times 100$$

Where Ac = absorbance of control

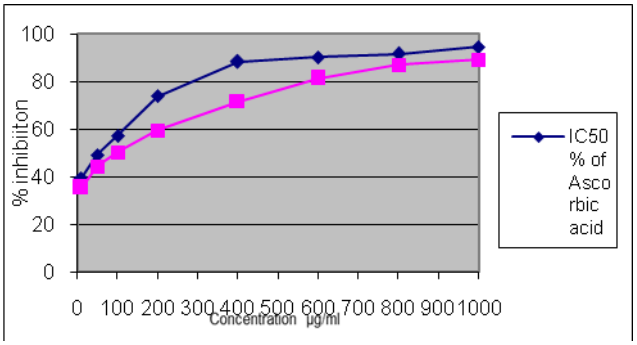
As = absorbance of sample.

RESULT AND DISCUSSION

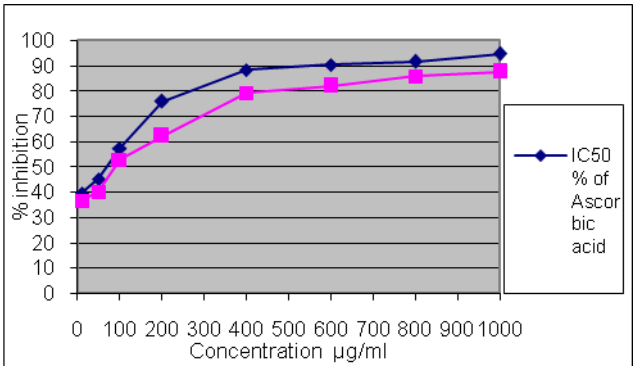
The antioxidant activity of methanolic extracts was investigated using DPPH assay, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. Ascorbic acid was chosen as the reference antioxidant for this test. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized as the colour change from deep violet to light yellow[18], which can be quantitatively measured from the changes in absorbance. The IC_{50} values of all the plant extracts have been calculated and depicted in the Figure 1 (A-D).



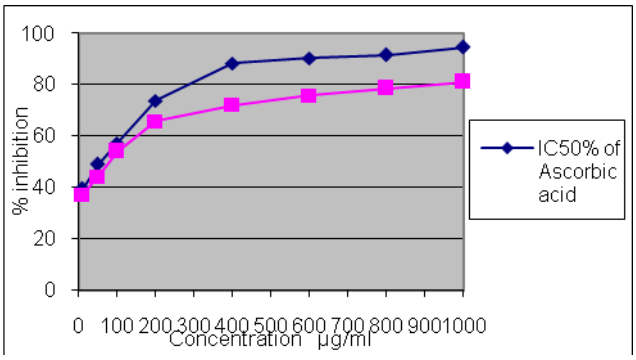
A: Evaluation of IC50 of leaf extract of T. indica compared with standard ascorbic acid



B: Evaluation of IC_{50} of stem extract of *T. indica* compared with standard ascorbic acid



C: Evaluation of IC_{50} of root extract of *T. indica* compared with standard ascorbic acid



D: Evaluation of IC_{50} of callus extract of *T. indica* compared with standard ascorbic acid

In *T. indica*, it is found that IC_{50} of the leaf extract was found to be 59.25 µg/ml which indicated the remarkable antioxidant activity of the extract as compared to standard ascorbic acid. However, callus was found to be moderate in respect to show IC_{50} value i.e. 95.15 µg/ml followed by stem with IC_{50} value i.e.112.50 µg/ml and root IC_{50} value i.e. 115.25 µg/ml (Fig. 1 A-D).

The free radical scavenging activity in the different plant part extracts decreased in the following order:

T. indica leaf (59.25 µg/ml) >*T. indica* callus (95.15 µg/ml) >*T. indica* stem (112.50 µg/ml) >*T. indica* root (115.25 µg/ml), respectively.

The maximum antioxidant effect of *T. indica* was seen in leaf extract (59.25 µg/ml) (Fig. A), during the present studies. Similarly, methanolic extracts of leaf of *Aloe vera*[19], *Thujaoccidentalis*L.[20]have also been reported to exhibit maximum antioxidant activity. However, antioxidant activities of *T. indica* plant parts have been studied earlier by many scientists [21, 22]. In contrast to the present results, ethanolic extract of leaves of *Thujaoccidentalis*exhibited least activity i.e. 202.45 µg/ml[23].

Beside, antioxidant activities of several other medicinal plants like *Rubusidaeus*L.[24],*Fragaria*x *ananassa*Duch.[25], *Clitoriaternatea* L. and *Eclipta prostrate* L.[26], *Cocosnucifera, Caesalpiniapulcherrima, Punicagranatum*and *Syzygium cumin* [27], *BuchananiaLanzan* Kernel[28], *Cissusrependa*[29], *Adhatodavasica*Nees and *Sesbaniagrandiflora* L.[30] have also been well studied and supported the present findings.

The present results suggested that all the tested plant extracts have showed variable antioxidant activity in different plant parts. Looking into the importance of this plant and the wide range of important constituents they bear, it will be further worthwhile to do same more assays and analysis.

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REFERENCE

1. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem. Pharm. Bull. 1988; 36: 1090-2097.
2. Auudy B, Ferreira F, Blasina L, Lafon F, Arredondo F, Dajas R, Tripathi PC. Screening of antioxidant activity of three Indian medicinal plants traditionally used for the management of neurodegenerative diseases. J. Ethanopharmacol, 2003; 84: 131-138.
3. Kuyvenhoven JP, Meinders AE. Oxidative stress and diabetes mellitus Pathogenesis of long-term complications. Eur. J. Intern. Med. 1999; 10: 9-19.
4. Kebapci N, Belgin EF, Fahrettin A, Emine S, Canan D. Oxidative stress and antioxidant therapy in Type-2 diabetes mellitus. Turk. JEM., 1999; 4: 153-162.

5. Maritim AC, Sanders RA, Watkins JB. Diabetes, Oxidative Stress, and Antioxidants: A Review. J. Biochem. Mol. Toxicol. 2003; 17: 24-38.
6. Devasagayam TPA, Tilak JC, Boloor KK. Review: Free radical and antioxidants in human health. Curr. Stat. Fut. Pros. JAPI. 2004; 53: 794-804.
7. Bhuiyan MAR, MZ Hoque, SJ Hossain.Free Radical Scavenging Activities of *Zizyphusmauritiana.* W. J. Agricult. Sci., 2009; 5 (3): 318-322.
8. Gutteridge JMC. Free radicals in disease processes, a complication of cause and consequence. Free Radic. Res. Comm. 1995; 19: 141-58.
9. Tiwari A. Imbalance in antioxidant defense and human diseases: Multiple approach of natural antioxidants therapy. Curr. Sci., 2001; 81: 1179-1187.
10. Buyukokuroglu ME, Oktay M, Kufrevioglu OI. In vitro antioxidant properties of dantrolene sodium. Pharmacol. Res., 2001; 44: 491-95.
11. Lobo VC, Phatak A, Chandra N..Antioxidant and Free Radical Scavenging Activity of Hygrophilaschulli (Buch.-Ham.) Almeida and Almeida Seeds. Adv. Biores., 2010; 1 (2) : 72 – 78.
12. Halliwell B, Gutteridge JMC, Cross CE. Free radicals, antioxidants, and human disease: where are we now. J. Lab. Clin. Med. 2002; 119(6) : 598-619.
13. Anonyms, the wealth of India, NISCAIR, CSIR, New Delhi, 1978, Page 398-399.
14. Bielory L, Lupoli K. Herbal interventions in asthma and allergy. J. Asthma. 1999; 36: 1-65.
15. Savithramma N, Sulochana C, Rao KN. Ethnobotanical survey of plants used to treat asthma in Andhra pradesh, India. J. Ethnopharmacol. 2007; 113(1): 54-61.
16. Gupta AK. Quality control of Indian medicinal plants, ICMR, 2003; 1: 219-220.
17. Gujrati V, Patel N, Rao VN, Nandakumar K, Gauda TS, Shalam M, Kumat SSM. Heptoprotective activity of alcoholic and aqueous extracts of leaf of *Tylophoraindica*in rats. Indian Pharmacol. 2007; 39(1): 43-47.
18. Marcel KK, Akhanovna MBJ, Yves-Alain B, Gabin DBM, Jérôme ZBT. *In vitro* antioxidant activities of total flavonoids extracts from leaves and stems of *Adenialobata* (Jacq.) Engl. (Passifloraceae). J. Pharmacog. Phytoth., 2011; 3(1) 8-12.
19. Miladi S, Damak M. *In vitro* antioxidant activities of *Aloe vera* leaf skin extracts. Journal de la SociétéChimique de Tunisie, 2008; 10, 101-109.
20. Dubey SK, Batra A. Antioxidant activities of *Thujaoccidentalis* Linn. As. J. Pharmace. Clin. Res. 2009; 2 (1): 73-76.
21. Mishra G, Singh P, Garg V K, Parvez N, Yadav S, Hwisa N, Molvi KI, AlSharif SM, Awen BZ, Khosa RL. Phytochemical Screening and Anticonvulsant Activity of WedeliaChinensis. IJPSR. 2011; 2(1): 25-29.
22. Gupta M, Mukhtar HM, Ahmad S. *In vitro* evaluation of antioxidant activity and total phenolic content of *Tylophorraindica* (Burm. f.) Merrill. IJPSR, 2011; 2(1): 121-126.
23. Dubey SK and Batra A. 2008. Hepatoprotective Activity from Ethanol Fraction of Thujaoccidentalis Linn. Asian J. Research Chem. 2008; 1(1): 32-35.
24. Buřičová L., Réblová Z. (2008): Czech medicinal plants as possible sources of antioxidants. Czech J. Food Sci., 26: 132–138.
25. Debnath SC and Ricard E. ISSR, anthocyanin content and antioxidant activity analyses to characterize strawberry genotypes. J. Appl. Hort., 2009; 11(2): 83-89.
26. Rao DB, Kiran RC, Madhavi Y, Rao KP, Rao TR. Evaluation of antioxidant potential of *Clitoriaternatea* L. and *Eclipta prostrate* L. Ind. J. Biochem. Biophy., 2009; 46: 247-252.
27. Hasan SMR, Hossain MM, Akter R, Jamila M, Mazumder MEH and Rahman S. J. Med. Plants Res., 2009; 3(11): 875-879.
28. Warokar AS, Ghante MH, Duragkar NJ, Bhusari KP.Anti-inflammatory and Antioxidant Activities of Methanolic extract of *BuchananiaLanzan* Kernel. Indian J.Pharm. Educ. Res. 2010; 44(4): 363-368.
29. Kardong D, Purkayastha B, UpadhyayaS,Chetia J. Antioxidant, antibacterial and hepatoprotective activities of *Cissusrependa*vahl on carbon tetrachloride (CCl4) induced liver damage in bird columbalivia. Asian J Pharm Clin Res. 2014; 7(2): 48-52.
30. Padmaja M, Sravanthi, Hemalatha KPJ. Evaluation of antioxidant activity of two Indian medicinal plants. J.Phytology. 2011; 3(3):86-91.