IJPSR (2011), Vol. 2, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 August, 2010; received in revised form 12 November, 2010; accepted 24 December, 2010

IN-VITRO EVALUATION OF ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF *TYLOPHORA INDICA* (BURM F.) MERILL.

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Keywords:

DPPH,

Antioxidant activity,

Total phenolic content,

Tylophora indica

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ABSTRACT

Tylophora indica is a perennial climbing plant native to India, found in plains, forests, hills of southern and eastern India. The portions of plant used medicinally are leaves. The Total phenolic content of methanolic leaves extract was estimated by Folin-Ciocalteu assay method, and was found to be 0.160 mg/CE/g (Catechine Equivalent per gram). However, antioxidant activity of methanolic leaves extract of *Tylophora indica* was determined by DPPH free radical scavenging method. The DPPH radical scavenging activity of methanolic extract of *Tylophora indica* was found to be highest at 100µl concentration which was 30.74%. Nevertheless, % DPPH scavenging activity of standard ascorbic acid at same concentration was found to be 45.43%. The % DPPH scavenging activity increases with the increasing concentration. The concentration of *Tylophora indica* needed for 50% inhibition (IC50) was found to be 199.58µg/ml whereas 194.58 µg/ml needed for ascorbic acid.

INTRODUCTION: Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce risk for chronic disease. The main ability of an antioxidant compound is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a variety of sources; these free radicals may oxidize nucleic acids, proteins, lipids, or DNA and can initiate degenerative disease. Antioxidant compounds scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanism that lead to degenerative disease. These are vital substances which posseses the ability to protect the body from damage caused by free radical induced oxidative stress [1]. Antioxidants are radical scavengers which protect the human body against free radical that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neuro-degenertion, parkinson's disease, mongolism, ageing process and perhaps dementias [2].

Oxidation is essential to many living organism for the production of energy to fuel biological processes. However oxygen centered free radicals and other reactive oxygen species (ROS), which are continuously, produced *in vivo,* result in cell death and tissue damage. The role of oxygen radical has been implicated in several diseases, including cancer, diabetes and cardiovascular diseases, ageing etc [3]. There is an increasing interest in natural anti-oxidants, eg., polyphenols, present in medicinal plants and dietary plants, which might help prevent oxidative damage [4]. In biological systems, lipid oxidation can produce toxic compounds and initiate other harmful reactions. Phenolic compounds can act as antioxidants by many potential pathways such as free radical scavenging [5].

The plant *Tylophora indica* (Burm. f.) Merr. is also known as "Antmul" is an perennial branching climber with long fleshy roots. It grows widely in plain and hilly places of India up to an altitude of 1,000 m in Bengal, Assam, Cachar, Orissa, and southern India [6]. The whole plant is of a pale yellow in colour and has no marked odour but has sweetish and acrid taste [7]. Traditionally plant is used in treatment of bronchial asthma; however alcoholic extract and total alkaloids produced CNS depression, myocardial depression, fall of blood pressure, non specific relaxation of smooth muscles and antagonized contractile effects of histamine and acetylcholine.

The active constituent phenanthroindolizidine alkaloid tylophorine caused CNS depression, potentiated pentobarbital sleeping time and showed anti-inflammatory effect. It inhibits systemic anaphylaxis and responses of adjuvant-induced arthritis. Tylophorine was marginally active against the leukaemia. Recently it was found that plant having good smooth muscle relaxant, antihistamine, hypotensive and antitumor activities [8]. The present investigations is undertaken to estimate the total phenolic content and antioxidant potential of *Tylophora indica* (Burm f.) merill. methanolic leaf extract though DPPH *in vitro* assay model.

MATERIALS AND METHODS:

Collection and authentication of plant: Fresh leaves of *Tylophora indica* were collected in the month of August-September (2009) from Herbal Garden of Jamia Hamdard University, New Delhi. The plant sample was identified and authenticated at Raw material, Herbarium and museum NISCAIR, CSIR, New Delhi, India. The sample was also submitted to museum for future reference (Ref: NISCAIR/RHMD/consult/-2009-10/1361/163).

Preparation of Methanolic Extract: The collected leaves were air-dried, pulverized in to a coarse powder by grinder and sieved. The dried powdered material was extracted with methanol in round bottom flask with soxhlet apparatus connected to it for 1 hour. The extract was filtered and the solvent was removed by distillation under vacuum, extract is subjected to evaporate to dryness in china dish. After dryness, by adding corresponding solvent to it, it is used for further *in-vitro* studies.

Total Phenolic Content: The total phenolic content of leaves of plant *Tylophora indica* was determined by using the Folin–Ciocalteu assay. An aliquot 1ml of extracts or standard solution of catchin (20, 30, 40, 50, 60, 70 mg/l) was added to 25ml volumetric flask, containing 9ml of distilled deionised water (dd H_2O). A reagent blank using dd H_2O was prepared. One millilitre of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5min 10 ml of Na_2CO_3 solution was added to the mixture. The solution was diluted to volume (25ml) with dd H_2O and mixed. After incubation for 90min at room temperature, the absorbance against prepared reagent blank was determined at 765nm with an UV-spectrometer. Total phenolic content of leaves of plant *Tylophora indica* was expressed as mg Catechin equivalents (CE)/g.

DPPH Free Radical Scavenging Assay: The free radical scavenging activity of the fractions was measured in vitro by 1, 1- diphenyl- 2-picrylhydrazyl (DPPH) assay. About 0.3mM solution of DPPH in 100% methanol was prepared and 1ml of this solution was added to 3ml of the fraction dissolved in methanol at different concentrations. The mixture was shaken and allowed to was measured at 517nm using a shimadzu spectrometer. The percentage scavenging inhibition was determined and was compared with that of ascorbic acid (AA), which was used as the standard [64].

Preparation of extract: 10g of the dried powdered leaves from plants *Tylophora indica* were taken separately in a paper cone and placed into soxhlet apparatus. 100ml of methanol a polar solvent was taken in the round bottom flak attached to the soxhlet apparatus. A condenser was attached to this set up. The temperature was set in the range of 25^{o}- 30^{o}C. Methanol gets vaporised and rises up to the condenser where it condenser where it condenses back into liquid. This liquid falls back into the round bottom flask. This process was continued till all the compounds that can be extracted from the plant by methanol gets extracted and finally only clear liquid of methanol falling into the round bottom flask. The extract got from the above process was evaporated over night stored in screw cap vials.

DPPH Radical Scavenging Activity: DPPH scavenging activity of the plant extract was carried out. DPPH chemical is brought from SIGMA ALDRICH, USA. The stock solution was prepared by dissolving plant extract with methanol at concentration of 1mg/ml. From this stock solution different concentrations were prepared 20, 40, 60, 80, 100 mg/ml. About 0.3mM solution of DPPH in 100% methanol was prepared and 2.5ml of this solution was added in different concentrations of plant extract which was prepared from stock solution.

The mixture was shaken vigorously and incubated for 30min in room temperature. Absorbance of the resulting was measured at 517nm UV-Visible spectrophotometer. Blank was prepared without the addition of DPPH. Absorbance of plant extract is compared with standard solution concentrations which is ascorbic acid.

Percentage of DPPH Scavenging Activity determined as follows:

% DPPH radical scavenging = [(absorbance of control – absorbance of test sample) ÷ (absorbance of control)] × 100

RESULTS AND DISCUSSION:

Total Phenolic Content: The phenolic content of leaf extract of *Tylophora indica* was measured by Folin-Ciocalteu assay method (**table 1**). The absorbance of sample was measured at 765nm using a UV-Spectrophotometer and was to be 2.194 Abs (**fig. 1**). The phenolic content calculated from calibration curve of catechin was found to be 0.160 mg CE/g.

TABLE 1: ABSORBANCE OF CATECHIN AT 765NM

Conc. (µg/ml)	Absorbance at 765nm	Mean Absorbance ± SD
20	0.121 0.123 0.125	0.123 ± 0.002
30	0.263 0.265 0.261	0.263 ± 0.002
40	0.540 0.542 0.541	0.541 ± 0.001
50	0.755 0.753 0.751	0.753 ± 0.002
60	0.970 0.974 0.975	0.973 ± 0.002
70	1.102 1.104 1.103	1.103 ± 0.001

TABLE 2: ABSORBANCE OF SAMPLE AT 765NM

Absorbance at 765nm	Mean Absorbance at 765nm ± SD
2.917 2.914 2.912	2.914 ±0.002

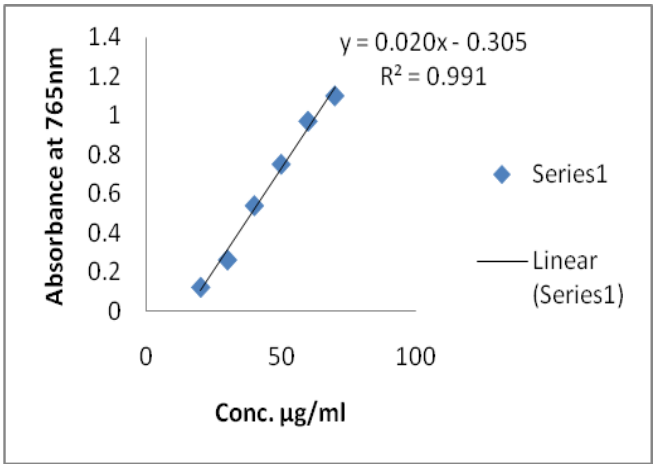

FIG. 1: CALIBRATION CURVE OF CATECHIN STANDARD

Antioxidant Activity: The %DPPH radical scavenging activity is presented in **table 2**. The DPPH radical scavenging activity of methanolic extract of *Tylophora indica* was found to be highest at 100µl concentration which was 30.74%. Nevertheless, %DPPH scavenging activity of standard ascorbic acid at same concentration was found to be 45.43% (**fig. 2**). The % DPPH scavenging activity increases with the increasing concentration. The concentration of *Tylophora indica* needed for 50% inhibition (IC_{50}) was found to be 199.58µg/ml whereas 194.58 µg/ml needed for ascorbic acid.

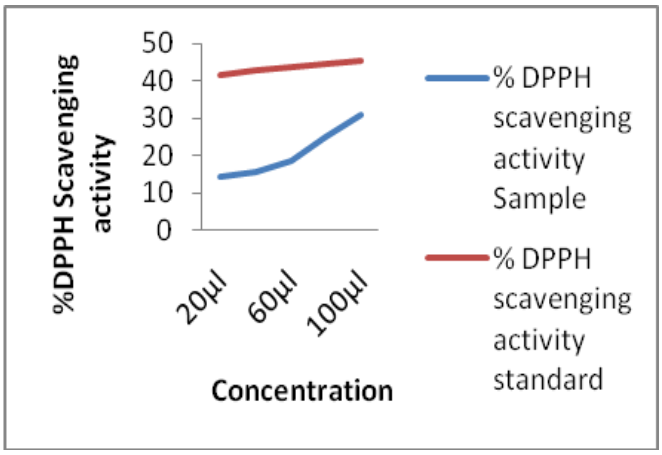

FIG. 2: % DPPH SCAVENGING ACTIVITY OF SAMPLE AND STANDARD

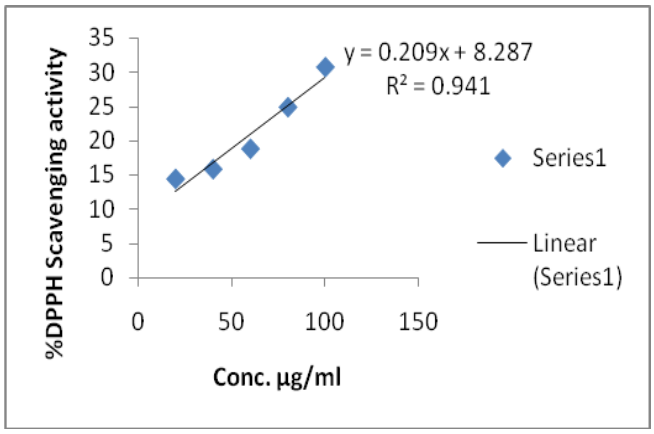

FIG. 3: CALIBRATION CURVE OF SAMPLE

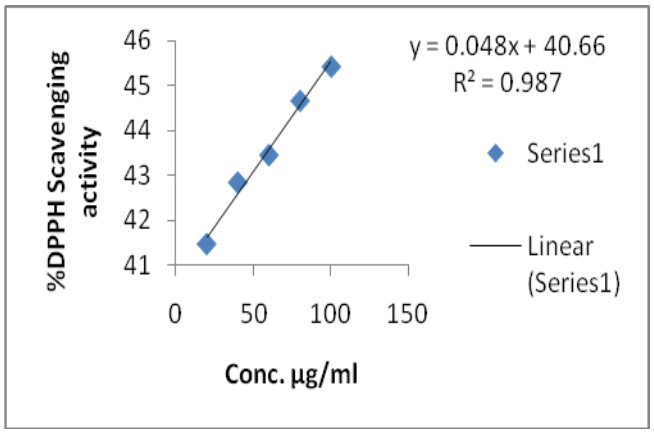

FIG. 3: CALIBRATION CURVE OF STANDARD ASCORBIC ACID

TABLE 2: ANTI-OXIDANT ACTIVITY OF METHANOLIC EXTRACT OF TYLOPHORA INDICA

Conc. µg/ml	Absorbance at 517nm		% DPPH scavenging activity		Mean % DPPH scavenging activity±SD		IC_{50} Value (µg/ml)	
	Sample	Standard	Sample	Standard	Sample	Standard	Sample	Standard
	0.560	0.386	14.98	41.33				
20	0.565	0.385	14.13	41.48	14.31±0.60	41.48±0.15		
	0.567	0.384	13.82	41.64				
	0.559	0.376	15.04	42.85				
40	0.553	0.374	15.95	43.16	15.75±0.63	42.85±0.30		
	0.551	0.378	16.26	42.55				
	0.537	0.372	18.38	43.46				
60	0.535	0.373	18.69	43.31	18.73±0.38	43.46±0.15	199.58	194.58
	0.532	0.371	19.14	43.61				
	0.494	0.365	24.92	44.52				
80	0.497	0.363	24.46	44.83	24.86±0.38	44.67±0.15		
	0.492	0.364	25.22	44.68				
	0.458	0.360	30.39	45.28				
100	0.453	0.358	31.15	45.59	30.74±0.38	45.43±0.15		
	0.456	0.359	30.69	45.44				

*Absorbance of Control at 517nm was 0.658

CONCLUSION: In the present investigational studies on important medicinal plant *Tylophora indica,* it is revealed that the phenolic content of leaf extract of *Tylophora indica* was measured by Folin- Ciocalteu assay method. The absorbance of sample was measured at 765nm using a UV-Spectrophotometer and was to be 2.194 Abs. The phenolic content calculated from calibration curve of catechin was found to be 0.160 mg CE/g. Phenolics posses a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic, as well as ability to modify the gene expressions. The DPPH radical scavenging activity of methanolic extract of *Tylophora indica* was found to be highest at 100µl concentration which was 30.74%. Nevertheless, % DPPH scavenging activity of standard ascorbic acid at same concentration was found to be 45.43%. The % DPPH scavenging activity increases with the increasing concentration. The concentration of *Tylophora indica* needed for 50% inhibition (IC50) was found to be 199.58µg/ml whereas 194.58

µg/ml needed for ascorbic acid. DPPH radical scavenging activity of *Tylophora indica* suggested that it may be used as antioxidant.

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