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Full Length Research Paper

Antioxidant activity of extract from the leaves of *Tylophora asthmatica*

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Extract from the leaves of *Tylophora asthmatica* were investigated for antioxidant activity. The methanolic extract of *T. asthmatica* had a 2, 2 diphenyl 1-1-picryl hydiazyl (DPPH) scavenging activity of 84.6% at 250 µg/ml and a reductive potential of 0.77% at 100 µg/ml. These values were comparable with those of Gallic acid, 91.4% at 250 µg/ml and ascorbic acid, 0.79% at 60 µg/ml as standards for DPPH scavenging activity and reductive potential, respectively. These findings suggest that the rich phytochemical content of *T. asthmatica* and its good antioxidant activity may be responsible for its popular and wide traditional use. The experiment was carried out with the leaves of the selected medicinal plants. The results are discussed with the available literature.

Key words: *Tylophora asthmatica,* antioxidant activity, reductive potential, 2, 2 diphenyl 1-1-picryl hydiazyl (DPPH).

INTRODUCTION

Plants show enormous versatility in synthesizing complex materials which have no immediate obvious growth or metabolic functions. These complex materials are retired to as secondary metabolites. Plants secondary metabolites have recently been referred to as phytochemicals. Phytochemicals are naturally, occurring and biologically active plant compounds that have potential disease inhibiting capabilities. It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect (Malliwell and Gutteridge, 1992). Antioxidant protects other molecules (*in vivo)* from oxidation when they are exposed to free radicals and reactive oxygen species which have been implicated in the etiology of many diseases and in food deterioration and spoilage (Kasaikna, 1997). Medicinal plants have been used for centuries before the advent of orthodox medicine. Leaves, flowers, stems, roots, seeds, fruit, and bark are all the constituents of herbal medicines. The medicinal values of these plants lie in their component phytochemicals which produce definite physiological actions on the human body. The most important of these phytochemicals are alkaloids, tannins, flavanoids and phenolic compounds (Hill, 1952). *Tylophora asthmatica*, a wild indigenous plant, belongs to the family Asclepidaceae and is commonly known as Indian ipecac. The powdered leaves, stems and root of *T. asthmatica* contain 0.2 to 0.3% alkaloids; of these, tylophorine and tylophorinidine are important alkaloids (Gopalakrishnan et al., 1979). Various studies have confirmed the anti-inflammatory activity (Gopalakrishnan et al., 1979), direct stimulate of adrenal cortex, anti-inflammatory activity, anti-asthmatic and the treatment of bronchitis, rheumatism and dermatitis. The present work has been designed to evaluate the antioxidant potential of extracts from the leaves of *T. asthmatica* and explore the basis for its traditional use.

MATERIALS AND METHODS

Chemicals

DPPH (2, 2 diphenyl 1-1-picryl hydiazyl) radical, gallic acid, ascorbic acid and Folin Ciocalteau reagent were obtained from sigma Aldrich, USA. All other chemicals are reagents used were of

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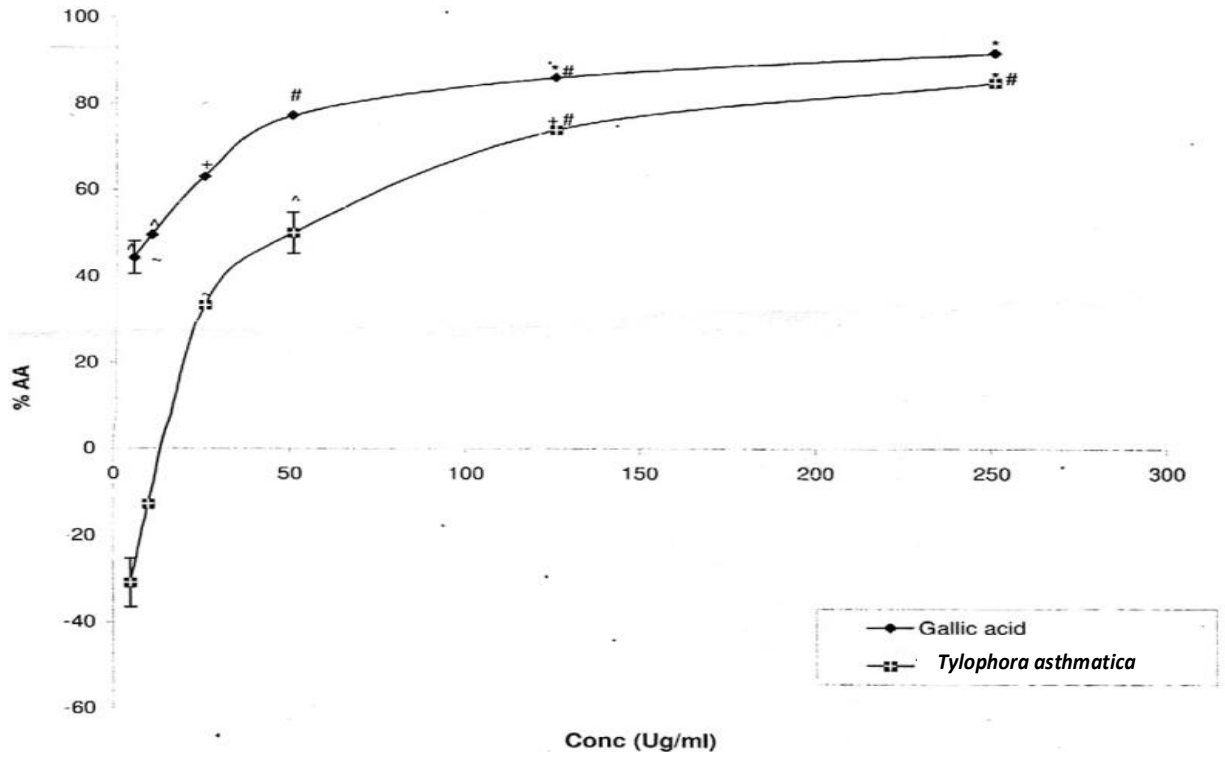

Figure 1. Dose-dependent DPPH scavenging activity of *T. asthamatica* leaf extract and gallic acid. Values sharing a common symbol are not significantly different (P > 0.05). Significantly different at P<0.01: GA_{250} vs Ta_{125}; GA_{10} vs Ta_{125}; GA_{10} vs Ta_{25}; Ta_{50} vs. Ta_{25}. Other values significantly different at P < 0.001.

analytical grade.

Collection and proceeding of plant material

The fresh leaves of *T. asthmatica* were collected during the month of February, 2008 in the Banks of Cauvery River, Tiruchirappalli, South India. It was botanically identified and authenticated. A voucher specimen (TAL-12) has been kept in our laboratory for future references. The leaves were shade dried, powdered, sieved through 410 meshes and stored in a tightly closed container for future use.

Preparation on of plant extract

The powdered plant material (500) was extracted with petroleum ether (60 to 80°C) using Soxhlet apparatus to remove lipids. It was filtered and the filtrate was discarded. The residue was extracted with methanol by soxhlet apparatus. The extract was completely dried in vacuum, stored in refrigerator at 4°C and protected from sunlight until the time for extract administration. The yield of methanolic dried extract was 8.63% (w/w).

Total phenolic content

Total phenolic content was determined using Folin-Ciocalteau reagent as previously described (McDonald et al., 2001). Total phenol value was obtained from the regression equation;

$y = 0.0055 x + 0.1139$

and expressed as mg/g gallic acid equivalent using the formula:

$C = cV/M$

where C = total content of phenolic compounds in mg/g GAE, c = the concentration of gallic acid (mg/ml) established from the calibration curve, V = volume of extract and m = the weight of pure plant methanolic extract (g).

DPPH radical scavenging activity

The ability of the extract to scavenge DPPH radical was determined according to the method described by (Mensor et al., 2001). Sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 250, 125, 50, 25, 10 and 5 µg/ml in methanol. 1 ml of a 0.3 mM DPPH methanol solution was added to 2.5 ml solution of the extract or standard and allowed to react at room temperature for 30 min. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA%) using the formula:

AA% = 100 – [(Abs sample – Abs blank) × 100] / Abs control Methanol (1.0ml) plus extract solution (2.5ml) was used as a blank. 1ml of 0.3 mM DPPH plus methanol (2.5ml) was used as a negative control. Solution of gallic acid served as positive control.

Reductive potential

This was determined according to the method of Oyaizu (1986).

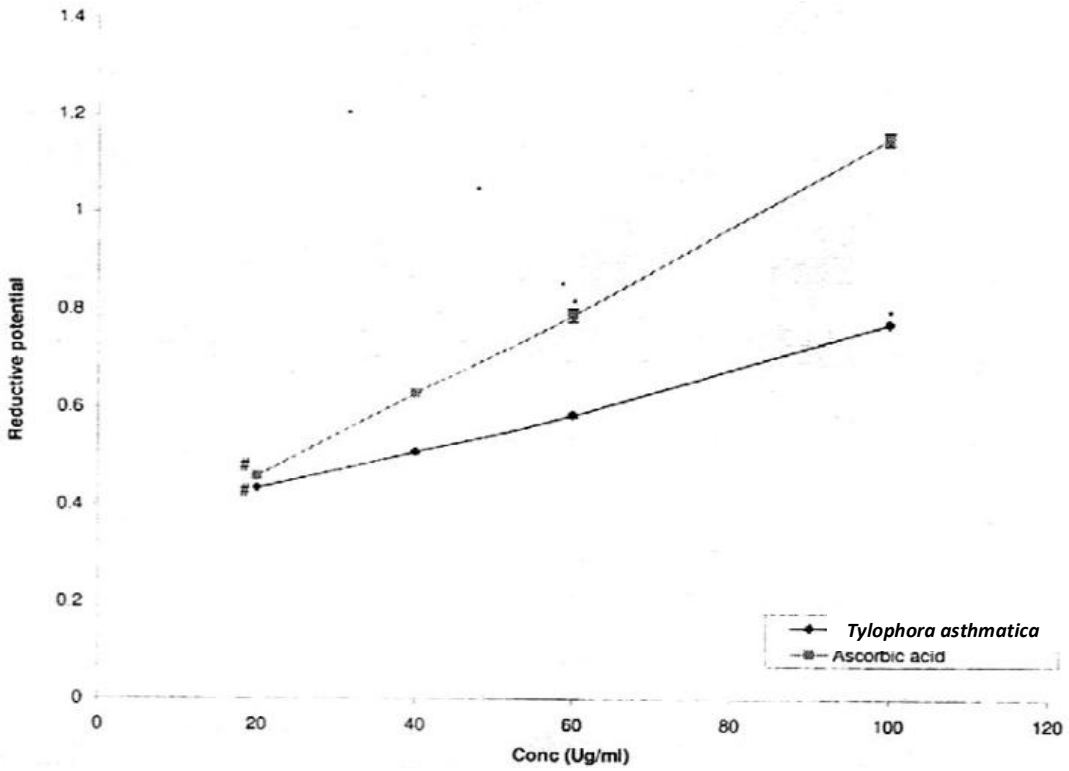

Figure 2. Dose-depedent reductive potential of *T. asthamatica* leaf extract and ascorbic acid. Values sharing a common symbol are not significantly different Ta_{60} significantly differ from AA40 ($P<0.05$); Ta_{60} significantly different from AA20 ($P<0.05$). All other values are significantly different ($P<0.001$).

Different concentrations of the methanolic extract of (20, 40, 60 and 100 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferric cyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Trichloro acetic acid (10%, 2.5 ml) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1%, 0.5 ml) and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicates higher reductive potential.

Statistical analysis

Data were expressed as mean ± SEM. A one-way analysis of variance was used to analyze data. $P < 0.5$ represented significant difference between means (Duncan's multiple range test).

RESULTS AND DISCUSSION

The total phenolic content in the methanolic extract was 5.68 ± 0.06 mg/g GAE. Phenolics are the largest group of phytochemicals and have been touted as accounting for most of the antioxidant activity of plants (or) plant products. The result of the DPPH scavenging activity of *T. asthmatica* extract compared to that of gallic acid, as shown in Figure 1, both showed a dose-dependent antioxidant activity. The AA% of GA was remarkable higher than that of *T.asthmatica* at lower concentrations but significant differences between them seem to be less conspicuous at higher concentrations.

The reductive potentials of *T. asthmatica* extract and ascorbic acid (AA) were also dose-dependent (Figure 2). The reductive potential of AA was clearly higher than that of *T. asthmatica* at all concentrations except the lease (20 µg/ml). However, it should be noted that the reductive potential of *T. asthmatica* was still appreciable. Malathi and Patrick Gomez (2007) reported that methanolic extract of *T. asthmatica* is rich in phytochemicals; specific biologically important compounds have been identified in extracts from the plant. The present works also reveal that the extract from the leaves of *T. asthmatica* possesses because of its phytochemical constituents (Thabrew et al., 1998). The DPPH scavenging activities of OG showed a good correlation with its reductive potentials. These facts justify the medicinal use of the plant for the treatment of various maladies (Dhawan et al., 1977; Oliver, 1980). However, further work is necessary to ascertain the clinical safety of the extracts from the plant (Effraim et al., 2001), and to determine appropriate concentration for therapy so as to safeguard the health of the teeming mass of traditional users who more often that not, do not take these factors into consideration.

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