In vitro antioxidant studies of the ethanolic extract of *Tephrosia purpurea* L.

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Abstract

The ethanolic extract of the root of *Tephrosia purpurea* was screened for in vitro antioxidant properties using standard procedures. The ethanolic extract exhibited IC_{50} values of 132.31±8.79 and 405.22±15.09 respectively in DPPH and nitric acid radical inhibition assay. These values were slightly more than those obtained for ascorbic acid and rutin used as standard. The findings justify the therapeutic application of the plant in the indigenous system of medicine, augmenting its therapeutic value.

Key words - Tephrosia purpurea, DPPH, Nitric oxide, Free radical scavenging.

Introduction

There is a potential role for antioxidants and antioxidant enzymes in the treatment and prevention of atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus and several other diseases . Antioxidants provide protection to living organisms from damaged caused by uncontrolled production of reactive oxygen species and the concomitant lipid peroxidation ,protein damage and DNA strand breaking. Ethnomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radical play a major role[1].

Tephrosia purpurea L.(TP) (Leguminosae) commonly known in Hindi as sharapunkha is a copiously branched sub erect herbaceous perennial with slender firm terete glabrescent branches. Leaves 6.5-12.5 cm long, short petiole. In ayurvedic texts, the root is considered alexipharmic ,useful in ulcer ,enlargement of spleen. Seeds are useful in poisoning due to rat bite. Whole plant bitter and acrid , anthelmintic, alexiteric, antipyretic, alternative, cures diseases of kidney, liver, spleen, heart, blood; cures tumours, ulcers, leprosy, asthma, bronchitis, piles and caries of teeth. Leaves are tonic to the intestine, improve appetite, are useful in diseases of lungs and chest, good in piles, syphilis and gonorrhoea[2].

A detailed review of literature afforded no information on the antioxidant potential of the plant . It was therefore thought worthwhile to investigate the antioxidant potential of TP.

Materials and Methods

All chemicals and solvents were of analytical grade obtained from Ranbaxy fine chemicals Mumbai,India.1,1-diphenyl,2-picrylhydrazyl(DPPH)were obtained from sigma chemicals, USA. Ascorbic acid and rutin were obtained from S.D. fine chem., Biosar, India.

Plant material

The roots of *T. purpurea* obtained from Mansi ayurvedic remedies, Mumbai, were identified and authenticated by Dr. S. Vyas, Departm-ent of Botany, Holkar Science College, Indore. A voucher specimen is preserved in our laboratory for further reference.

Extraction

Powdered root of *T. purpurea* were exhaustively extracted with 95% ethanol using a soxhlet apparatus. The residue was filtered and concentrated in vacuo(yield12.5%).

Phytochemical analysis

Phytochemical investigation on *T. purpurea* have revealed the presence of glycoside, rotenoids, isoflavone, flavanones, flavonols and sterols[3].

Preparation of extract and standard

A weighted quantity of the extract was dissolved in distilled dimethyl sulphoxide (DMSO) and used. Solutions of ascorbic acid and rutin used as standards for these studies were prepared in distilled DMSO.

In-vitro antioxidant activity

1.DPPH method

The antioxidant activity of the plant extract and the standards were assessed on the basis of the radical scavenging effect of the stable DPPH free radical.Ten microlitre of extract (from5ìg/ml to500ìg/ml)was added to 200 ìl of DPPH in methanol solution (100 ìM)in a microtitre plate. After incubation at 37^0 c for 30 min., the absorbance of each solution was determined at 517nm using ELISA micro plate reader.The corresponding blank reading were also taken and the

remaining DPPH was calculated[4] .IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radical.

2. Nitric oxide radical inhibition assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of Griess Illosvoy reaction[5].In the present investigation , Griess Illosvoy reagent is modified by using naphthyl ethylene diamine dihydrochloride (0.1%w/v) instead of 1-napthylamine(5%).Scavengers of nitric oxide complete with oxygen leading to reduced production of nitric oxide[6] .The reaction mixture containing sodium nitroprusside (10 mM, 2 ml) phoshate buffer saline (0.5ml) and extract or standard solution was (0.5ml) incubated at 25^0 c for 150 min. After incubation, 0.5ml. of the reaction mixture containing nitrite was pipetted and mixed with 1ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min. for complete diazotization.Then ,1 ml. of naphthyl ethylene diamine dihydrochloride was added,mixed and allowed to stand for 30 min.at 25^0 c. A pink colored chromophore is formed in diffused light. The absorbance of this solution was measured at 540 nm against the corresponding blank solutions in microtitre plate using ELISA reader. IC_{50} value is the concentration of sample required to inhibit 50% of nitric oxide radical.

Stasistical Analysis- Linear regression analysis was used to calculate the IC_{50} values.

Results

The qualitative phytochemical analysis indicates that T. purpurea root contained glycoside, rotenoids, isoflavone, flavanones, flavonols and sterols. Several concentration ranging from 5-500ìg/ml of the ethanolic extract of TP root were tested for their antioxidant activity. It was observed that free radicals were scavenged by the extract in a concentration dependent manner.

The ethanolic extract of TP scavenged the DPPH with IC_{50} value of 132.31±8.79. Incubation of solution of sodium nitroprusside in PBS at 25^0c for 150 min resulted in linear time dependent nitrite production, which was maintained by the extract with IC_{50} value 405.22±15.09 (table 1). These values were found to be slightly more than those obtained for the reference standards.

Discussion

Free radical oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders ,resulting usually from deficient natural antioxidant defences[7]. Antioxidant activity using DPPH method , DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH· DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses colour

stoichometrically depending on the number of electrons taken up[8]. From the present results it may be postulated that TP reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles [9].

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases[10-11]. In the present study the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25°C was reduced by the ethanolic extract of TP. This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide[12] thereby inhibiting the generation of nitrite.

Roots of TP are rich in flavonoids like rutin and quercetin[13]. Flavonoids are natural products, which have been shown to possess various biological properties related to antioxidant mechanisms [14-16].

Conclusion

In conclusion, TP has the potential to be a rich source of flavonoids. Consideration of the antioxidant properties of ethanolic extract of TP reported here and the potential disease preventive properties, suggests that it is appropriate for further work with this, to be directed at exploration of its chemopreventive properties.

Table - 1

***I n vitro* antioxidant activity of the ethanolic extract of *T. purpurea*.**

S.No.	Tested Material	IC_{50}(µg/mL)±S.E.*	
		DPPH Method	Nitric oxide Method
1.	Ethanolic extract	132.31±8.79	405.22±15.09
2.	Ascorbic acid	89.7±6.3	122.67±8.2
3.	Rutin	46.25±3.2	308.12±9.8

* Average of six determinations.

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