



IN VITRO ANTIOXIDANT ACTIVITY OF ROOTS OF *TEPHROSIA PURPUREA* LINN

RUMIT SHAH¹, HEENA KATHAD², RAJAL SHETH³, NAVEEN SHETH⁴

¹Vidyabharti Trust College of Pharmacy, Umrakh, Bardoli, Gujarat, India, ²Saraswati School of Pharmacy, Ranela, Mehsana, Gujarat, India.

³Department of Bioscience, Saurashtra University, Rajkot, Gujarat, India, ⁴Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India. Email: rumit_herbal@yahoo.com

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ABSTRACT

Tephrosia purpurea is a polymorphic, much - branched, sub erectm perennial herb, 30 - 60 cm. high, found through out India, ascending to an altitude of 1,850 m. in the Himalayas. The primary phytochemical study and in vitro anti oxidant study was performed on hydroalcoholic extract of shade dried roots. The hydroalcoholic extract of *Tephrosia purpurea* were prepared and evaluated for its primary phytochemical analysis for total phenolic content and in vitro anti oxidant activity study by DPPH free radical scavenging activity, super oxide free radical activity and nitric oxide scavenging activity. Results indicate that hydroalcoholic root extract of *Tephrosia purpurea* have marked amount of total phenols which could be responsible for the anti oxidant activity of hydroalcoholic extract of *Tephrosia purpurea* but the mechanism remains unclear and could be further investigated by detailed phytochemical investigation.

Keywords: *Tephrosia purpurea*, In-vitro anti-oxidant activity.

INTRODUCTION

Antioxidant compounds in food play an important role as a health-protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Antioxidants are compounds that inhibit or setback the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Exogenous chemical and endogenous metabolic processes in the human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing bio molecules, resulting in cell death or tissue damage. Oxidative damage plays a significantly pathological role in human disease. Free radicals lead to cellular necrosis, which is implicated in some membrane pathophysiological conditions including atherosclerosis, rheumatoid arthritis as well as toxicity of many xenobiotics, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, ageing, inflammatory response syndrome, respiratory diseases, liver diseases, cancer and AIDS.¹⁻³ Many herbal plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite.^{4,5} The antioxidants can neutralize the sick effects of free radicals by scavenging or chain breaking (like vitamin A, C, β -carotene, etc.) or some other mechanism of action. These antioxidants must be constantly replenished since they are 'used up' in the process of neutralizing free radicals.⁶

Flavonoids are natural product and can be regarded as C₆ - C₃ - C₆ in which each C₆ moiety is a benzene ring. The variation in the state of oxidation of connecting C₃ moiety determines the properties and class of each such compound. Flavonoid compounds and the related

coumarins usually occur in plants as glycosides in which one or more phenolic hydroxyl group are combined with sugar residues. The hydroxyl group are nearly always found in position 5 and 7 in ring A, whole ring B commonly carries hydroxyl or alkoxy group at the 4' position or at both 3' and 4' positions. Glycosides of flavanoids compounds may bear the sugar on any of the available hydroxyl groups.^{4,6}

Many of the natural antioxidants, hence, especially flavanoids exhibit a wide range of biological effects, including antibacterial, antiviral, and anti-inflammatory, anti allergic, antithrombotic, and vasodilatory actions. Antioxidant activity is an essential for many biological functions such as antimutagenicity, anticarcinogenicity and antiangiogenicity originate from this property. *Tephrosia purpurea* Linn. (Family: Fabaceae) is a polymorphic, much - branched, suberectm perennial herb, 30 - 60 cm. high, found through out India, ascending to an altitude of 1,850 m. in the Himalayas. This plant is known as wild indigo, Sarphonk, Sharpunkha. Previous phytochemical screening shows that plant contains coumarins, flavanoids and rotenoids, flavanones, isoflavanones and quercetins,⁷ so we have assessed roots of *Tephrosia purpurea* for its in-vitro antioxidant activities.

MATERIALS AND METHODS

Collection of plant material

The roots of *Tephrosia purpurea* Linn., were collected from the river bank of Karjan, at Rajpipla, Gujarat, in the month of the November ending of year 2008. The plant was authenticated by National survey office of botany at Jodhpur, Rajasthan and voucher specimen was deposited in the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot. The plant specimen number is SU/DPS/Herb/13.

Plant material and preparation of extract

The roots of *Tephrosia purpurea* were shade dried and made to a fine powder (particle size ~0.25mm). The powder was first defated with petroleum ether and then it was further extracted with 50% hydroalcohol. Then dry powder of hydroalcoholic extract obtained was then used for the preliminary phytochemical screening, assessment of total phenolic content and antioxidant activity through various *in vitro* assays.

Chemicals

L-Ascorbic acid, Gallic acid, curcumin, 1, 1 - diphenyl-2-picrylhydrazyl (DPPH), Ethylene diamine tetra acetic acid (EDTA), Nitro blue tetrazolium (NBT), and all other chemicals and reagents used were of analytical grade.

Preliminary phytochemical screening

The hydroalcoholic extract was taken for various qualitative chemical tests to determine the presence of various phyto constituents like alkaloids,⁹ glycosides,¹⁰ carbohydrates,¹¹ phenolics and tannins,¹² phytosterols,¹³ fixed oils,¹⁴ protein and amino acids,¹⁵ flavanoids,¹⁵ saponins,¹⁵ gums and mucilage¹⁵ using reported method.

Total phenolic content

Total Phenolic content was determined using Folin - Ciocalteu method. The powdered extract of plant was dissolved in methanol to obtain a concentration of 1 mg/ml. The 100 μ l of this solution was taken in to 25ml volumetric flask, to which 10ml of water and 1.5ml of Folin Ciocalteu reagent were added. The mixture was then kept for 5 min and to it 4 ml of 20% w/v sodium carbonate solution was added the volume was made up to 25ml with double distilled water. The mixture was kept for 30 minute until blue colour develops. The samples were then observed at 765 nm in UV- visible spectrometer Shimadzu, UV-1601, Japan. The % of total phenolic was calculated from calibration curve of Gallic acid plotted by using similar procedure.^{16,20}

The powdered extract of plant was dissolved in methanol to obtain a concentration of 1 mg/ml and taken as a test sample to study various *In-vitro* activities i.e. the DPPH free radical scavenging activity, Super oxide free radical scavenging activity and Nitric oxide scavenging activity.

The result of Total phenolic content, DPPH free radical scavenging activity, Super oxide free radical scavenging activity, Nitric oxide scavenging activity were compared with standard by using paired t-test.

Determination of DPPH radical scavenging activity

4.3mg of DPPH (1, 1-Diphenyl -2-picrylhydrazyl) was dissolved in 3.3 ml methanol; it was protected from light by covering the test tubes with aluminium foil. 150 μ l DPPH solution was added to 3ml methanol and absorbance was taken immediately at 516 nm for control reading. Different volumes of plant extract i.e., 30 μ l, 60 μ l, 90 μ l, 120 μ l, 150 μ l were taken and the volume was made uniformly to 150 μ l using methanol. Each of the samples was then further diluted with methanol up to 3ml and to each 150 μ l DPPH was added. Absorbance was taken after 15 min at 516 nm using methanol as blank on UV-visible spectrometer Shimadzu, UV-1601, Japan. IC₅₀ values for HATP were then calculated and compared with value of Ascorbic acid taking it as a positive control.^{21,22}

Determination of nitric oxide radical scavenging activity

50 μ l, 60 μ l, 70 μ l and 90 μ l of the samples of plant extract were taken in separate tubes and the volume was uniformly made up to 150 μ l with methanol to each tube 2.0 ml of sodium nitroprusside (10 mM) in phosphate buffer saline was added. The solutions were incubated at room temperature for 150 minutes. The similar procedure was repeated with methanol as blank which served as control. After the incubation, 5 ml of Griess reagent was added to each tube including control. The absorbance of chromophore formed was measured at 546 nm on UV-visible spectrometer Shimadzu, UV-1601, Japan.

Curcumin was used as positive control IC₅₀ values were compared by paired t-test.^{23,24}

Super oxide free radical scavenging activity

100 μ l riboflavin solution [20 μ g], 200 μ l EDTA solution [12mM], 200 μ l methanol and 100 μ l NBT (Nitro-blue tetrazolium) solution [0.1mg] were mixed in test tube and reaction mixture was diluted up to 3ml with phosphate buffer [50mM]. The absorbance of solution was measured at 590 nm using phosphate buffer as blank after illumination for 5min. This is taken as control. Different volumes of 50 μ l, 70 μ l, 100 μ l, 125 μ l and 150 μ l of samples of plant extract were taken and volume was made up to 150 μ l with methanol, to each of this, 100 μ l riboflavin, 200 μ l EDTA, 200 μ l methanol and 100 μ l NBT was mixed in test tubes and further diluted up to 3ml with phosphate buffer. Absorbance was measured after illumination for 5min at 590nm on UV visible spectrometer Shimadzu, UV-1601, Japan. IC₅₀ value were calculated and compared with ascorbic acid which was used as positive control in this assay.^{25,26}

RESULT AND DISCUSSION

Preliminary phytochemical screening

It was found that 50% hydroalcoholic extract of *Tephrosia purpurea* extract contained alkaloid, carbohydrate, proteins, amino acids, phenolic compounds and flavanoid.

Table 1: Preliminary phytochemical screening

Class	Hydroalcoholic extract of <i>Tephrosia purpurea</i>
Alkaloid	+
Glycosides	+
Carbohydrates	+
Phytosterols	+
Protein and Amino Acid	+
Flavanoids	+
Saponins	-
Phenolic compound	+

(+) Indicate Present and (-) Indicate Absent

Total phenolic content

The total phenolic content of hydroalcoholic extract of *Tephrosia purpurea* was 113.46 μ g/ml calculated as Gallic acid equivalent of phenols was detected.

Free radicals are produced under certain environmental conditions and during normal cellular function in the body; these molecules are missing in an electron, giving them an electric charge. To neutralize this, charge, free radicals try to withdraw an electron from, or donate an electron to, a neighbouring molecule. The newly create free radical, in turn, looks out for another molecules and withdraws or donates an electron, setting off a chain reaction that can damage hundred of molecules. Antioxidants halt this chain reaction. Some antioxidants are themselves free radical, donating electrons to stabilize and neutralize the dangerous free radicals. Other antioxidants work against the molecules that form free radicals destroying them before they can begin the domino effect that leads to oxidative damage.²⁶

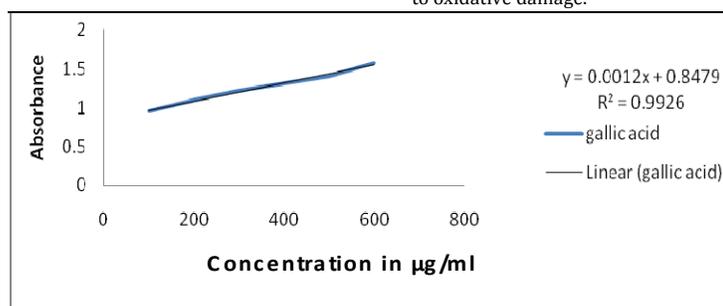


Fig. 1: The total phenolic content

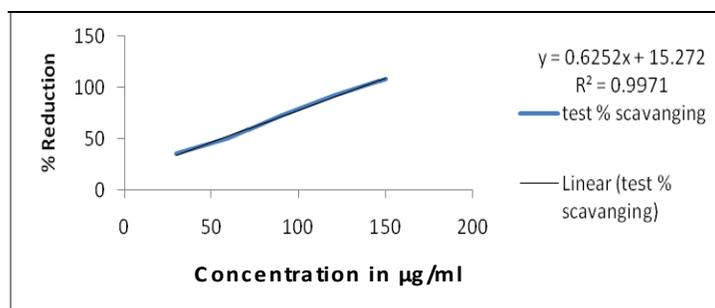


Fig. 2: DPPH free radical scavenging activity

DPPH free radical scavenging activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH both transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1 - 1 diphenyl - 2 - picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug. The reduction capacity of DPPH radical is determine by the decrease in its absorbance at 516 nm induced by antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants because of the

reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. Hence DPPH is usually used as a substance to evaluate the antioxidant activity.

The activity of hydroalcoholic extract of *Tephrosia purpurea* was compared with ascorbic acid as standard 18.53 µg/ml ($y = 0.6252x + 15.272$, 0.9971) and result is 92.6 µg/ml. Data were compared by student t - test and t- value found to be 32.355 which is considered extremely significant.

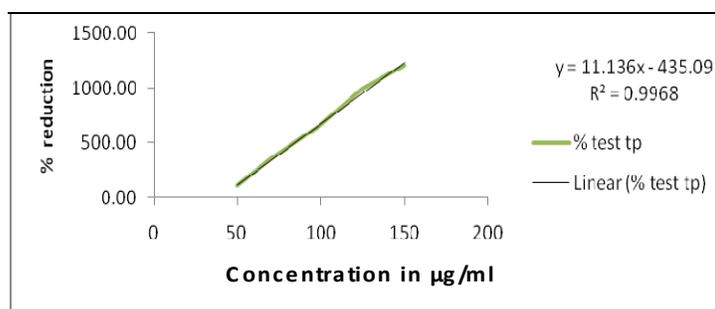


Fig. 3: Super oxide free radical scavenging activity

Super oxide free radical scavenging activity

Super oxide radical known to be very harmful to the cellular component. Superoxide free radical was formed by alkaline DMSO which reacts with nitro blue tetrazolium (NBT) to produced coloured diformazan. The HATP scavenge superoxide radical and thus inhibit foamazan formation.

The activity of hydroalcoholic extract of *Tephrosia purpurea* was compared with ascorbic acid as standard 18.16 µg/ml ($y = 11.136x - 435.09$, 0.9968) and result is 93.58 µg/ml. Data were compared by student t - test and t- value found to be 46.185 which is considered extremely significant.

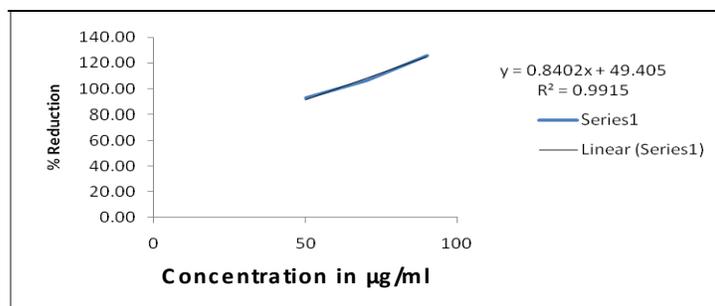


Fig. 4: Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by the greiss reagent reduction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrate ions that can

be estimated by use of greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by hydroalcoholic extract of *Tephrosia purpurea*. The activity of hydroalcoholic extract of

Tephrosia purpurea was compared with Curcumin as standard 10.52 µg/ml ($y = 0.8402x + 49.405, 0.9915$) and result is 114.953 µg/ml. Data were compared by student t – test and t- value found to be 96.297 which is considered extremely significant.

CONCLUSION

The hydroalcoholic extract of *Tephrosia purpurea* showed antioxidant activity by inhibiting DPPH and hydroxyl radical, nitric oxide and super oxide anion scavenging, hydrogen peroxide scavenging, and reducing power activities. In addition, the hydroalcoholic extract of *Tephrosia purpurea* found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. The results of this study show that the hydroalcoholic extract of *Tephrosia purpurea* can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. However, the components responsible for the antioxidant activity of hydroalcoholic extract of *Tephrosia purpurea* are currently unclear. Therefore, further works should be performed on the isolation and identification of the antioxidant components in hydroalcoholic extract of *Tephrosia purpurea*.

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