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Research Article……!!!

ANTIOXIDANT ACTIVITY AND QUANTITATIVE ESTIMATION Of *TEPHROSIA PURPUREA* LEAF

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KEYWORDS:

Quantifative Estimation, Antioxidant, *Tephrosia purpurea* Medicinlal plants.

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ABSTRACT

Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. *Tephrosia purpurea* is a species of flowering plant in the pea family, *Fabaceae,*a common waste land weed and grows in poor soils. *Tephrosia purpurea* has played an important role in the traditional medicine. Thus, the modern pharmacological and clinigal investigation of T*ephrosia purpurea* is a valuable herbal therapy that has an antioxidant, antimicrobial, anti inflammatory, anti-viral and antiulcer properties.

INTRODUCTION:

Tephrosia purpurea Leguminoseae family, Sanskrit as sharapunkha is a highly branched, sub,erect, herbaceous such as "Tephroli" and yakritit used for liver disorders (1,2). The roots and seeds are reported to have insecticidal and piscicidal properties and also used as vermifuge. The roots are also reported to be effective in leprosy wound and their juice, to the eruption on skin (3). The aqueous extract of seeds has shown significant in vivo hypoglycaemic activity in diabetic rabbits (4). The ethanolic extracts of *Tephrosia purpureai* possessed potential antibacterial activity. The total flavonoids were extracted from plant found to have antimicrobial activity (5).

Antioxidant molecules have beeb shown to counteract these oxidative stress. Natural antioxidant substances (ie phytochemicals, vitamins, carotenoids and phenolic compounds) are presumed to tbe safe since they occur in plant roots and are seen as more desirable (han their synthetic counter parts. Natural antioxidants occur in all parts of the plant bark, stem, pods,leaves, pollen roots, flowers and seeds (6,7). Pointed our clearly that medicinal plants constitute the main source of raw pharmaceuticals and health care products while (8). Also reported that extraction and characterization of several active phytocompounds from green plants have given birth to some high activity profile drugs. Such phytochemical screening of various plants had been reported to many workers (9,10).

MATERIALS AND METHODS:

PREPARATION OF ETHANOL EXTRACT:

The leaves of *Tephrosia purpurea* was shade dried at room temperature. The dried material was then homogenized to obtain coarse powder and stored in air-tight bottles for further analysis. The shade dried, powdered leaves were extracted with ethanol by lot extraction using soxhlet apparatus collected and stored in a vial for further analysis.

CHEMICALS:

All chemicals used including the Solvents, were analytical grade. Folin-ciocalteu phenol reagent, 1,1-diphenyl-2-picryl hydrazyl (DPPH), polyvinyl polypyrrolidone (PVPP), Ammonium acetate, glacial aceticacid, 2,4,6- tripyridyl-s-triazine(TPTZ) and Ethanol were purchased from merck co (Germany).

Determination of total phenolics and tannins

The total phenolic content was determined according to the method described by Siddhuraju and Becker (2003). Ten microlitre aliquots of the extracts (2mg/2ml) were taken in test tubes and made up to the volumes of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol

reagent and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents

Using the same extracts the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP) (Siddhuraju and Manian,2007). One hundred milligrams of PVPP was weighed into a 100*12 mm test tube and to this 1 ml distilled water and then 1 ml of the sample extracts were added. The content was vortexed and kept in the test tube at 4^{o} C for 4h. Then the sample was centrifuged (3000 rpm for 10 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other tannins. The phenolic content of the supernatant was measured as mentioned above and expressed as the results, the tannin contenat of sample was calculated as follows:

Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%)

Determination of total flavonoid content

The flavonoid content was determined by the use of a slightly modified method 5% $NaNO_2$ solution. After 6 min, 0.15ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2ml of 4% NaOH solution was added to the mixture. Immediately, water eas added to bring the final volume to 5ml, and then the mixture was thoroughly mixed and allowed to stand for another 15min. Absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate and the results were expressed as rutin equivalent.

DPPH radical scavenging activity (Blois, 1958)

The sample extracts at various concentrations was added to 5ml of a 0.1 mM methanolic solution of DPPH and allowed to stand for 20 min at 27^{0} C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was excpressed as the inhibition percentage of free radical by the sample and was calculated using the formula

% DPPH radical scavenging activity = (Control OD-Sample OD / Control OD) × 100

IC_{50} values of the extract i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

Nitric oxide radical scavenging activity (Sreejayan and R1997)

3ml of 10mM sodium nitroprusside in 0.2 M phosphate buffered saline ($p^{H\ 7.4}$) was mixed with different concentrations of extract and incubated at room temperature for 150 min. After incubation time, 0.5 ml of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride

in 2% H_3 PO_4) was added. The absorbance of the chromophore formed was read at 546 nm. Percentage radical scavenging activity of the sample was calculated as follows:

% NO radical scavenging activity = (control OD-sample OD / control OD) × 100

Superoxide radical scavenging activity (Beauchamp and Fridovich, 1971)

Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (p^H 7.6) , 20 mg riboflavin, 12mM EDTA, 0.1 mg NBT and various concentrations of sample extracts. Reaction was started by illuminating the reaction mixture with sample extract for 90 seconds. Immediately after illumination the absorbance was measured at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil Identical tubes with reaction mixture kept in dark served as blank. The percentage inhibition of superoxide anion generation was calculated as: % Inhibition = control OD – sample OD / control OD) × 10

Hydroxyl radical scavenging activity (Klein et al., 1991)

Various quantities of extract were added with 1ml of iron-EDTA solution (0.13% ferrous ammojium sulfate and 0.26% EDTA), 0.5 ml of EDTA soluation (0.018%) and 1ml of DMSO (0.85%V/V in 0.1M Phosphare buffer pH 7.4). The reaction initiated by adding 0.5ml of ascorbic acid (0.22%) and incubated at 80-90^o C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1ml of ice cold TCA (17.5 w/v). Three milliliters of Nash reagent (75.0g of ammonium acetate, 3ml of glacial acetic acid were mixed and raised to IL with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of color formed was measured spectroscopically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity by the following formula

% HRSA = 1 – (difference in absorbance of sample / difference in absorbance of blank) × 100

Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacities of extracts of samples were estimated according to the procedure described by Pulido et al. (2000). FRAP reaget (900) µl), prepared freshly and incubated at 37^0 C, was mixed with 90 µl of distilled water and 30µl of test sample. The test sample was incubated at 37^0 C min in a water bath. The final dilution of the sample in the reaction mixture was 1/34. The FRAP reagent contained 2.5 ml of 20 mmol/l 2,4,5-tripyridyl-S-triazine (TPTZ) solution in 40 mmol/l HCL plus 2.5 mL of 20 mmol/l $FeCl_3$. 6H2O and 25ml of 0.3 mol/l acetate buffer (pH 3.6) described by Siddhuraju and Beaker, (2003). At the end of incubation, the absorbance readings were taken immediately at 593 nm, using a spectrophotometer. The values are expressed as mmol Fe (II) per milligram extract.

TABLE 1. Total phenol, Flavonoids and tannin content of Ethanolic leaf of *Tephrosia purpurea*

Tephrosia purpurea	Total phenolics content (Mg/g extract)	Total flavonoid content (Mg/g extract)	Total tannin content (Mg/g extract)
Leaf	63.22 ± 0.33	1.44 ± 0.07	*19.19 ± 0.38*

TABLE-2. Antioxidant activity of *Tephrosia purpurea* leaf

S.L.NO	Antioxidant	Sample concentration(mg)	Percentage activity	IC 50(mg)
1.	DPPH radical scavenging activity	60 120 180 240 300	28.65 ± 0.19 37.69 ± 0.09 53.33 ± 0.09 71.21 ± 0.63 83.23 ± 0.19	171.23
2.	Nirtic oxide radical scavenging activity	60 120 180 240 300	9.42 ± 0.83 13.30 ± 0.23 18.76 ± 0.47 29.29 ± 0.36 36.95 ± 0.56	420.16
3.	Superoxide radical scavenging activity	60 120 180 240 300	5.25 ± 0.37 10.30 ± 0.27 15.35 ± 0.31 20.67 ± 0.21 25.30 ± 0.16	588.23
4.	Hydroxyl radical scavenging activity	60 120 180 240 300	8.26 ± o.72 12.41 ± 0.61 16.71 ± 0.55 25.24 ± 0.68 32.62 ± 0.61	476.19

TABLE 3. Ferric reducing / antioxidant power (FRAP) assay of *Tephrosia purpurea leaf*

(FRAP) assay of *Tephrosia purpurea* leaf	744.52 ± 3.64

RESULTS AND DISCUSSION:

Antioxidants are free radical scavengers and other reactive oxygen species (Ros) within the body, hence hindering the process of oxidation. Many oxidative stress related diseases are as a results of accumulation of free radicals in the body. Some molecules distributed widely in the biological system and capable of scavenging free radicals. The total phenol, flavonoids, and rennin content results are presented in Table-1.

Oxidative damage has beeb suggested to be occurasa conseauence of reactive oxygen species (Ros) produced by a product of ETC in mitochondria. A number of studies have been suggested that Ros can affect critical events associated with many disorders. It gets special attention due to many factors such as drought, gold, heat, herbicides and heavy metals, because they harm the cells by raising the oxidative level through loss of cellular strcture and function. Tephrosia purpurea leaf extracts for free radical scavenging activity by DPPH, Nitric oxide, Super oxide, Hydroxyl and FRAP assay values of IC50 (mg) (Table-2,3).

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