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In vitro Antioxidant Potential of Ethanol Extract and Different Fractions of whole plant of *Tephrosia Purpurea* (Linn.) Pers.

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ABSTRACT

The Antioxidant potential of ethanol extract as well as petroleum ether, chloroform, ethyl acetate and aqueous soluble fractions of *Tephrosia purpurea* (Linn.) Pers., which is widely used in indigenous system of medicine for different purposes. The ethanol extract as well as petroleum ether, chloroform, ethyl acetate and aqueous soluble fraction were analysed for total phenolics content, total flavonoid content and free radical scavenging activity using DPPH (1,1- diphenyl-2- picryl hydrazyl) radical. The ethanol extract was found more effective than four different fractions of ethanol extract of *Tephrosia purpurea*. Total phenolics content and total flavonoid content are found to be highest in ethyl acetate fraction and least in petroleum ether and more or less similar in aqueous and chloroform fractions. In general, the results indicate that the ethanol extract and ethyl acetate fraction are rich in phenolic content and flavonoid content with potent free radical scavenging activity implying their importance to human health.

Key Words: Antioxidant, *Tephrosia purpurea*, DPPH.

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INTRODUCTION

Uncontrolled generation of free radicals together with reduced level of antioxidative vitamins and enzymes is considered to be the main contributor to oxidative stress. Free radicals attack membrane lipids, protein and DNA, which is believed to be involved in many health disorders such as diabetes mellitus, cancer, neurodegenerative and inflammatory diseases. Growing knowledge about the health promoting impact of antioxidants in everyday foods, combined with the assumption that a number of common synthetic preservatives may have hazardous effects has led to multiple investigations in the field of natural antioxidants. They are known to have beneficial effects on the prevention or progression of diseases related to oxidative stress on account of their high antioxidant activity[1].

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as by products of biological reaction or from exogenous factors[2]. *In vivo*, some of these ROS play an important role in cell metabolism including energy production, phagocytosis and intercellular signalling[3]. However, these ROS produced by sun light, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects such as DNA damage, carcinogenesis and various degenerative disorders such as cardio vascular diseases, aging and neuro-degenerative diseases[4,5,6]. A potent broad spectrum scavenger of these species may serve as a possible preventive intervention for free radical mediated cellular damage and diseases[7].

Mechanisms of antioxidant action can include suppressing reactive oxygen species formation, scavenging reactive oxygen species or up regulating or protecting antioxidant defences. Sources of natural antioxidants include plant phenolics which occur in all parts of plants like fruits, vegetables, seeds, leaves, roots and bark. In the field of nutrition, health and medicine, crude extracts of these parts rich in phenolics are under extensive research, because they retard oxidative degradation of lipids and thereby improve the quality and nutritive value of foods[8].

Tephrosia purpurea Linn. Figure: 1 is commonly known as wild indigo in English, Kolanji in Tamil, Sharapunkha in Sanskrit, Sarponkha in Hindi, Thila in Gujarati, Vempali in Telugu[9,10,11]. According to Ayurveda literature this plant has also given the name of *wranvishapaka* which means that it has the property of healing all types of wounds[12]. It has been used in Ayurvedic system, Siddha, Unani system of medicine for the treatment of various diseases. Experimental studies suggest that *Tephrosia purpurea* Linn. exerts anti-ulcer, anti-oxidant, hepatoproductive and hypoglycaemic activities. It has been reported to possess hepatoprotective and mast cell

stabilising effect in various experimental models[13]. The present study was aimed to evaluate the total phenolic content, total flavonoid content and antioxidant activity of extract and fractions of the whole plant of *Tephrosia purpurea*. The antioxidant activity was assessed in terms of the DPPH radical scavenging activity of the extract and fractions.

Figure : 1 *Tephrosia purpurea*

MATERIALS AND METHODS

Collection of Plant material and Authentication:

The whole plant of *Tephrosia purpurea* was collected from Mandya district, Karnataka. The plant material were identified and authenticated by Dr. Raj Singh Saini, Head of the department of biotechnology, IIMT College of Medical Sciences, Meerut. A specimen number IIMT/SL/2011/11 is kept in herbarium for further reference in college.

Successive extraction:

The accurately weighed 250 g of dried powder of drug (*Tephrosia purpurea*) in three successive batches was packed in thimble flask and 750ml of ethanol was added in 1 litre round bottom flask. The Soxhlet assembly was set up to complete 15 cycles. The same procedure repeated for two times to get sufficient amount of extract. After that the extract was filtered and distilled under reduced pressure. The obtained extract was kept in a desiccators over calcium chloride for 3 days. The extracts were subjected to phytochemical investigations by qualitative chemical tests.

Fractionization of ethanol extract with different solvents in increasing order of their polarity.

60gm of *Tephrosia purpurea* was suspended in 100ml of distilled water. This suspension was transferred to the separating funnel, to this suspension added the 100ml of petroleum ether per

batch into three successive times and shaking for 15 min in separating funnel. The petroleum ether soluble fraction was separated and washed with 3ml of water, this washings transferred to the aqueous soluble fraction. To this aqueous soluble fraction again added the 100ml of chloroform per batch into three successive times and shaking for 15 min in separating funnel. The chloroform soluble fraction was separated and washed with 3ml of water, this washings transferred to the aqueous soluble fraction. To this aqueous soluble fraction again added the 100ml of ethyl acetate per batch into three successive times and shaking for 15 min in separating funnel. The ethyl acetate soluble fraction was separated and washed with 3ml of water, this washings transferred to the aqueous soluble fraction. The obtained fractions (Petroleum ether, chloroform, ethyl acetate, aqueous) were distilled under reduced pressure and kept in a desiccator over calcium chloride to obtain the dry extract of respective fractions.

***In vitro* antioxidant activity of EETP and its different fractions**

Estimation of total phenolic content by Spectrophotometer

By Folin–Denis Method. The method is based on the oxidation of molecule containing a –OH groups. The tannins and tannin like compounds reduce Phosphotungustomolybdic acid in alkaline solution to produce a highly blue colored solution. 1ml of the ethanol extract and different fractions (Petroleum ether, chloroform, ethyl acetate, aqueous) of ethanol extract that has adjusted to come under the linearity range *i.e.* (50 µg/ml) of all the extracts (Different fractions) was transferred in 10 ml volumetric flask separately. To each flask 0.5 ml of Folin-Denis reagent and 1 ml of Sodium carbonate was added and volume is made up to 10 ml with distilled water. The absorbance was measured at absorption maxima 700 nm within 30 min of reaction against the blank. The total phenolic content was determined by using calibration curve (5 to 30 µg/ml).Three readings were taken for each and every solution for checking there reproducibility and to get accurate result. The intensity of the solution is proportional to the amount of tannins and can be estimated against standard gallic acid, the total phenolic content, expressed as mg gallic acid equivalents per 100 gm dry weight of sample.

Total Flavonoid Content by Spectrophotometer

Aluminium chloride colorimetric assay method

Total flavonoid contents were measured with the aluminium chloride colorimetric assay. The ethanol extract and different fractions (Petroleum ether, chloroform, ethyl acetate, aqueous) of ethanol extracts that has been adjusted to come under the linearity range *i.e.* (400 µg/ml) and different dilution of standard solution of Quercetin (10-50 µg/ml) were transferred to 10 ml volumetric flask containing 4 ml of water. To the above mixture, 0.3ml of 5% $NaNO_2$ was

added. After 5 min, 0.3 ml of 10% $AlCl_3$ was added. After 6 min, 2 ml of 1 M NaOH was added and the total volume was made up to10 ml with distilled water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. Total flavonoid content of the extract and fractions were expressed as percentage of Quercetin equivalent per 100 gm dry weight of sample.

DPPH –RSA method

The free radical scavenging activity of ethanol extract and different fractions was evaluated using standard L-Ascorbic Acid (Vitamin C) was measured in terms of hydrogen donating or radical scavenging ability with stable radical DPPH. Here 0.1 mM solution of DPPH in alcohol was prepared and it must be protected from light influence by maintaining the dark condition and also fold by aluminium foil and 3 ml of this solution was added to 1 ml various concentrations of ethanol extract (50 µg/ml) and different fractions (100-500 µg/ml) (Petroleum ether, chloroform, ethyl acetate, aqueous) of ethanol extract or standard solution of (10-50 µg/ml). Absorbance was taken after 30 min at 517 nm. The percentage inhibition activity was calculated using formula $[(A_0-A_1)/A_0]$ x 100, where A_0 is the absorbance of the control and A_1 is the absorbance of ethanol extract and different fractions of ethanol extract/standard taken as Ascorbic acid[14].

RESULT AND DISCUSSION

There are different models available for evaluation of antioxidant activities. The chemical complexity of different extract and mixture of compounds present could lead to scattered results, depending on the test employed. Therefore, an approach with multiple assays for evaluating the antioxidant potential of extracts would be more informative and even necessary. In this study, different free radical scavenging activities were measured and all results were compared with standard antioxidant.

Total Phenolic content

Phenolic compounds are known as powerful chain breaking antioxidants. The concentration of phenol in the extract and fractions expressed as µg of gallic acid (figure. 2) as per mg of the sample shown in Table 1. The total phenolic content was determined in EETP and different fractions (Petroleum ether, chloroform, ethyl acetate, aqueous) of EETP. Total phenolics are found to be least in petroleum ether fraction (9.13 % w/w) and more or less in chloroform fraction (10.45 % w/w) and ethyl acetate fraction (11.10 % w/w) and highest in aqueous fraction (18.13 % w/w) and ethanol extract (20.16 % w/w). These above results showed that ethanol extract and aqueous fraction contain more tannins compared to other fractions of EETP

(Petroleum ether, chloroform and ethyl acetate). The high concentration of poly-phenolics in the ethanol extract and aqueous fraction may be due to purification and concentration of phenolics and it is probably responsible for its high free radical scavenging activity. It was reported that presence of hydroxyl groups contribute directly to antioxidant effect of the system and it also has an important role in preventing lipid oxidation[15].

Table 1: Total phenolic content

SI No	Concentration of extract and Fractions	% w/w of total gallin
1	Ethanol extract 50 µg/ml	20.16 ± 0.005
2	Petroleum ether fraction 50 µg/ml	9.13± 0.01
3	Chloroform fraction 50 µg/ml	10.45 ± 0.02
4	Ethyl acetate fraction 50 µg/ml	11.10 ± 0.02
5	Aqueous fraction 50 µg/ml	18.13 ± 0.005

Values are mean ± SD, n=3

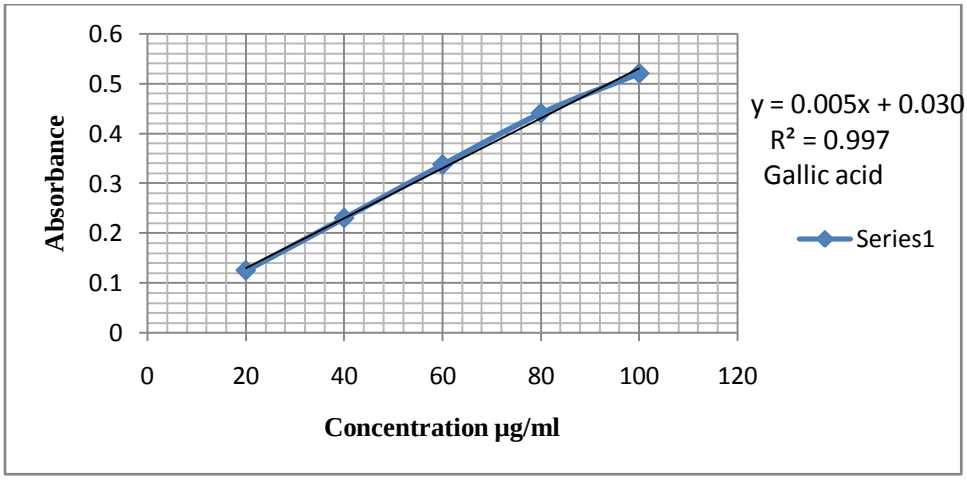

Figure. 2: Total Phenolic content: Gallic acid standard

Total Flavonoid content

The flavonoids, which are the largest and most studied poly phenols, are gaining interest as antioxidants because of their high capacity to scavenge free radicals. There are reports regarding the anti-inflammatory, anti-allergic, antiviral and anti-carcinogenic properties of flavonoids. Flavonoids prevent hydroxyl radical induced damage by donating an electron to neutralize the species[16]. The concentration of flavonoid in the extract and fractions expressed as µg of Quercetin (figure. 3) as per mg of the sample shown in Table 2. The flavonoid content was determined in EETP and different fractions (Petroleum ether, chloroform, ethyl acetate, aqueous) of EETP. Total flavonoids are found to be least in petroleum ether fraction (2.50 % w/w) and more or less in chloroform fraction (3.40 % w/w) and ethyl acetate fraction (4.18 % w/w) and highest in aqueous fraction (5.13 % w/w) and ethanol extract (7.24 % w/w). The above results

show that ethanol extract and aqueous fraction contain more flavonoid compared to other fractions of EETP (Petroleum ether, chloroform and ethyl acetate).

Table 2: Total flavonoid content

SI No	Concentration of extract and Fractions	% w/w of total Flavonoid
1	Ethanol extract 400 µg/ml	7.24 ± 0.01
2	Petroleum ether fraction 400 µg/ml	2.50 ± 0.005
3	Chloroform fraction 400 µg/ml	3.40 ± 0.004
4	Ethyl acetate fraction 400 µg/ml	4.18 ± 0.002
5	Aqueous fraction 400 µg/ml	5.13 ± 0.03

Values are mean ± SD, n=3

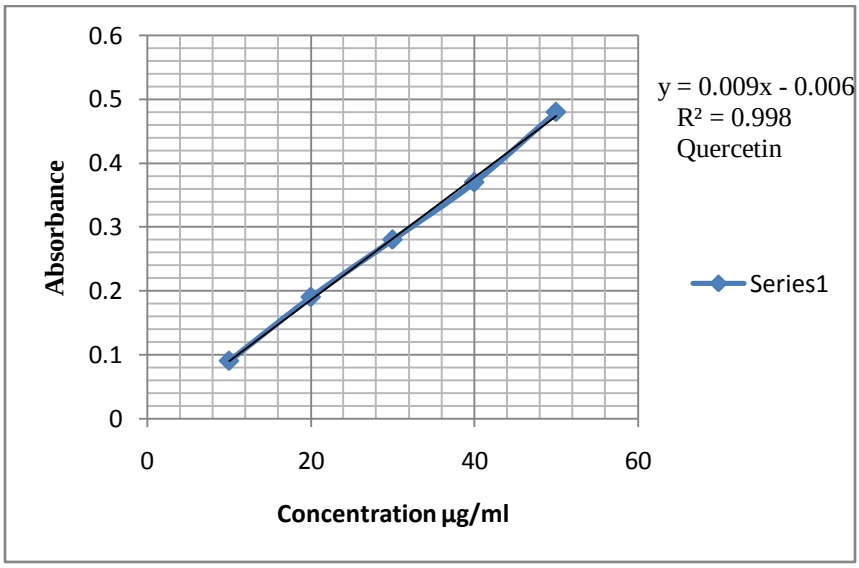

Figure. 3: Total Flavonoid Content: Quercetin standard

Capacity of DPPH- RSA

The results of the free radical scavenging potential of extract and different fractions tested by DPPH method. Antioxidant reacts with DPPH, which is a nitrogen centred radical with a characteristic absorption at 517 nm and converts it to 1,1-diphenyl-2-picryl hydrazine, due to its hydrogen accepting ability at a very rapid rate . The degree of discoloration indicates the scavenging potentials of the antioxidant[17]. The ethanol extract (382.0 µg/ml) (figure. 5) and aqueous fraction (395.13 µg/ml) of EETP showed highest DPPH radical scavenging activity comparing to other fractions (Petroleum ether (500.18 µg/ml), chloroform (479.45 µg/ml), ethyl acetate fraction (464.54 µg/ml)) of EETP (figure. 6) . Ascorbic acid has taken as reference which showed (54.39 µg/ml) (figure. 4). This assay provides information on the reactivity of different fractions with a stable free radical. The bleaching of DPPH absorption is representative of the capacity of tested compounds to scavenge free radicals independently from any enzymatic activity. The low IC_{50} value of ethanol extract and aqueous fraction is due to presence of high

poly phenolics and flavonoids. The above results showed that EETP are more effective than aqueous fraction and other fractions (Petroleum ether, chloroform, ethyl acetate) of EETP. The results of the free radical scavenging potential of extract/different fractions tested by DPPH method. The IC_{50} value of ethanol extract and different fractions described in Table 3.

Table 3: IC_{50} values of extract and its different fractions and standard anti-oxidants by DPPH free radical scavenging methods.

SI	Description	IC_{50} value µg/ml
1	Standard (Ascorbic acid)	54.39 µg/ml
2	Ethanol extract	382.0 µg/ml
3	Petroleum ether fraction	500.18 µg/ml
4	Chloroform fraction	479.45 µg/ml
5	Ethyl acetate fraction	464.54 µg/ml
6	Aqueous fraction	395.13µg/ml

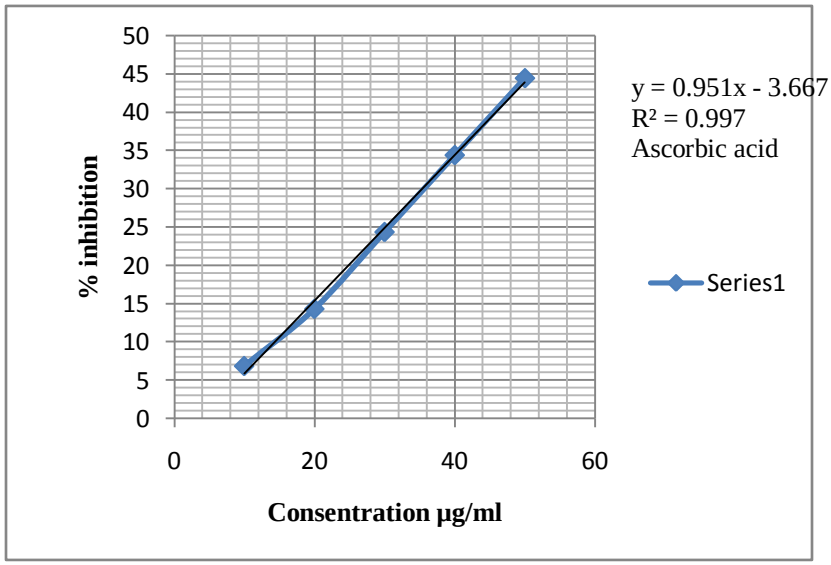

Figure. 4: DPPH Radical Scavenging assay: STD Ascorbic acid

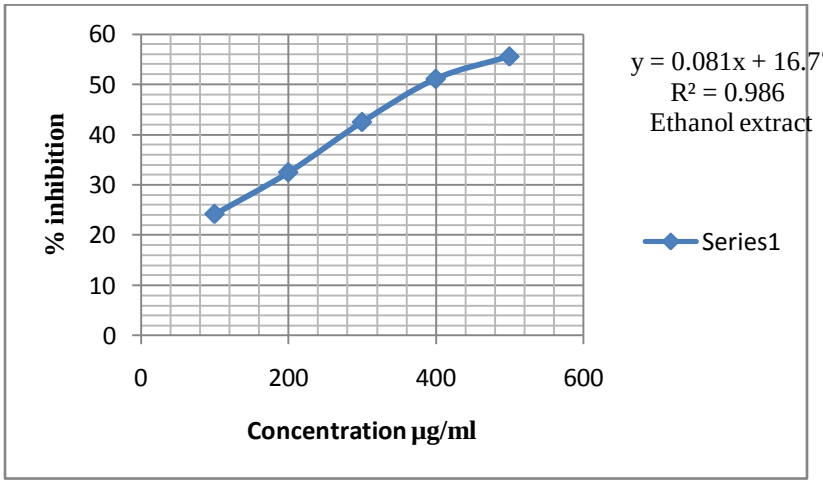

Figure. 5: DPPH Radical Scavenging assay: Ethanol extract

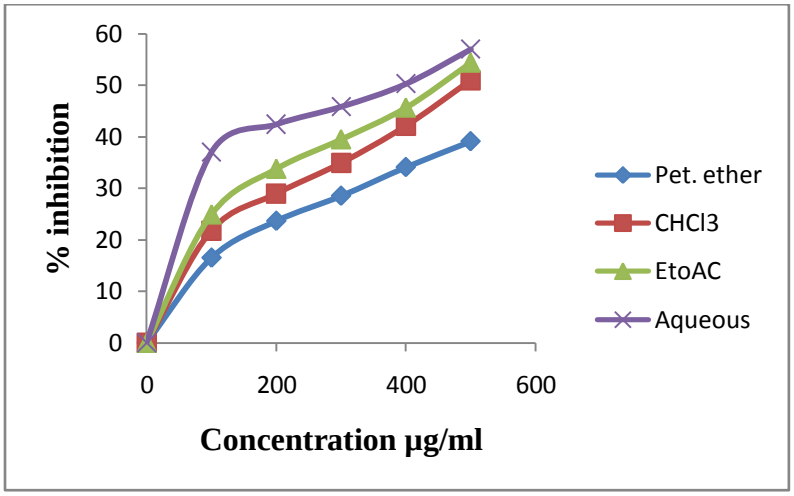

Figure. 6: Radical scavenging potential of different fractions of EETP by DPPH method at different concentration (µg/ml)

CONCLUSION

The results expressed in this study are the first information on the antioxidant activities of *Tephrosia purpurea*. The extract and all the fractions showed free radical scavenging activity when tested. Ethanol extract exhibited the highest free radical scavenging activity and fraction the aqueous fraction showed better activity than other fractions of EETP. The ethanol extract was found to contain flavonoids, saponins and tannins. The highest scavenging activities of the aqueous fraction can be ascribed to its flavonoids, which was concentrated in the fraction due to fractionation. The scavenging effect on DPPH and superoxide radicals represents the fraction direct radical scavenging activity. However, in the hydroxyl radical scavenging assay, hydroxyl radicals are generated by the Fenton reaction and the inhibition of deoxyribose degradation could be attributed to the inhibition of radicals. It is well documented that free radicals are responsible for several diseases. The present result confirms the free radical scavenging activity of the plant which can be accounted for the traditional uses of the plant in treating several diseases.

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