Estimation of Flavonoid, Polyphenolic Content and In-vitro Antioxidant Capacity of leaves of *Tephrosia purpurea* Linn. (Leguminosae)

[a]Avani Patel*, [b]Amit Patel, [a]Amit Patel, [a]Dr. N. M. Patel

[a]Department of Pharmacognosy & Phytochemistry, Shri B.M.Shah college of Pharmaceutical Education & Research, Modasa -383315, North Gujarat, India, affiliated to Hemchandracharya North Gujarat University, Patan, North Gujarat, India.

[b]Shri Sarvajanik Pharmacy College, Mehsana-384001, Gujarat

ABSTRACT

Introduction

Leaves of *Tephrosia purpurea* Linn (sarpankh), belonging to the family Leguminosae being used for the treatment of jaundice and claimed to be effective in many other diseases. The present research work was under taken to investigate the in-vitro antioxidant activity of aqueous and ethanolic extracts.

Method

The therapeutic effects of tannins and flavonoids can be largely attributed to their antioxidant properties. So that the quantitative determinations were undertaken. All the methods are based on UV-Spectrophotometric determination.

Result

The total Phenolic content of aqueous and ethanolic extracts showed the content values of 9.44 ± 0.22% w/w and 18.44 ± 0.13% w/w and total flavonoids estimation of aqueous and ethanolic extract showed the content values of 0.91 ± 0.08 % w/w and 1.56 ± 0.12%w/w for Quercetin and for 1.85 ± 0.08 % w/w and 2.54 ± 0.12 % w/w Rutin respectively. Further investigation were carried out for In-vitro antioxidant activity and Radical scavenging assay by calculating its % inhibition by means of IC_{50} values, all the extracts concentration has been adjusted to come under the linearity range and here many reference standards like Tannic acid, Gallic acid, Quercetin, Ascorbic acid have been taken for the method suitability.

Conclusion

The results revealed that leaves of this plant have antioxidant potential .Among these results ethanolic extract has more potent than traditionally claiming aqueous decoction. In conclusion that *Tephrosia purpurea* Linn. (Leguminosae) leaves possesses the antioxidant substance which may be potential responsible for the treatment of jaundice and other oxidative stress related diseases.

KEY WORDS: *Tephrosia purpurea* Linn**.** (Leguminosae), Total Phenolic Content (TPC), Ferric reducing antioxidant power (FRAP), radical scavenging assay (DPPH – RSA)

INTRODUCTION

Tephrosia purpurea Linn. (Leguminosae) commonly known as Sarpankh called as Thila in Gujarati, Sarponkha (Hindi), Vempali (Telugu) which is not official in Ayurvedic Pharmacopoeia[1]. It is one of the excellent plant for human being made and gifted by the nature having composition of all the essential constituents that are required for normal and good human health. Leaves of *Tephrosia purpurea* Linn. (Leguminosae) are taken as emetic in the form of leaf juice or decoction. This by adding sugar also used in Jaundice. *Tephrosia purpurea* (Linn.) (Leguminosae) commonly known in Sanskrit as Sharapunkha is a highly branched, sub-erect, herbaceous perennial herb[2]. 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[3]. It is an important component of some preparations such as Tephroli and Yakrifit used for liver disorders[4]. In

Ayurvedic system of medicine various parts of this plant are used as remedy for impotency, asthma, diarrhoea, gonorrhoea, rheumatism, ulcer and urinary disorders. The plant has been claimed to cure diseases of kidney, liver spleen, heart and blood[5]. The dried herb is effective as tonic laxative, diuretics and deobstruents. It is also used in the treatment of bronchitis, bilious febrile attack, boils, pimples and bleeding piles. 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 leprous wound and their juice, to the eruption on skin. An extract of pods is effective for pain, inflammation and their decoction is used in vomiting[6]. The ethanolic extract of seeds has shown significant in vivo hypoglycaemic activity in diabetic rabbits[7]. The ethanolic extracts of *Tephrosia purpurea* (Linn.) (Leguminosae) possessed potential antibacterial activity. The flavanoids were found to have antimicrobial activity[8]. The phytochemical investigations on TP have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, and sterols[9]. It is also good source of minerals and amino acids. HPLC analysis of extract of flowering plant of *Tephrosia purpurea* (Linn.) (Leguminosae) shows presence of rutin and quercetin. Although the *Tephrosia purpurea* (Linn.) (Leguminosae) plant leaves carries high potential uses especially for the treatment of jaundice, but the proper scientific studies have not been much reported for the leaves of this plant especially for an antioxidant perspective. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols[10]. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful. In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics[11]. So that we got an interest to fulfill the paucity of studies by means of preliminary In-vitro antioxidant work which we have carried out in leaves portion of *Tephrosia purpurea*, here is our initiation for the future drug.

MATERIAL AND METHODS

Chemicals and reagents

Chemicals used in this study were 1, 1-diphenyl-2-picrylhydrazyl (DPPH) obtained from Sigma-Aldrich, U.S.A, Phosphomolybdic acid, sodium tungustate, potassium ferricyanide and sodium nitroprusside, naphthylethylenediamine dihydrochloride, sodium nitrite, trichloroacetic acid, ascorbic acid, ethylenediamine tetraacetic acid, phosphoric acid, nitro bluetetrazolium, phenazine methosulfate, ferrous ammonium sulfate are obtained from Sd Fine Chemicals Ltd, India. All other reagents and solvents used in the study were of analytical grade.

Plant material

Leaves of *Tephrosia purpurea* (Linn.) (Leguminosae) were collected with flowering top during the month of August from local cultivating field area of Mehsana district, Gujarat, India. The plant material was authenticated at the Department of Botany, Govt. Arts and science College Modasa, Gujarat. A voucher specimen as a herbarium (NMP/08/28082009) has been kept in our museum for future reference. The leaf parts were chopped and dried at room temperature for 10 days and used as raw material. The dried leaves of the drug have powdered using mechanical method and resulting powder was passed through the 40 # sieve and stored in the airtight container.

Preparation of Raw Material

Preparation of crude aqueous extract

Then weighed accurately 100 g of powder was taken in stainless steel vessel and mixed with 2000 ml (1:20) of distilled water. Then the mixture was boiled for about 2 hours using gas burner. After that, the mixture was filtered through cotton bag and then using vacuum filter assembly, here the filtrate must be poured in a borosilicate 500ml beaker. Then the filtrate was evaporated on hot plate until it reaches the concentrated quantity (do not be in viscous state)

To prepare dry powder form of extract

The dry powder of this extract was prepared by using the simple saloon water sprayer by spraying the extract on stainless steel evaporating plate, after the predetermined flow conditioned consistency thick solution was poured into the sprayer (here the above concentrated extract solution varies to nature of plant material), by which it was heated on hot plate at constant temperature of about 60°C. The clumpy dry powder obtained was scraped by the knife and made into fine powder form by using pretreated mortal and pestle glass type from the plate and packed in air tight plastic container every steps must be carried out at above the room temperature and stored in vacuum desiccators as such or in the form of stock solution prepared by the same solvent until further use. The preconditioned set method can be optimized by evaluating the quantitative test of any existed constituents like tannins, flavonoids or any existed markers by suitable validated methods. This present study was undertaken by the spectrophotometrical method

Preparation of ethanolic extracts

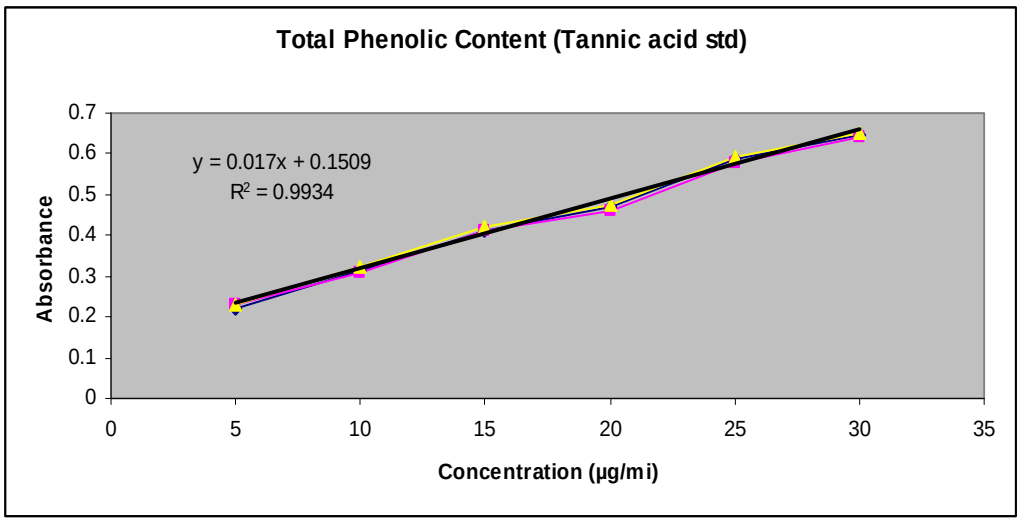
Then weighed loading limit amount of 55 g of powder of drug was packed in thimble flask and 550ml of ethanol (70%) was added in 1 liter round bottom flask. Then the Soxhlet assembly was set up to complete 10 to 15 cycles. After that the extract was filtered and filtrate was concentrated up to 50 ml using water bath. From the concentrated 10ml of extract was taken in evaporating dish (Borosilicate glass) which is previously weighed. The total weigh of evaporating dish containing 10ml extract was recorded and the extract was evaporated till thick liquor was obtained. After then calculate the difference in weight was noted at every 10 min until the constant weigh was obtained. The residue at the constant weigh (it can be obtained from the graph %L.O.D) is used as dry extractives[12], which can be used to prepare the stock solutions (w/v) and the % yield would be 11.2% w/w and stored in the freezer until further use

Estimation of TPC By Spectrophotometer

By Folin – Denis Method

The method is based on the oxidation of molecule containing a –OH groups. The tannin and tannin like compound reduce Phosphotungustomolybdic acid in alkaline solution to produce a highly blue colored solution[13, 14]. 1ml of the aqueous and ethanolic extract that has adjusted to come under the linearity range i.e. (50µg/ml) of both the drugs was withdrawn in 10ml volumetric flask separately. To each flask 0.5ml of Folin-Denis reagent and 1ml of Sodium carbonate was added and volume is made up to 10ml with distill water. The absorbance was measured at absorption maxima 700nm within 30 minute of reaction against the blank. The total phenolic content was determined by using calibration curve (5 to 30µg/ml).Three readings were taken for each and every solution for checking the reproducibility and to get accurate result. Results are provided in (Table 1 and Figure 1).The intensity of the solution is proportional to the amount of tannins and can be estimated against standard tannic acid, the total phenolic content, expressed as mg tannic acid equivalents per 100 g dry weight of sample.

Figure 1: Results of Total Phenolic content



R^2 values represented mean data set of n=3

Table 1: Results of Totalphenolic content

No	conc. of extracts	%w/w of total tannin
1	L.S Aqueous. 50µg/ml	9.44 ± 0.22
2	L.S Ethanolic. 50µg/ml	18.44 ± 1.23

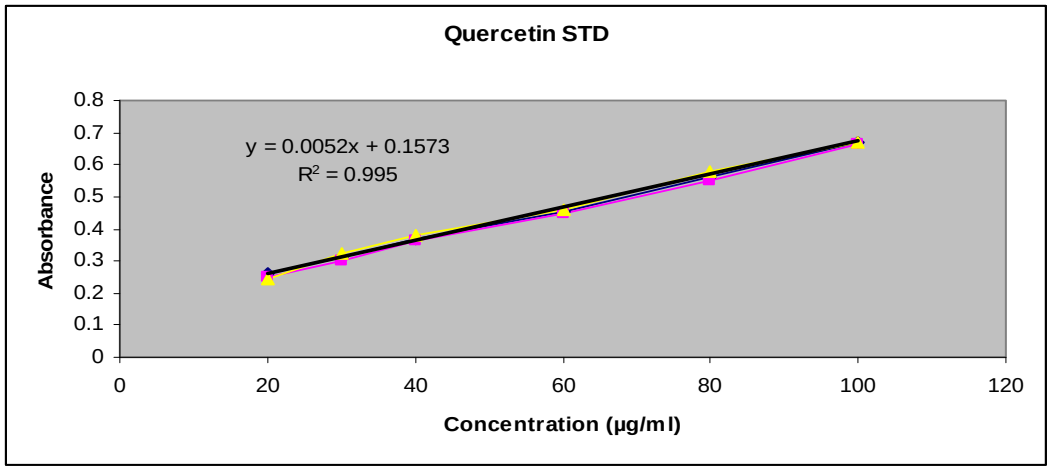
Values are mean ± S.E.M, n=3

Total Flavonoid Content By Spectrophotometer

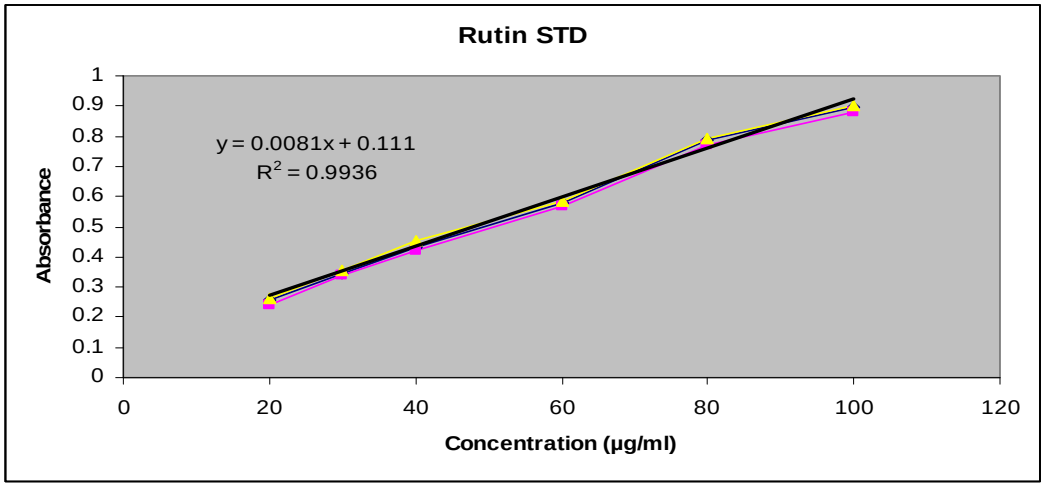
Aluminum chloride colorimetric assay method

Total flavonoid contents were measured with the aluminum chloride colorimetric assay[15]. Aqueous and ethanolic extracts that has been adjusted to come under the linearity range i.e. (400µg/ml) and different dilution of standard solution of Quercetin (10-100µg/ml) were added to 10ml volumetric flask containing 4ml of water. To the above mixture, 0.3ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3ml of 10% $AlCl_3$ was added. After 6 min, 2ml of 1 M NaOH was added and the total volume was made up to 10ml with distill water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. Results are provided in (Table 2 and Figure 2). Total flavonoid content of the extracts was expressed as percentage of Quercetin equivalent per 100 g dry weight of sample.

Figure 2: Results of Flavonoid content



R^2 values represented mean data set of n=3



R^2 values represented mean data set of n=3

Table 2: Results of Flavonoid content

No	conc. of extracts	%w/w of total Flavonoid	
		Quercetin	Rutin
1	L.S Aqueous. 500µg/ml	0.91 ± 0.08	1.85 ± 0.23
2	L.S Ethanolic. 500µg/ml	1.56 ± 0.32	2.54 ± 0.68

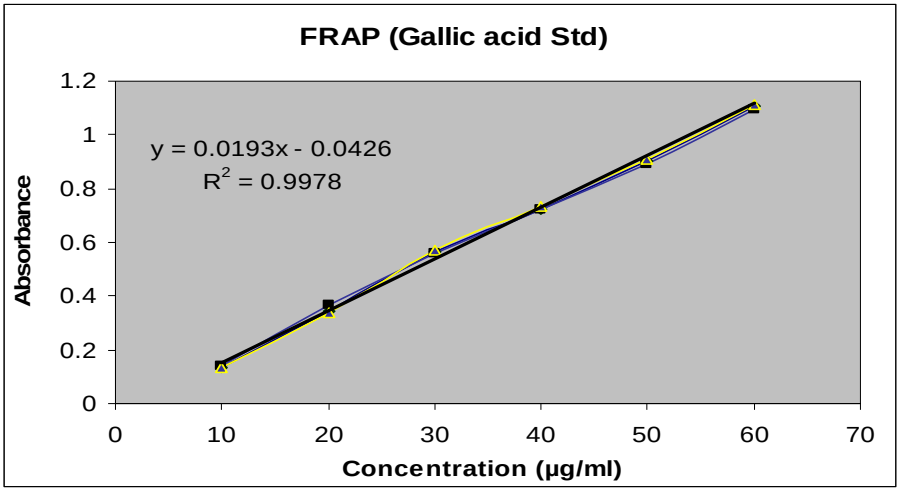
Values are mean ± S.E.M, n=3

In-Vitro Antioxidant Study

FRAP method[16]

The ferric reducing property of the extract was determined by taking 1ml of different dilutions of standard solutions of Gallic acid (10 -100 µg/ml) or aqueous and ethanolic extract that has adjusted to come under the linearity range (500µg/ml) was taken in 10ml volumetric flasks and mixed with 2.5ml of potassium buffer (0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20min. Then 2.5ml of 10% trichloroacetic acid was added to the mixture to stop the reaction. To the 2.5ml of above solution 2.5ml of distill water is added and then 0.5ml of 0.1% of $FeCl_3$ was added and allowed to stand for 30 min before measuring the absorbance at 593 nm. Results are provided in (Table 3 and Figure 3).The absorbance obtained was converted to Gallic acid equivalent in mg per gm of dry material (GAE/g) using Gallic acid standard curve.

Figure 3: Results of FRAP



R^2 values represented mean data set of n=3

Table 3: Results of FRAP

No	Conc. of extracts	mg GAE/g of extracts
1	T.P Aqueous. 500µg/ml	64.94 ± 0.28
2	T.P Ethanolic. 500µg/ml	76.56 ± 0.54

Values are mean ± S.E.M, n=3

Scavenging Activity Assays

Nitric oxide scavenging assay

Nitric oxide radical inhibition was estimated by the use of Griess Illosvory reaction[17, 18]. In this investigation, Griess Illosvory reagent was generally modified by using Napthyl ethylene diamine dihydrochloride (0.1%w/v) instead of the use of 1-napthylamine (5%). The reaction mixture (3ml) containing 2ml of 10 mM sodium nitroprusside, 0.5ml saline phosphate buffer and 0.5ml of standard

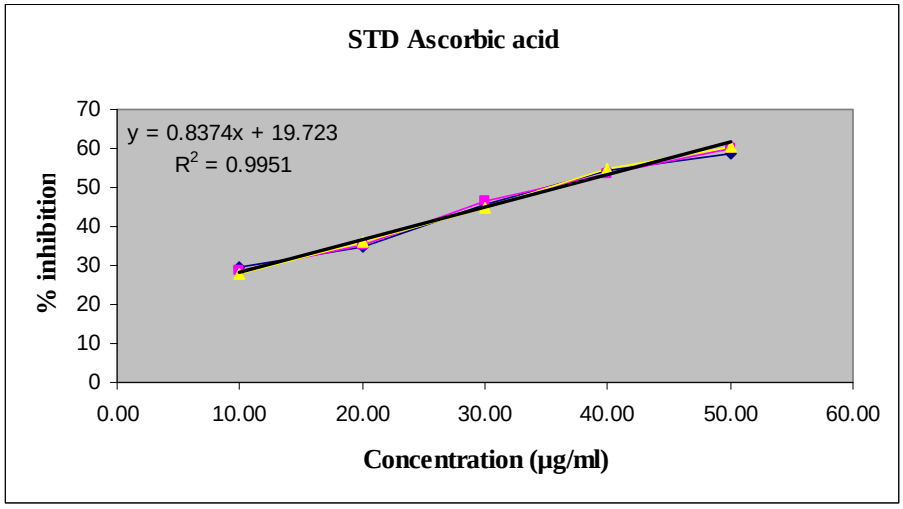
solution or aqueous and ethanolic extract of (500 -1000µg/ml) were incubated at 25˚C for 150min. After incubation, 0.5ml of the reaction mixture was mixed with 1ml Sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5min for the completion of the reaction of diazotization. After that further 1ml of the Napthyl ethylene diamine dihydrochloride was added, mixed and was allowed to stand for 30min at 25˚C. The concentration of nitrite was assayed at 546nm and was calculated with the control absorbance of the standard nitrite solution (without extracts or standards, but the same condition should be followed).Here the blank is taken as the buffer and make up solvents and the Ascorbic acid and Quercetin (10 -50 µg/ml) was taken as standard. Results are provided in (Figure 4 -7) .The percentage inhibition was calculated using the formula:

$$\% \text{ Scavenging Activity} = \frac{A_{control} - A_{test} \text{ or } A_{Std}}{A_{control}} * 100$$

Where, $A_{control}$ = absorbance of control

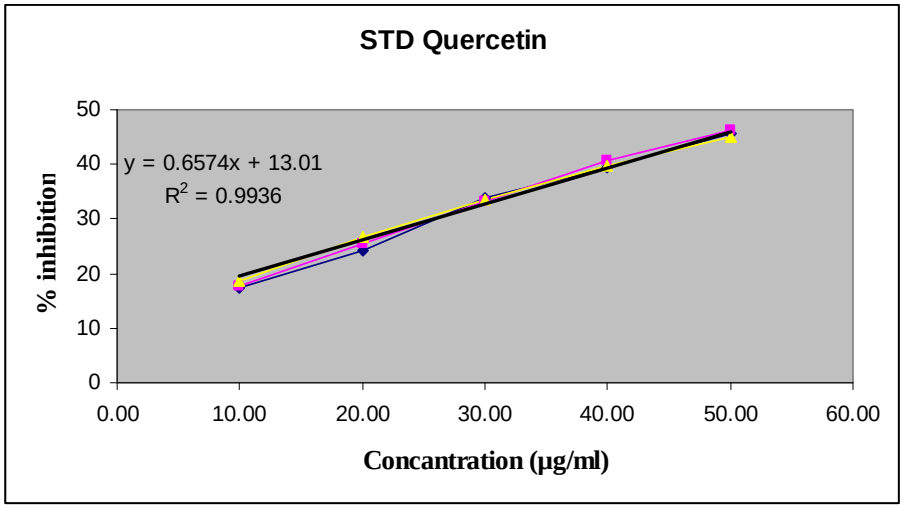
A_{test} or A_{Std} = absorbance of test or std

Figure 4: Results of Nitric oxide radical scavenging assay



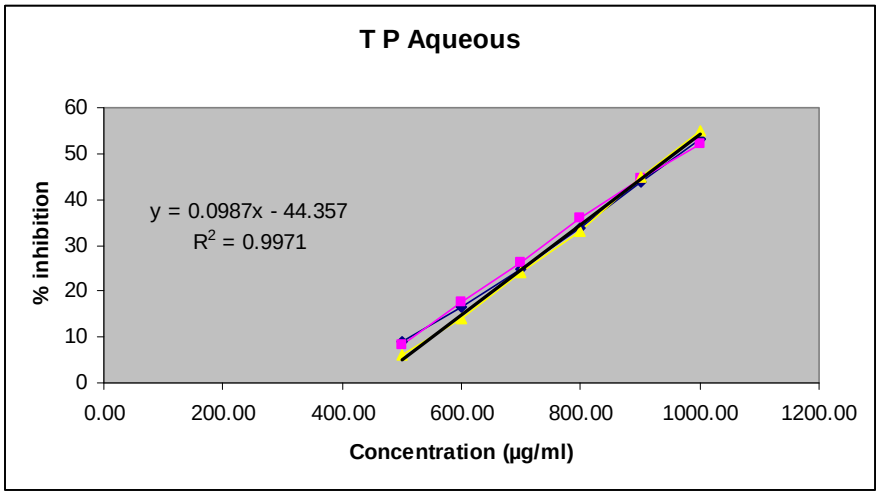
R^2 values represented mean data set of n=3

Figure 5: Results of Nitric oxide radical scavenging assay



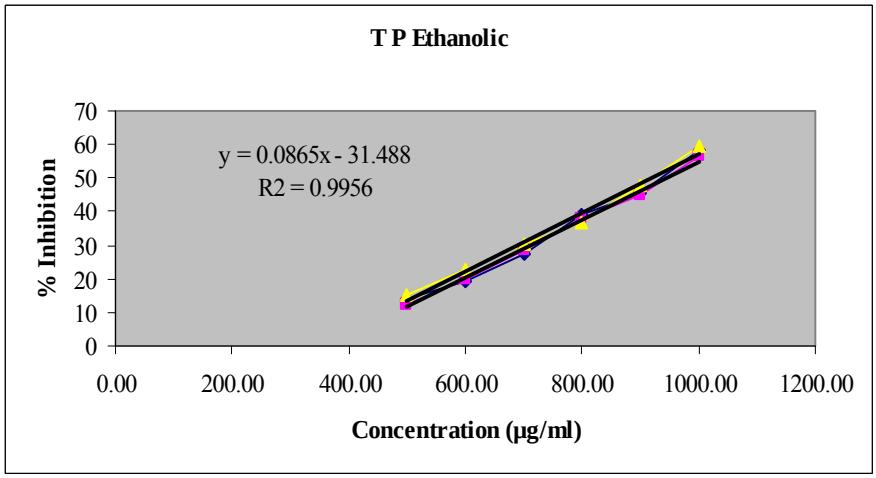
R^2 values represented mean data set of n=3

Figure 6: Results of Nitric oxide radical scavenging assay



R^2 values represented mean data set of n=3

Figure7: Results of Nitric oxide radical scavenging assay

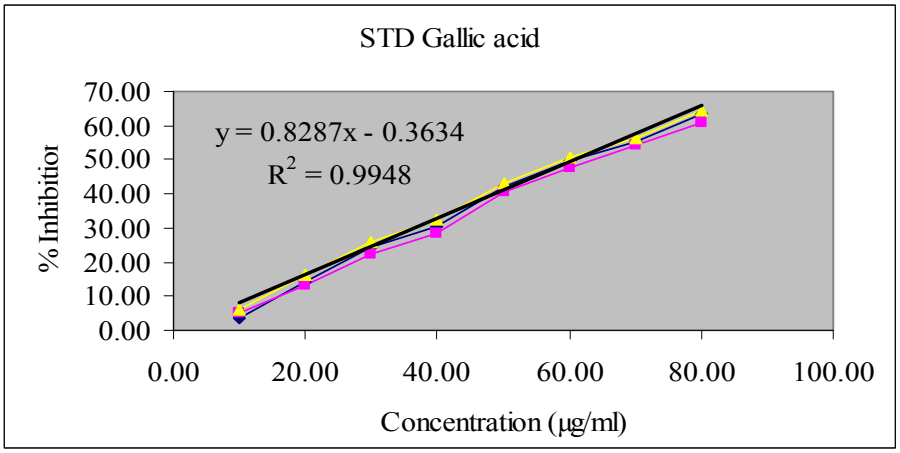


R^2 values represented mean data set of n=3

Hydrogen Peroxide scavenging Assay[19]

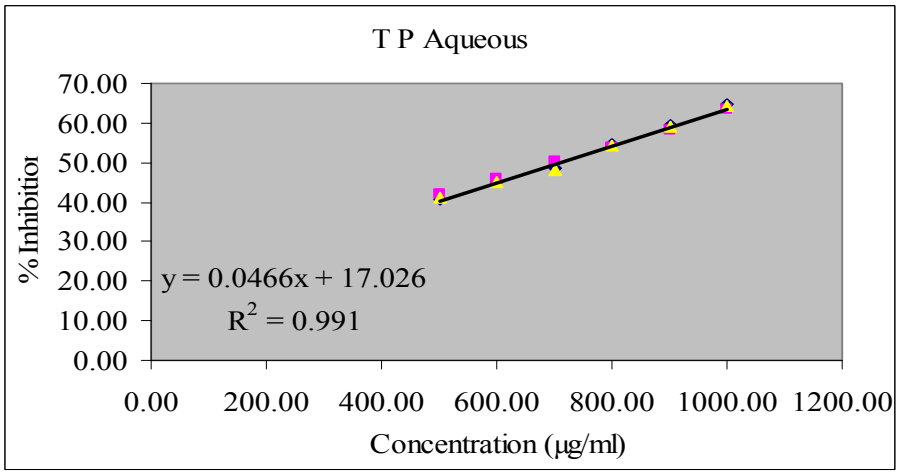
The ability of extracts to scavenge hydrogen peroxide was determined by little modification here the solution of hydrogen peroxide (100mM) was prepared instead of 40mM in phosphate buffer saline of (PH 7.4), at various concentration of aqueous and ethanolic extract (100 -1000 µg/ml) were added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for back ground subtraction. In case of control takes absorbance of hydrogen peroxide at 230 nm without sample extracts. Results are provided in (Figure 8-10).The percentage inhibition activity was calculated from $[(A_0-A_1)/A_0]$ x 100, where A_0 is the absorbance of the control and A_1 is the absorbance of extract/standard taken as Gallic acid (10 -100 µg/ml).

Figure 8: Results of Hydrogen Peroxide scavenging Assay



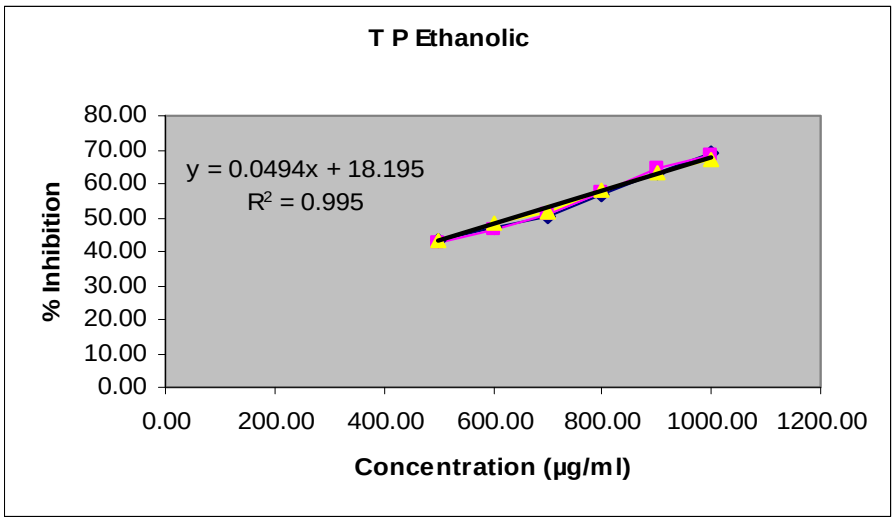
R^2 values represented mean data set of n=3

Figure 9: Results of Hydrogen Peroxide scavenging Assay



R^2 values represented mean data set of n=3

Figure 10: Results of Hydrogen Peroxide scavenging Assay

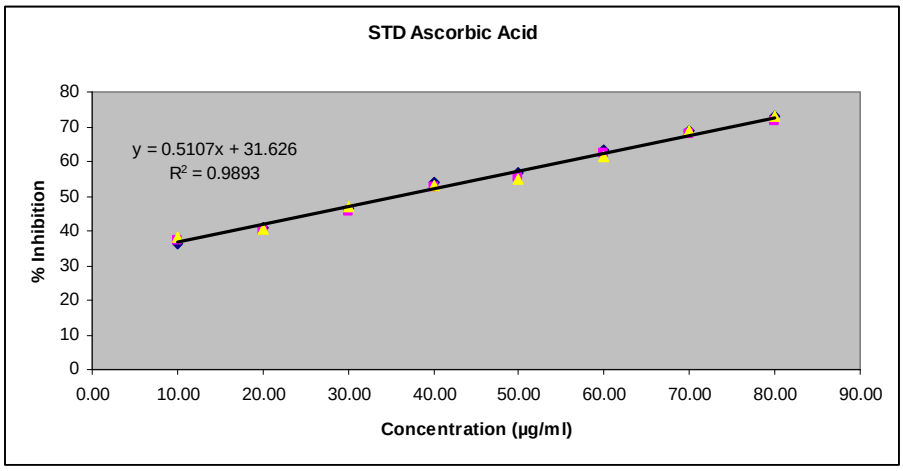


R^2 values represented mean data set of n=3

DPPH –RSA method

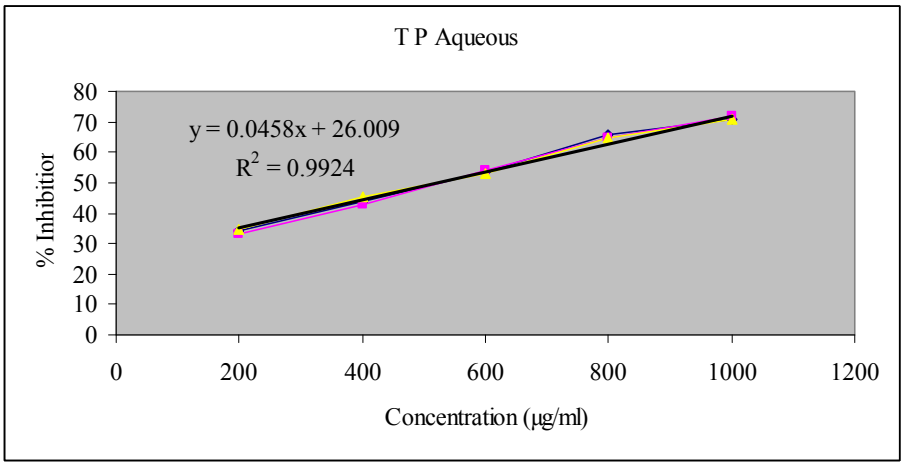
The free radical scavenging activity of aqueous and ethanolic extracts and the standard L-Ascorbic Acid (Vitamin C) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH[20, 21]. Here,0.1mM solution of DPPH in alcohol was prepared and it must be protected from light influence by maintaining the dark condition and also fold by aluminum foil and 3ml of this solution was added to 1ml various conc.(100-2000 µg/ml) of extracts or standard solution of (10-100 µg/ml). Absorbance was taken after 30min at 517nm. Results are provided in (Figure11-13).The percentage inhibition activity was calculated from $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of extract/standard taken as Ascorbic acid.

Figure 11: Results of DPPH radical scavenging assay



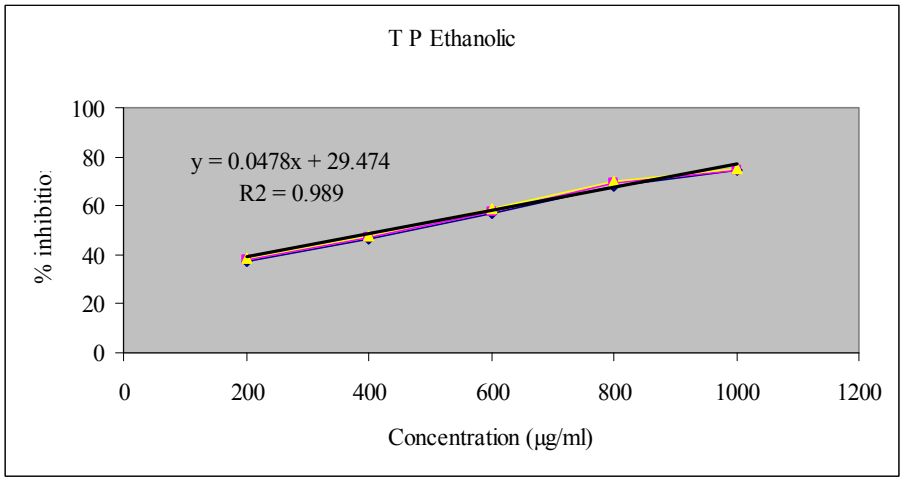
R^2 values represented mean data set of n=3

Figure 12: Results of DPPH radical scavenging assay



R^2 values represented mean data set of n=3

Figure 13: Results of DPPH radical scavenging assay



R^2 values represented mean data set of n=3

STATISTICAL ANALYSIS

Values were represented as mean ±S.E.M of three parallel data's.

RESULTS AND DISCUSSION:

Effect of TPC & Flavonoid content

The quantitative determination of the total phenolic content, expressed as mg tannic acid equivalents and per 100 g dry weight of sample TPC of L.S aqueous and ethanolic extracts showed the content values of 9.44± 0.22%w/w and 18.44± 1.23%w/w and total flavonoid content of the extracts was expressed as percentage of Quercetin & Rutin equivalent per 100 g dry weight of sample the total flavonoids estimation of aqueous and ethanolic extracts showed the content values of 0.91 ± 0.08%w/w and 1.56 ± 0.32%w/w for quercetin and 1.85 ± 0.28%w/w and 2.54 ± 0.43%w/w for rutin respectively. The above results showed that aqueous contain less tannins and flavonoid content than the alcoholic extract. It may due to the solubility of principle contents presence be higher incase of alcoholic solvent, thus it has been accepted that it is a universal solvent for the extraction of plant constituents.

Capacity of FRAP method

At low pH, measuring the change in absorption at 593 nm can monitor reduction of a ferric complex to the ferrous form, which has an intense bluish green color. The change in absorbance is directly related to the combined or "total" reducing power of the electron-donating antioxidants present in the reaction mixture. Here the FRAP showed the results of aqueous and ethanolic extracts that of 64.94 ± 0.28 mg equivalent to Gallic acid(GAE)/g of sample and 76.56 ± 2.54 mg GAE/g of sample respectively.

Capacity of Nitric oxide scavenging assay

Nitric oxide is a very unstable species under the aerobic condition. It reacts with O_2 to produce the stable product nitrates and nitrite through intermediates through NO_2, N_2O_4 and N_3O_4. It is estimated by using the Griess reagent. In the presence of test compound, which is a scavenger, the amount of nitrous acid will decrease. The extent of decrease will reflect the extent of scavenging, the %inhibition of aqueous and ethanolic extract of three parallel readings of (r^2=0.9971) showed that IC_{50} values 956.00µg/ml and 942.06µg/ml (r^2=0.9956) respectively as compared to the standard of Ascorbic acid and Quercetin of 36.16µg/ml (r^2=0.9951) and 56.27µg/ml (r^2=0.9936) respectively.

Capacity of Hydrogen Peroxide scavenging

H_2O_2 itself is not very reactive, but it can sometimes be toxic to the cell because it may give rise to hydroxyl radical in the cells. Thus, removal of H_2O_2 is very important for protection of food systems. Scavenging of Hydrogen per oxide and its %inhibition of aqueous and ethanolic extract showed that IC_{50} values 707.60µg/ml (r^2=0.991) and 834.21µg/ml (r^2=0.995) respectively. Gallic acid has taken as reference which showed 62.33µg/ml. (r^2=0.9948)

Capacity of DPPH –RSA

The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1- Diphenyl-2- Picryl Hydrazine. The ability to scavenge the free radical, DPPH was measured at an absorbance of 517 nm. So the DPPH – RSA and its %inhibition of aqueous and ethanolic extract showed that IC_{50} values 523.82 µg/ml (r^2=0.9924) and 429.41µg/ml (r^2=0.989) respectively. Ascorbic acid has taken as reference which showed 35.98 µg/ml. (r^2=0.9893) among these results ethanolic extract has more potent than traditionally claiming decoction.

The overall results of % inhibition as shown in the (Table 5) respective to IC_{50} values and regression r^2 is the mean value of (n=3).

Table 5: Results and discussion of all the % Inhibition studies

Results	Samples	Equation	* r^2 values	I_C 50 values
% Inhibition by Nitric oxide assay	Standard Ascorbic acid	y = 0.8374x +19.723	0.9951	43..71µg/ml
	Standard Quercitin	y = 0.6574x +13.01	0.9936	28.73µg/ml
	L.S Aqueous	y = 0.0987x - 44.357	0.9971	938.92µg/ml
	L.S Ethanolic		0.9956	805.85µg/ml
% Inhibition by Hydrogen Peroxide assay	Standard Gallic acid	y = 0.8287x - 0.3634	0.9948	62.28µg/ml
	L.S Aqueous	y = 0.0466x +17.026	0.9932	653.28µg/ml
	L.S Ethanolic	y = 0.5107x +31.626	0.991	341.55µg/ml
% Inhibition by DPPH-RSA	Standard Ascorbic acid	y = 0.0494x +18.195	0.995	58.96µg/ml
	L.S Aqueous	y = 0.0458x +26.009	0.9924	831.36µg/ml
	L.S Ethanolic	y = 0.0478x +29.474	0.989	561.18µg/ml

*Data set of n=3 and mean r^2 values obtained from the graphs.

CONCLUSION:

In conclusion that *Tephrosia purpurea* Linn. (Leguminosae) leaves possesses the antioxidant substance which may be potential responsible for the treatment of jaundice. So there are many scopes are there in leaves portion and more number of studies can be undertaken like oxidative stress hepatoprotective,

anticancer activities and etc. In future we look forward to check the potency of the leaves by means of In-vivo antioxidant studies.

REFERENCES

[1] Khatri A, Garg A, Agrawal SS. Evaluation of Hepatoprotective activity of areal parts of *Tephrosia purpurea* and Stem bark of *Tecomella undulate.* Journal of Ethnopharmacology. Elsevier publication, Ireland. 2009, 122, 1-5.
[2] Chopra RN, Nayer SL, Chopra IC. Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research. New Delhi, India. 1956.
[3] Despande SS, Shah GB, Parmar NS. Indian Journal of Pharmacology, 2003, 35:168–172.
[4] Sankaran JR. The Antiseptic, 1980, 77:643-646.
[5] Kirtikar KR, Basu BD. Indian Medicinal Plants. Lalit Mohan Basu. Allahabad, India, 1956.
[6] The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Product. Publication and Information Directorate, CSIR. New Delhi, India: 1976.
[7] Rahman H, Kashifudduja M, Syed M.et al. Indian Journal of Medical Research, 1985, 81:418-422.
[8] Gokhale AB, Saraf MN. Indian Drugs 2000, 37 :553-557.
[9] Pelter A., Ward RS, Rao EV, et al. Journal of Chemical Society. Perkin Trans, 1981, 1: 2491-2495.
[10] Hyoung Lee S. Antioxidant activity of browing reaction product isolated from storage-aged orange juice. *J Agric Food Chem.*1992, 40 (4):550-552.
[11] Garrat DC. *The quantitative analysis of drugs,* Chapman and Hall Ltd., Japan. 1983, 2.
[12] The *Ayurvedic Pharmacopoeia of India* 1st edition, 1990, 233-240.
[13] Cetkovic G, Canadanovic J, et al., Assessment of Polyphenolic content and in vitro antiradical characteristics of apple pomace, *Food Chemistry*. 2008, 109:340-347.
[14] Jain U.K. and Dixit V.K spectrophotometrical estimation of tannins from the Chyavanprash, *Journal of Indian drugs.*2004, 41:469-472.
[15] S.Kumar et al., Antioxidant free radical scavenging potential of Citrullus colocynthis (L.) Schrad. Methanolic fruit extract, *Acta pharma*. 2008, 58:215-220.
[16] Y.Y. Lim, J. Murtijaya/*LWT*. 2007, **40**:1664-1669.
[17] Chakraborthy G.S.,Free radical scavenging activity of Costus speciosus leaves, *Indian Journal of Pharmaceutical Education and Research*. 2009, 43(1):96-98.
[18] Hyoung Lee S., Antioxidant activity of browing reaction products isolated from storage aged orange juice, *J agric Food Chem.*1992, 40(4): 550-552.
[19] I.Gülçin, H. A. Alici and M. Cesur., Determination of in vitro antioxidant radical scavenging activities of propofol *Chem, Pharm. Bull*. 2005, 53:281–285.
[20] Sreejayan and M. N. A. Rao, Free radical scavenging activity of curcuminoids. *Drug Res* .1996, 46:169–171.
[21] Mohammad T.A., Determination of Polyphenolic content and free radical scavenging activity of Flemingia strobilifera, *Advances in Pharmacology and Toxicology.* 2009, 10 (1):89-95.