COMPARATIVE ANTIOXIDANT STUDIES OF ETHANOL EXTRACT AND FRESH AQUEOUS EXTRACTS OF TEPHROSIA PURPUREA

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ABSTRACT
Tephrosia purpurea (Linn.) Pers., a popular Indian medicinal plant, has long been used commonly in Ayurvedic and unani system of medicine. In the present review, an attempt has been made to explore a literature survey on its traditional uses, established phytochemical studies and pharmacological properties. The whole plant as well as specific parts such as roots, leaves, seeds and bark have been widely used and shows the different pharmacological activities such as antimicrobial, anti-inflammatory, hepatoprotective, anti-ulcer, anti-epileptic, antibiotic, anticarcinogenic, antilipidperoxidative, wound healing, antidiabetic, antioxidant etc.

Keywords: Tephrosia purpurea, Traditional uses, Phytoconstituents, Pharmacological activities.

INTRODUCTION
Tephrosia purpurea Linn. (Leguminosae) (Figure No : A) commonly known as Sarpankh called as Thila in Gujarati, Sarponkha (Hindi), Vempali (Telugu). Leaves of Tephrosia purpurea are taken as emetic in the form of leaf juice or decoction. This by adding sugar also used in Jaundice. Tephrosia purpurea commonly known in Sanskrit as Sharapunkha is a highly branched, sub-erect, herbaceous perennial herb[1]. 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[2]. It is an important component of some preparations such as Tephroli and Yakrifit used for liver disorders[3]. 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[4]. The dried herb is effective as tonic laxative and diuretics. 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 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. The ethanolic extract of seeds has shown significant in vivo hypoglycaemic activity in diabetic rabbits\textsuperscript{[5]}. The ethanolic extracts of Tephrosia purpurea possessed potential antibacterial activity. The flavonoids were found to have antimicrobial activity \[6\]. The phytochemical investigations on Tephrosia purpurea have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, and sterols\textsuperscript{[7]}. It is also good source of minerals and amino acids. HPLC analysis of extract of flowering plant of Tephrosia purpurea shows presence of rutin and quercetin\textsuperscript{[8]}

We decide to study by comparison of In-vitro antioxidant studies which we carried out on whole plant of Tephrosia purpurea, in both aqueous extract of fresh and ethanolic extract of dried plant. To established that in which form of plant is most suitable of best use.

**MATERIAL AND METHODS**

**Chemicals and reagents**

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

**Plant material**

Whole Plant of Tephrosia purpurea (Linn.) (Leguminosae) was collected with flowering top during the month of August from Salem district, Tamilnadu, India. The plant material was authenticated at the Department of Botany, Karpagam University, Coimbatore and Tamilnadu. A voucher specimen as a herbarium (037/09KU/2009) has been kept in museum for future reference. The plants were chopped and dried at room temperature for 10 days and used as raw material. The dried plants were powdered using mechanical method and resulting powder was passed through the 40 # sieve and stored in the airtight container.
Preparation of crude aqueous extract

Fresh plants were chopped in small pieces and then weighed accurately 250 gm. transferred in a stainless steel vessel and mixed with 2 litre of distilled water. Then the mixture was boiled for about 2 hours, then mixture was filtered by using vacuum filter assembly. Then the filtrate was evaporated on hot plate until it reaches the concentrated quantity.

Preparation of ethanolic extracts

Then weighed accurately 250 g of dried powder of drug was packed in thimble flask and 750ml of ethanol (70%) was added in 1 litre 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 using water bath. The obtained extract was kept in a desiccator for 3 days, to carry out the antioxidant studies.

Preliminary phytochemical screening

The aqueous and alcoholic extract were taken for various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids[9], glycosides[10], carbohydrates[11], phenolic and tannins[12], phytosterols[13] fixed oils, protein and amino acids, flavanoids, saponins, gums and mucilage[14] using reported method. The aqueous extract shows the presence of glycosides[10], carbohydrates[11] phenols, flavanoids, saponins, gums and mucilage[14].

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 tannin and tannin like compound reduce Phosphotungustomolybdic acid in alkaline solution to produce a highly blue colored solution[15,16,17] 1ml of the aqueous and ethanolic extract that has adjusted to come under the linearity range i.e. (50 μg/ml) of both the extract 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 distilled 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 30 g/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.

**Total Flavonoid Content by Spectrophotometer**

**Aluminum chloride colorimetric assay method**

Total flavonoid contents were measured with the aluminum chloride colorimetric assay[18]. Aqueous and ethanolic extracts that has been adjusted to come under the linearity range i.e. (400 μg/ml) and different dilution of standard solution of Quercetin (10-100 g/ml) were added to 10ml volumetric flask containing 4ml of water. To the above mixture, 0.3ml of 5% NaNO₂ was added. After 5 minutes, 0.3ml of 10% AlCl₃ was added. After 6 min, 2ml of 1 M NaOH was added and the total volume was made up to 10ml with distilled water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. Results are tabled 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.

**In-Vitro Antioxidant Study**

**FRAP method[19]**

The ferric reducing property of the extract was determined by taking 1ml of different dilutions of standard solutions of Gallic acid (10 -100 g/ml) or aqueous and ethanolic extract that has adjusted to come under the linearity range (500 g/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 distilled water is added and then 0.5ml of 0.1% of FeCl₃ 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.

**Scavenging Activity Assays**

**Nitric oxide scavenging assay**
Nitric oxide radical inhibition was estimated by the use of Griess Illosvory reaction\cite{20,21} \cite{20,21}. 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 (50 -500 μg/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 (10 -50 μg/ml) was taken as standard. Results are represented in (Figure 4 and 5) .The percentage inhibition was calculated using the formula:

\[
\text{% Scavenging Activity} = \left(\frac{A_{\text{control}} - A_{\text{test or std}}}{A_{\text{control}}}\right) \times 100
\]

Where, \(A_{\text{control}}\) = absorbance of control

\(A_{\text{test or std}}\) = absorbance of test or std

**Hydrogen Peroxide scavenging Assay\cite{22}**

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 (50 -500 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 6 and 7). The percentage inhibition activity was calculated
from \[\frac{(A0-A1)}{A0} \times 100\], where A0 is the absorbance of the control and A1 is the absorbance of extract/standard taken as Gallic acid (10 -100 g/ml).

**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\(^{[23,24]}\). 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 g/ml) of extracts or standard solution of (10-100 g/ml). Absorbance was taken after 30min at 517nm. Results are provided in (Figure 8 and 9). The percentage inhibition activity was calculated from \[\frac{(A0-A1)}{A0} \times 100\], where A0 is the absorbance of the control and A1 is the absorbance of extract/standard taken as Ascorbic acid.

**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 18.13±0.21%w/w and 15.40±0.31%w/w and total flavonoid content of the extracts was expressed as percentage of Quercetin equivalent per 100 g dry weight of sample the total flavonoids estimation of aqueous and ethanolic extracts showed the content values of 1.21±0.21%w/w and 0.92±0.31%w/w. The above results showed that aqueous contain more tannins and flavonoid content than the alcoholic extract. It may due to the solubility of principle contents presence be higher in case of aqueous solvent because of higher polarity in comparison with alcohol.

**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 56.87±0.43mg equivalent to Gallic acid(GAE)/g of sample and 51.21±0.26mg 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 O₂ to produce the stable product nitrates and nitrite through intermediates through NO₂, N₂O₄ and N₃O₄. 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 (r²=0.9933) and (r²=0.9964). Aqueous and ethanolic extract showed that I₅₀ values 238.00μg/ml and 252.06μg/ml respectively as compared to the standard of Ascorbic acid of 34.16μg/ml (r²=0.9999).

Capacity of Hydrogen Peroxide scavenging

H₂O₂ 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 H₂O₂ is very important for protection of food systems. Scavenging of Hydrogen peroxide and its %inhibition of aqueous and ethanolic extract showed that I₅₀ values 238.60μg/ml (r²=0.9997) and 244.21μg/ml (r²=0.9991) respectively. Gallic acid has taken as reference which showed 60.00μg/ml. (r²=0.9997)

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 I₅₀ values 213.24μg/ml (r²=0.9995) and 241.11μg/ml (r²=0.9993) respectively. Ascorbic acid has taken as reference which showed 51.12μg/ml. (r²=0.9998) among these results fresh aqueous extract has more potent than dried plants alcoholic extract.
Figure 1
Total Phenolic Content (Tannic acid)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration of extract</th>
<th>% w/w of total tannin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aqueous extract 50µg/ml</td>
<td>18.13±0.21</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol extract 50µg/ml</td>
<td>15.40±0.31</td>
</tr>
</tbody>
</table>

Values are mean ±S.E.M, n=3
TABLE 2:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration of extract</th>
<th>% w/w of total Flavonoid (Quercetin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aqueous extract 50µg/ml</td>
<td>1.21±0.21</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol extract 50µg/ml</td>
<td>0.92±0.31</td>
</tr>
</tbody>
</table>

Values are mean ±S.E.M, n=3
TABLE 3:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration of extract</th>
<th>Mg GAE/g of extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aqueous extract 500µg/ml</td>
<td>56.87±0.43</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol extract 500µg/ml</td>
<td>51.21±0.26</td>
</tr>
</tbody>
</table>

Values are ± S.E.M, n=3

Figure 4
Nitric oxide radical scavenging assay: Ascorbic acid STD

Figure 5
Nitric oxide radical scavenging assay
Series 1 shows Aqueous extract  
Series 2 shows Ethanolic extract
Figure 6
Hydrogen Peroxide Scavenging Assay: STD Gallic acid

Figure 7
Hydrogen Peroxide Scavenging Assay
Series 1 Aqueous extract
Series 2 ethanolic extract
CONCLUSION

It can be concluded that *Tephrosia purpurea* Linn. (Leguminosae) plant possesses the antioxidant substance which may be potential responsible for the treatment of various
diseases. But it is evident from the above study that fresh plant aqueous extracts are more potent antioxidant in comparison of ethanolic extract.

The fresh plant extract contain more antioxidant substance which are highly soluble in Water because of higher polarity. The total flavonoids content in fresh aqueous extract is more than other extract flavonoids are well known for their antioxidant properties. More over there may be some change in the chemical nature after drying so change in solubility of substance differ in ethanol and cannot be extracted out, same may be present in aqueous solvent.

Our further investigation is going on to isolate the responsible component in fresh aqueous extract due which the extract showing very good antioxidant properties.

ACKNOWLEDGEMENTS

Author is thankful to Dr.T.Tamiz mani, Principal Bharthi College of Pharmacy, Mandya for granting permission to use the lab and chemicals for above study.

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