IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
**Research Article**
February 2021 Vol.:20, Issue:3


# Qualitative and Quantitative Phytochemical Analysis of *Tephrosia villosa* per Herb

IJPPR
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ISSN 2349-7203
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**Submitted:** 10 January 2021
**Revised:** 30 January 2021
**Accepted:** 19 February 2021


**www.ijppr.humanjournals.com**

**Keywords:** *Tephrosia villosa*, phytochemicals hydro-alcoholic, Flavonoids, qualitative and quantitative

**ABSTRACT**

Present study was aimed to find out the presence of phytochemicals in 70% alcoholic extract of *Tephrosia villosa per* herb by both qualitative and quantitative screening methods, in qualitative analysis, the phytoconstituents like alkaloids, tannins flavonoids etc are identified and further total phenol, flavonoid and total tannin content were quantified, total phenolic content and total tannin content was assessed using Folin-Ciocalteu's method, estimation of total flavonoids was carried out by aluminium chloride colorimetric method, tannic acid and quercetin were used as a standard and absorbance was measured using spectrophotometer, these studies showed that hydroalcoholic extract have significant amount of phenolic, flavonoid and tannin content.

## INTRODUCTION:

The traditional medicinal plants are used throughout the world for the treatment of various diseases including diabetes mellitus, plant based drugs have been known to be safe, cheaper and play a major role to manage various diseases, Indian traditional health care system uses number of medicinal plants traditionally over 1000 years, India is the largest producer of medicinal herbs and is called as 'Botanical garden of the world'. Plants consists of many non-nutrient bioactive compounds referred as phytoconstituents which are synthesized by primary and secondary metabolic pathways reveals the medicinal values, as they are effective, non toxic and have less or no side effects (1), *Tephrosia villosa* (L.) Pers traditionally known as "Sharpunkha" of Genus *Tephrosia* Pers, which is a large seasonal pantropic genus of about 400 species belongs to family *Fabaceae*,( 2), *Tephrosia villosa* has a large distribution found in southern and Eastern Africa, the Arabian Peninsula and across southern Asia(3).

Traditionally *Tephrosia villosa* has been used in different parts of the world. Ethnobotanical studies revels that root powder and paste has been used for stomach ache, fever and in typhoid(4), used for various skin disorders in Karnataka(5), used for dental pain in Tamilnadu(6), used for the treatment of dropsy and enlargement of viscera(7). In Ethopia it is used for respiratory tract disorders(8), literature survey reveals that *Tephrosia villosa (per)* has been found to possess anti-microbial property(9), anti-diabetic (10), anthelmintic property (11), anti-oxidant (12), used as potential bio-insecticide (13), and green corrosion inhibitor (14). Considering the medicinal importance of this widely available plant, the present study was aimed to identify the qualitative and quantitative estimation of phytoconstituents present in alcoholic extract of the plant.

## MATERIALS AND METHODS

**Plant materials:** The *Tephrosia villosa* (L.) Pers herb was collected in the month of September to November from fields of Harapanahalli and authenticated by Professor K. Prabhu, Department of Pharmacognosy, S.C.S College of Pharmacy, Harapanahalli. The herbarium specimen is deposited in S C S College of pharmacy museum for future reference.

**Preparation of plant extract:** The cleaned, healthy plant materials are cut in to small sections and dried under shade for three to four weeks. The dried material was ground into fine powder. The *Tephrosia villosa* (L.) Pers herb extract was prepared by successive soxhlation i.e. extracting dried powder with the solvents of increasing order of polarity i.e.

Petroleum Ether (60-80°), chloroform (59.5-61.5°), 70% ethanoloic (64.5-65.5°), extracts were concentrated under reduced pressure and stored in airtight container in refrigerator below 10°C.

**Calculation of percentage yield:**

The percentage yield was calculated for the extracts with reference to the crude material taken using the formula given below. The percentage yield of each extract is tabulated,

$$\% \text{ yield of extract} = \frac{\text{Weight in grams of extracts obtained}}{\text{Weight in grams of plant material taken}} \times 100$$

**Preliminary phytochemical screening**;

The solution of extract was prepared using distilled water and subjected the obtained extract was subjected to preliminary phytochemical screening following the standard procedures described in the practical Pharmacognosy by C.K. Kokate(15) and R.K. Khandelwal(16),

**1. Detection of carbohydrates**

The extract are dissolved in 5mL distilled water and filtered. The filtrates are used to test for the presence of carbohydrates.

**Molisch's test**: One mL of filtrate solution is treated with 2 drops of alcoholic alpha_-naphthol solution in a test tube. 2mL of concentrated sulfuric acid is added on the side of the test tube. Formation of the violet coloured ring at the junction indicates the presence of carbohydrates.

**2. Detection of proteins and amino acids**

**Ninhydrin test**: To the extract, 0.25% w/v ninhydrin reagent is added and boiled for few minutes. Formation of blue-violet color indicates the presence of amino acids or protein.

**3. Detection of alkaloids**

The crude extract powder is dissolved in 2N Hydrochloric acid and filtered. The filtrate is divided into four portions to achieve the following tests.

**Dragendroff's test**: One filtrate portion is treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

**4. Detection of flavonoids**

**Alkaline reagent test**: Extract sample is treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

**Shinoda's test**: The alcoholic extract is treated with magnesium turning and concentrated HCl gives red colour which indicates the presence of flavonones. Orange-red color indicates the presence of flavonols.

**5. Detection of tannins**

**Gelatin test**: To the extract, 1% gelatin solution containing NaCl is added. Formation of white precipitate indicates the presence of tannins.

**6. Detection of diterpenes**

**Copper acetate test:** Extracts is dissolved in water and treated with a few drops of copper acetate solution, Formation emerald green color indicates the presence of diterpenes.

**7. Detection of steroids and triterpenoids**

**Libermann-Burchard test**: The extract sample is dissolved in 2mL of chloroform in a dry test tube. 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid are then added. If the solution becomes red, then blue and finally bluish green in color, it indicates the presence of steroidal nucleus while formation of purple or red color indicates the presence of triterpenoidal nucleus.

**8. Detection of saponins**

**Froth test**: Crude dry powder of extract is vigorously shaken with 2mL of distilled water and is allowed to stand for 10 min. If stable froth appears, it indicates the presence of saponins.

**9. Detection of cardiac glycoside**

**Keller-Kiliani's** test: A portion of dry extract is treated with 1mL of $FeCl_3$ reagent (1 volume of 5% $FeCl_3$ and 99 volume of glacial acetic acid). To this solution a few drops of

concentrated $H_2SO_4$ is added. The presence of greenish blue color within few minutes indicates the presence of deoxy sugar of cardiac glycosides.

## QUANTITATIVE ANALYSIS

Depending on the above qualitative results 70% hydroalcoholic extract was selected for further quantitative assay.

### Estimation of Total Phenolic Content (17)

The total phenolic content of *Tephrosia villosa* (L.) Pers herb was estimated by Folin-Ciocaltaeu assay method described by Kavitha chandran et al, 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin Ciocalteu's phenol reagent. After 5 minutes,10 ml of 7% sodium carbonate solution was added to the mixture followed by the addition of 13ml of deionized distilled water and mixed thoroughly, volume was made upto 25ml and the mixture was kept in the dark for 90 minutes at 23°C, after which the absorbance was read at 760nm. The blue colouration in the tube is due to the formation of molybdenum blue as a result of complex redox reaction between phenols and phosphomolibdic acid in Folin ciocalteau reagent in alkaline medium. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. TPC was expressed as milligrams of Gallic acid equivalents (GAE)/g of dried sample.

### Estimation of Total Tannin Content (17)

The total tannin content of *Tephrosia villosa* (L.) Pers herb was determined by Folin-Ciocalteu (Folin dellin 1% $K_3Fe(CN)_6$ and 1ml 1%$FeCl_3$) method explained by Kavitha chandran et al, (2016)About 1 ml of the sample extract was added to a volumetric flask (10ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. a set of reference standard solutions of tannic acid (100, 200, 300, 400, 500ug/ ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/ Visible spectrophotometer. The tannin content was expressed in terms of mg of tannic acid (Gallic acid) equivalents/ g of dried sample.

**Estimation of Total Flavonoid Content (17)**

The total flavanoids content of *Tephrosia villosa* (L.) Pers herb extract was estimated by aluminum chloride colorimetric assay method described by Kavitha Chandran *et al*. Based on this method, each sample (1.0 ml) was mixed with 4 ml of distilled water and subsequently with 0.30ml of $NaNO_2$ solution (10%). After 5 min, 0.30ml $AlCl_3$ solution (10%) was added followed by 2.0ml of NaOH solution (1%) to the mixture. Immediately the mixture was thoroughly mixed and absorbance was then determined at 510 nm versus blank and results were expressed as quercetin equivalents (mg quercetin/ g) dried extract.

**Preparation of standard solution**

10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with Methanol to get 100mcg/ml Quercetin standard solution (stock solution). From the stock solution, concentration of 100, 200, 300, 400, 500, mcg/ml were prepared. To each of these 4ml water was added followed by 0.3ml of 5% sodium nitrite. After 5min, 0.3ml of 10% Aluminium chloride solution and at the 6th minute 2ml of 1M Sodium hydroxide was added. The total volume was made up to 10ml with distilled water, a blank was prepared without addition of aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 510nm using UV-Visible spectrophotometer, a standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.

**RESULTS**

**Preliminary phytochemical screening**

Percentage yield of crude extract of *Tephrosia villosa* herb are shown in table 1 and Results of the preliminary phytochemical investigation of *Tephrosia villosa* herb in table no. 2.

**Table no 1. Percentage yield of crude extract of *Tephrosia villosa* herb**

| Sl. No. | Solvent | Colour and Consistency | Percentage yield |
|---|---|---|---|
| 1 | Pet. Ether | Greenish black sticky | 1.92% |
| 2 | Chloroform | Brownish black and sticky | 3.95% |
| 3 | 70% Ethanol | Brownish black and nonsticky | 18.31% |

Preliminary phytochemical screening of ***Tephrosia villosa per*** **herb**.

It is observed from the preliminary phytochemical screening of *Tephrosia villosa per* herb Alkaloids, Glycosides, Flavonoid, Tanin, Saponin, Protein and Carbohydrate are present in 70% Ethanolic extract and except Alkaloids, Glycosides and steroids all the phytoconstituents are found absent in petroleum ether and chloroform extract,it was qualitatively observed that 70% ethanolic extract contain higher concentration of polyphenolic components and previous literature revels the same hence 70% ethanolic extract selected for further studies.

**Table no 2.** Phytochemical constituent of *Tephrosia villosa per* herb in different extract.

| Types of Phytochemical constituents | Petroleum ether Extract | Chloroform Extract | 70/ Alcoholic Extract |
|---|---|---|---|
| Alkaloids | + | + | +++ |
| Carbohydrates | + | _ | +++ |
| Flavonoids | _ | _ | +++ |
| Glycosides | + | + | + |
| Tannins and Poly phenol | _ | _ | +++ |
| Protein | + | - | + |
| Steroids | + | - | ++ |
| Saponin | - | _ | + |

- Absent ++ More clarity

\+ Indicates presence +++ Better response

**III. Quantitative determination of total polyphenolic, flavonoid and tannin content:**

The total phenolic content of 70% Ethanolic extract of ***Tephrosia villosa per*** **herb** was 1.08 mg/G expressed as equivalent to catechol. Similarly, flavonoid content was found to be 3.21 mg/G expressed as equivalent to quercetin and total tannin content found to be 5.10 mg/G expressed as equivalent to tannic acid.

**Table no. 3: Quantitative determination of secondary metabolites in 70% EETVH**

| Sl.No. | Name of the secondary metabolites. | Absorbance At | In ethanolic extract (mg/g) |
|---|---|---|---|
| 1 | Total Phenol | 650nm | 1.08 mg/g Catechol |
| 2 | Flavonoid | 510nm | 3.21 mg/g Quercetin |
| 3 | Tannin | 700nm | 5.10 mg/g Tannic acid |

From the obtained data it is concluded that 70% EETVH shows diversified phytoconstituents and quantification has proved higher content of phytochemicals.

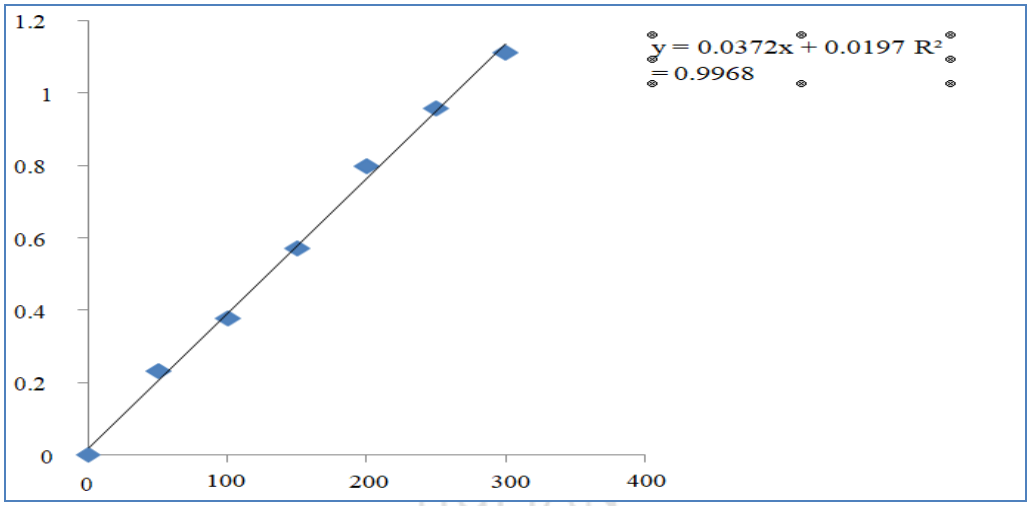


**Figure No.1: Standard graph of catechol**

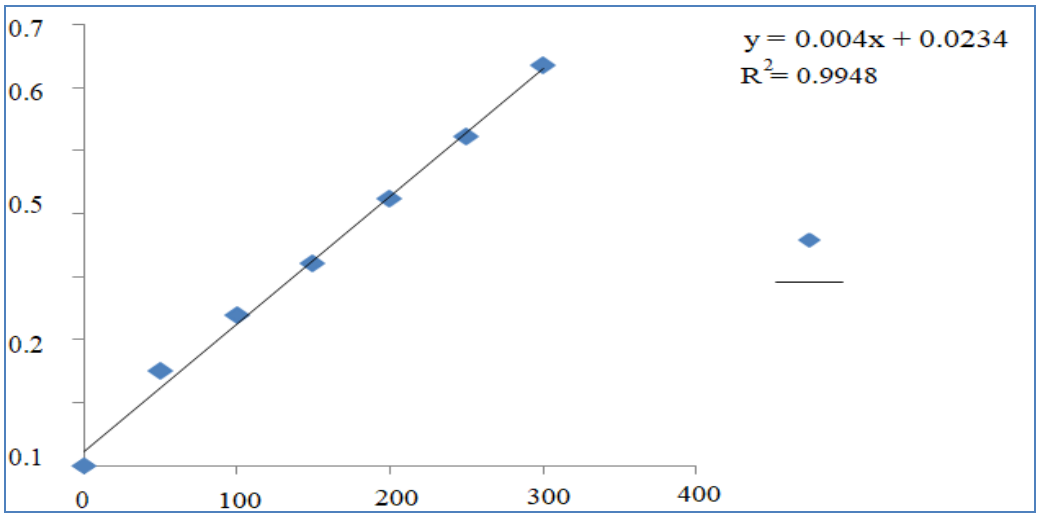


**Figure No.2: Standard graph of Quercetin.**

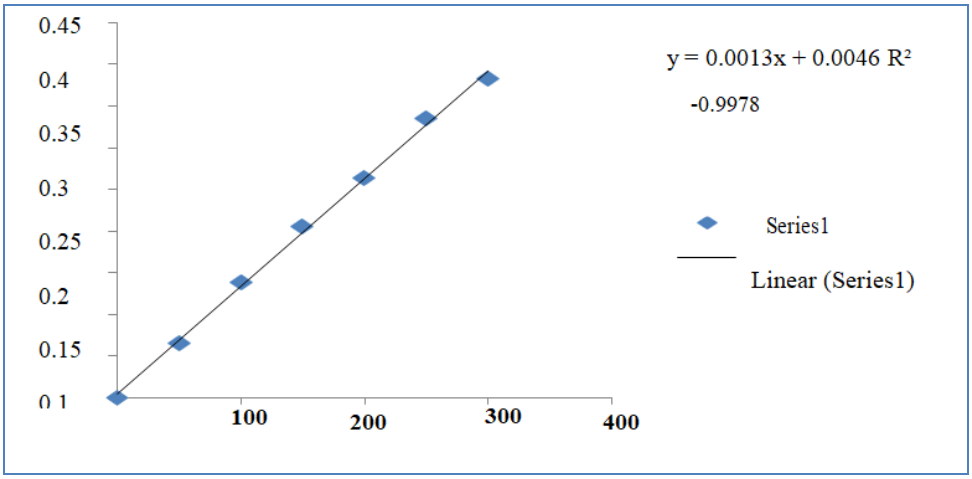


**Figure No.3: Standard graph of Tannic acid**

## CONCLUSION:

It was qualitatively observed that 70% ethanolic extract contain higher concentration of polyphenol, flavonoid, alkaloid, protein and tannin components and the total phenolic content of 70% EETVH was 1.08 mg/G expressed as equivalent to catechol. Similarly, flavonoid content was found to be 3.21 mg/G expressed as equivalent to quercetin and total tannin content found to be 5.10 mg/G expressed as equivalent to tannic acid. from the obtained data it is concluded that 70% EETVH shows diversified phytoconstituents and quantification has proved higher content of phytochemicals, and previous literature revels the same hence 70% ethanolic extract may be selected for further study and Phytochemical studies to isolate components are also required to be done to locate the molecules responsible for pharmacological activity, further spectral characterization of the isolated compounds can yield promising drugs of future use.

## Acknowledgement

I thank Dean, College of pharmacy SSSUTMS Sehore for the guidance and valuable suggestion and management SCS College of pharmacy Harapanahalli for their support to carry out this research work.

## Conflict of interest

There is no conflict of interest associated with this work.

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