INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

QUANTITATIVE AND QUALITATIVE CHARACTERIZATION OF PHYTOCONSTITUENTS FROM LEAVES OF *TEPHROSIA VILLOSA* PERS.

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Abstract: **Objective**: Medicinal plants are considered as a factory for the synthesis of many primary and secondary metabolites. These primary and secondary metabolites are composed of various simple and complex chemical substances. Secondary metabolites play very important role for the treatment of various disorders. The main objective of present study was to find out the active principles from the leaves of *Tephrosia villosa* Pers. **Methods**: Different extracts were subjected for preliminary phytochemical screening for the identification of active functional groups**. Results:** It showed presence of many active phytoconstituents like carbohydrates, phytosterols, saponins, glycoside, phenolic compound and flavonoids. Further powdered drug was used for the detection of inorganic elements from the ash of the powdered drug and it showed presence of many inorganic elements like iron, chloride and nitrates. For quantitative estimations total phenolic content, total flavonoid content and total triterpenoids content was determined. **Conclusion:** Purification of identified active phytoconstituents, TLC was performed by using two different standard samples i.e. beta sitosterol and lupeol. It showed significant result which is given in the figure.

Keywords: *Tephrosia villosa*, Leaves, Qualitative estimation, Quantitative estimation

PAPER-QR CODE

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Access Online On:

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How to Cite This Article:

Sufiyan Ahmad, IJPRBS, 2016; Volume 5(4): 247-256

INTRODUCTION

In many of the developing countries the use of plants drugs is increasing because modern life – saving drugs are beyond the reach of three quarters of the third world population, although many such countries spend 40 – 50% of their total health budget on drugs because of natural origin and lesser side effect or dissatisfaction with result of synthetic drugs [1]. Standardization of plant based medicine is a difficult task, because plants synthesizes not only single compounds but it may vary even up to hundreds of compounds may be present in plant. Hence it is difficult to standardize herbal medicines as compared to other medicines. Correct identification and quality assurance of the starting material is therefore an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy [2, 3].

The quality and quantity of safety and efficacy information on traditional medicines are not sufficient to meet the criteria to support its use worldwide. The reason behind lack of research data are not only due to health policies but also due to lack of methodologies for the evaluation of herbal medicines. The plants possess many active therapeutically active chemical constituents associated with many inert substances such as cellulose, lignin and coloring agents etc. The active constituents are extracted from plants and purified for their pharmacological utility. So the quality control of herbal drugs is important for their active chemical constituents in modern system of medicine. To meet new thrust of inquisitiveness, standardization of herbal drug is mandatory [4-8]. *Tephrosia villosa* Pers. has many medicinally active compounds in it hence, present study deals with the qualitative and quantitative analysis of the leaves part of the plant.

MATERIALS AND METHODS

Plant material: The plant specimens for the proposed study were collected from the Salem Dist. (T.N.) and authenticated by Dr. Marimuthu, Arts, science college Attur, Tamil Nadu (India). After drying 2kg leaves and stems of *Tephrosia villosa* milled and pass from sieve # 40. The milled powder material kept in air tight container up to further use. The collected, cleaned and powdered leaves of *Tephrosia villosa* Pers. was used for the extraction purpose. 200gm of powdered material was evenly packed in the soxhlet apparatus. It was then extracted successively with various solvents of increasing polarity [9-12].

Qualitative Estimations

Preliminary phytochemical screening of extracts [13-14]. The above extracts obtained from the leaves were subjected for the various chemical test for the identification of active phytoconstituents groups by following standard procedure. Elemental analysis of ash for

detection of inorganic elements [15]. The powdered drug was incinerated in muffle furnace to obtain ash. The ash was treated with 50% hydrochloric acid for 30 minutes and filtered. The filtrate was used for the detection of elements (calcium, iron, magnesium, potassium, sulphate, phosphate, chloride, carbonate and nitrate) by specific test.

Thin layer chromatography

For thin layer chromatography analysis, the method used was taken from quality standards of Indian medicinal plants ICMR (volume 4, 2006) for β-sitosterol. Improvements were made to the sample preparation and the mobile phase used in the method.

Sample preparation

0.2g of *Tephrosia villosa* leaves methanolic extract was diluted with 10ml methanol. Steroids are non-polar in nature so the methanol extract of *Tephrosia villosa* leaves was then partitioned with petroleum ether (PE) so that the steroids separate in the petroleum ether layer and all the other polar components remain in the methanol layer. The petroleum ether layer was further used for TLC.

Standard preparation

1. Dissolve 10 mg of β-sitosterol (available from Total Herb solution) in 10 ml of methanol.

2. Dissolve 10 mg of lupeol (available from Total Herb solution) in 10 ml of methanol.

Reagent preparation

Prepare anisaldehyde-sulfuric acid reagent by slowly adding 9 ml of 98% H_2SO_4 to an ice cooled mixture of 85 ml of methanol and 10 ml of glacial acetic acid. To this solution add 0.5 ml of anisaldehyde and mix well. The anisaldehyde-sulfuric acid reagent is colorless and should be stored in a refrigerator. If a color develops, the reagent must be discarded

Chromatographic conditions

Silica gel 60F$_{254}$ pre-coated TLC plate (Merck)

Toluene: Ethyl Acetate (80: 20)

Spray plate with the anisaldehyde-sulfuric acid reagent, heat to 105ºC for 15 minutes. Inspect plate in visible light.

QUANTITATIVE ESTIMATIONS

Dtermination of total phenolic content

To determine total phenolic content from the methanolic extract of leaves of *Tephrosia villosa*, caliberation curve of standard gallic acid of 20, 40, 60, 80 and 100 mg/ml was prepared in water and img/ml of methnolic extract of leaves of *Tephrosia villosa*, was prepared simultaneously. Each sample was mixed with 0.25 ml of Folin ciocalteu reagent and 1.25 ml sodium carbonate solution. The mixtures were allowed to react for 40 minutes at room temperature. After the reaction period the blue color ease measured at 725 nm on UV visible spectrophotometer of LABINDIA 3000+ and calculated the amount of total phenolic content from calibration curve as gallic acid.

Determination of total flavonoid content

An aliquot (l ml) of standard solution of quercetin (20, 40, 60, 80,100 μg/ml) was added to 10 ml volumetric flask containing 4 ml of 5% $NaNO_2$ into it. After 5 minute 0.3 ml of 10% $AlCl_3$ was added. Then 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. Same dilutions were also prepared for the test solution. Blank determination was done by using methanol in place of test or standard solutions.

Mixed well and taken the absorbance at 358 nm against blank. From the obtained standard curve of quercetin the total flavonoids content of methanolic extract of leaves of *Tephrosia villosa*, was determined.

Determination of total terpenoids

5 g of powder extracted with 50 ml distilled water by heating on water bath for 30 min. then the extract was allow to cool and then filter. 75 ml chloroform and diethyl ether was added in 1:2 concentrations by continuous stirring for 30 min. after 5 gm of sodium carboxyl methyl cellulose was added to forms lumps and sticky mass and then separated. Further marc subjected to extraction with 75 ml chloroform: diethyl ether (1:2) for four times. The obtained residue was dissolved in 50 ml of neutral absolute alcohol. Then the mixture were titrated with 0.1 N NaOH using phenolphthalein as an indicator. Similarly blank readings were taken without addition of sample. Percentage of triterpenoids content was calculated as per the given factor [16-17].

Factor for the calculation: each ml of 0.1N NaOH = 48.8 mg of triterpenoids

RESULTS AND DISCUSSION

Extraction of plant material: The powdered leaves of *Tephrosia villosa* Pers. was extracted in soxhlet extractor by using different solvents of increasing polarity. 200gm of drug was used for extraction purpose and the quantities of extracts obtained are given in [Table 1].

Qualitative   Estimation: Extraction of powdered leaves of *Tephrosia villosa* Pers. was carried out by using various solvents of increasing polarity and then the methanolic extract was subjected to preliminary phytochemical screening for the identification active major functional groups. And ash of powdered drug was utilized for the detection of inorganic elements. Results for both the studies are given [Table 2]. After identification of major active functional groups the drug was subjected for the comparative TLC for further purification purpose by using two different standard compounds namely β-sitosterol and lupeol. It has been observed that the bands in TLC obtained of the test drug have same Rf values as standard. The results for TLC are given in [Fig. 1, 2].

Quantitative Estimations: For quantitative estimations parameters like total flavonoid content, total phenol content and toal triterpenoid content have been determined from the leaves of *Tephrosia villosa* Pers. It was found to be 72.5%, 70.53% and 0.35% respectively. Results for quantitative estimation are given in [Table 3, 5 and Fig. 3, 4].

Table 1: Showing results of extraction of leaves of *Tephrosia villosa*

Sr. No.	Solvents	Estimated percentage in gm
1	Petroleum Ether	2.18 % w/w
2	Chloroform	3.68 % w/w
3	Ethyl acetate	2.88 % w/w
4	Acetone	1.46 % w/w
5	Methanol	12.66 % w/w
6	Aqueous extract	8.50 % w/w

Table 2: Showing results of phytochemical study and detection of inorganic elements.

Test for organic elements			Test for inorganic elements	
Sr. No.	Test	Inference	Test	Inference
1	Carbohydrate	Positive	Calcium	Negative
2	Amino acid & Protein	Negative	Iron	Positive
3	Glycoside	Positive	Magnesium	Negative
4	Saponin	Positive	Potassium	Negative
5	Coumarin	Positivee	Sulphate	Positive

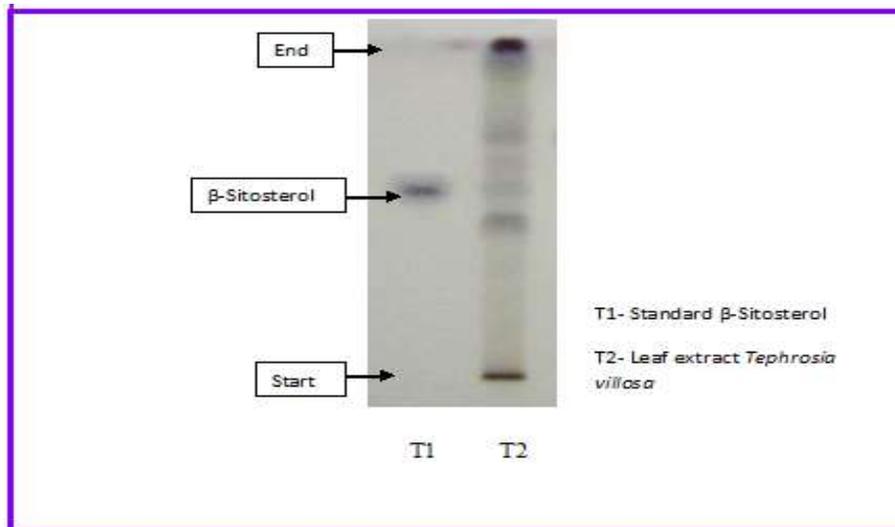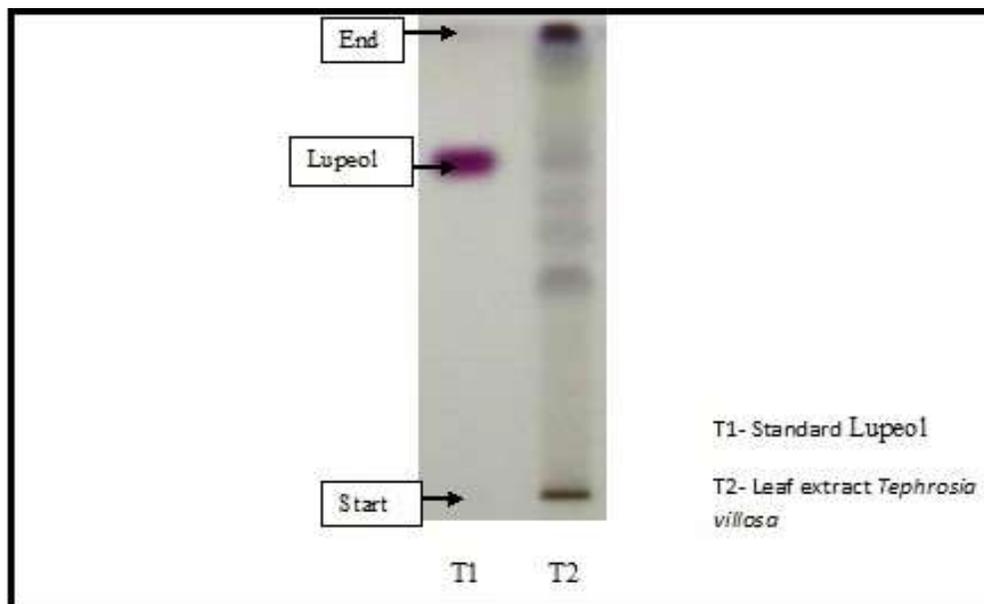| 6	Flavonoid	Positive	Phosphate	Negative
7	Anthraquinone glycoside	Negative	Choride	Positive
8	Phytosterol	Positive	Carbonate	Negative
9	Phenolic Compound	Positive	Nitrate	Positive
10	Alkaloids	Negative	-----	-----
11	Lipid	Negative	-----	-----
12	Tanins	Positive	-----	-----

Fig. 1: TLC of β-Sitosterol

Fig. 2: TLC of Lupeol

Table 3: Showing results of Total Flavonoid content

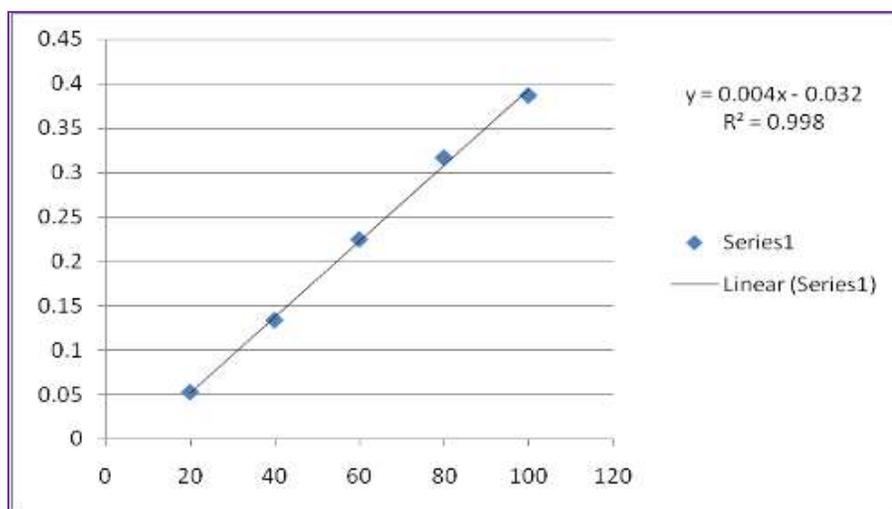| Sr. No.	Standard (Quercetin mg/ml)	Absorbance (nm)			Mean
		I	II	III	
1	20	0.049	0.053	0.056	0.053
2	40	0.131	0.137	0.134	0.134
3	60	0.228	0.221	0.225	0.225
4	80	0.319	0.315	0.318	0.317
5	100	0.387	0.384	0.389	0.387
Sample	72.5	0.316	0.324	0.322	0.320

$y = 0.004x - 0.032$
$R^2 = 0.998$

Fig. 3: Calibration curve of standard Quercetin

Table 4: showing result of Total Phenolic content

Sr. No.	Standard (Gallic acid mg/ml)	Absorbance (nm)			Mean
		I	II	III	
1	20	0.278	0.274	0.273	0.275
2	40	0.592	0.553	0.5421	0.562
3	60	0.876	0.888	0.897	0.887
4	80	1.189	1.193	1.183	1.188
5	100	1.453	1.463	1.459	1.458
Sample	70.53	0.276	0.270	0.274	0.273

y = 0.015x - 0.0236
R² = 0.9992
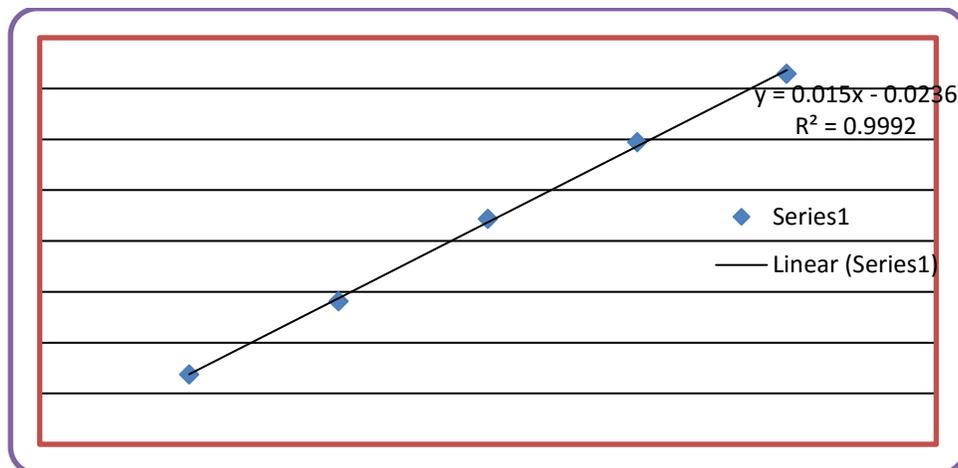
◆ Series1
—— Linear (Series1)

Fig. 4: Calibration curve of standard Gallic acid

Table 5: Showing result of total triterpenoids

Sr. No.	Sample	Burette reading (ml)			Mean
		I	II	III	
1	Methanolic extract of leaves of *TV*.	0.4	0.4	0.3	0.36

Calculation

Factor for the calculation:

Each ml of 0.1N NaOH = 48.8 mg of triterpenoids

0.36 ml of 0.1N NAOH = X mg of triterpenoid

Therefore X= 48.8 X 0.36 X 0.1 / 0.1

X=17.56 mg of triperpenoids

Hence, 5 g *TVP* leaves containing 17.56 mg of triterpenoids, therefore percentage of triterpenoids in TVP leaves was found to be 0.35 % w/w.

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