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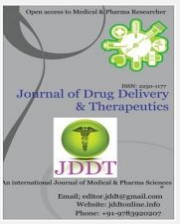

Open Access Research Article

PHYTOCHEMICAL INVESTIGATION AND QUANTITATIVE ESTIMATION OF FLAVONOID AND PHENOLIC CONTENTS OF THE ROOT, STEM AND LEAVES OF *TEPHROSIA PURPUREA* LINN

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ABSTRACT

Herbal drugs are traditionally used in various parts of the world to cure different diseases. The purpose of present study is to characterize phytoconstituents in the various part of *Tephrosia purpurea*. The root, stem and leaves of *Tephrosia purpurea* were washed, air dried and then powdered. The aqueous and ethanolic extracts of various part of *Tephrosia purpurea* were used for the phytochemical investigation to find out the qualitative and quantitative phytochemical constituents in the plant. The result of the phytochemical analysis of *Tephrosia purpurea* showed presence or absence in addition to quantitative (mg/100mg) contents of flavonoid and Phenol in the plant.Present study will help to identify the different parts of the plant from which higher quantities of the phytochemical can be derived and for the development of new herbal drugs from *Tephrosia purpurea*.

Keywords: Phytoconstituents, Phytochemical, *Tephrosia purpurea*, Aqueous ethanolic extract, Flavonoid, Phenol.

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INTRODUCTION

India is sitting on a gold mine of well-recorded and traditional well practiced acquaintance of herbal medicine[1]. Herbal medicines deals with plant and plant extracts in treating diseases. These medicines are considered safer because of natural ingredients with lesser side effects[2].The demand for herbal products are rising exponentially throughout the world. Finding healing power in plants is a primitive idea. Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say Return to Nature[3]. The herbal drug products are prepared from renewable resources of raw materials by ecofriendly processes and will bring economic prosperity to the masses growing these raw materials[4]. World Health Organization (WHO) estimates that eighty percent of total world's population presently uses medicines of herbal origin for primary health care[5].*Tephrosia purpurea*, known as "Sarapunkha" is belonging to pea family has subfamily papillonaceae. It is indigenous to India[6]. *Tephrosia purpurea* Pers. (Leguminosae), is a wild plant known as "Sarapunkha" in Sanskrit, 'Purple Tephrosia' or 'Wild indigo' in English[7]. There are approximately 400 species included in this genus[8]. The plants of genus *Tephrosia* of family *Leguminosae* are widely distributed in many tropical and subtropical countries of the world and have been used in folk medicine for the treatment of large number of diseases[9]. Different parts of the plant are traditionally claimed to be used for the treatment of ailments including diarrhoea, bronchitis, asthma, inflammation, boils, pimples, enlargement of the spleen, diseases of liver, heart, kidney and blood, in tumors, ulcers, leprosy and asthma[10]. *Tephrosia purpurea* root have good cytotoxic activity[11], anticancer properties chemopreventive efficacy, antilipidperoxidative effect[12] and antiulcers properties[13]. Whole plant of *Tephrosia purpurea* produced significant ($p<0.01$) antiulcer

activity in the ethanol induced ulcer model[2]. The ethanol extract of whole plant *Tephrosia purpurea* showed significant antibacterial activity against pathogens namely *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginos*a, and *Bacillus subtitils*[7,12] , leafs of *Tephrosia purpurea* are reported to have antibacterial activity against *Staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram negative) and *Candida albicans* (Fungal pathogen) [15]. It has shown resistance against arsenic induced toxicity by its protective effect[16]. The leaves of *Tephrosia purpurea* has also been reported to have appreciable quantum of antioxidant properties[17-19]. Many herbs have a long history of traditional use in revitalizing the liver[20]. Hepatoprotective activity reported in whole plant[21], leaf[22], root[23-25] of *Tephrosia purpurea***.** As many as 10,000 different phytochemicals which have the potential to cure diseases like cancer, stroke or metabolic syndrome has been estimated[26-27]. The extract of *Tephrosia purpurea* seeds showed significant antihyperglycemic and antilipid peroxidative effects[28]. Many studies have been carried out that strongly support the contribution of polyphenols to the prevention of cardiovascular diseases, cancer, osteoporosis, neurodegenerative diseases and diabetes mellitus. Phenolic compounds baicalein, cinnamic acid, oleuropein, rutin, quercetin, and tephrosin, reported in *Tephrosia purpurea*[29]. The methanol, ethanolic and aqueous extracts of *Tephrosia purpurea* are reported to have high phytochemicals[30]. The purpose of present study is to characterize phytoconstituents in the various part of *Tephrosia purpurea* and, so this will help to identify the parts of the plant from which higher quantities of the phytochemicals can be derived. In India, there has been increase of interest in *Tephrosia purpurea* regarding their healing potentials for the management of a number ailment.

MATERIAL AND METHODS

Chemicals and reagents

All chemicals and solvents used in this study were of analytical grade obtained from Himedia lab. Pvt. Limited.

Plant collection

Plant material of *Tephrosia purpurea* L. were collected from vidhya region. The plant material was authenticated at the Department of Botany, Govt. Girls College, Rewa, (M.P.), A voucher specimen as a herbarium has been kept for future reference.

Preparation of aqueous and ethanolic extract

The plants were washed, separated into roots, stems and leaves with knife. Each part was spread on clean table top and shade dried under the room temperature around 25°C used as raw material. The dried plant material was coarsely powdered using mechanical method. The individual species parts were stored in air tight labeled specimen bottles analysis and resulting powder subjected to extraction with petroleum ether (60-80°C). The extraction was continued till the defatting of the material had taken place. 100 g. of *Tephrosia purpurea* L. dried material were exhaustively extracted with ethanol and aqueous using maceration process. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts and stored at 4 °C in labeled sterile bottles until further use[31].

Qualitative phytochemical analysis

The powdered plant extracts were subjected to qualitative phytochemical tests using standard procedure[32-33].

Estimation of TPC by spectrophotometer

Estimation of phenol was done according to Folin-Ciocalteu method[34]. 2 ml of extract or standard (1mg/ml) was withdrawn in 10ml volumetric flask separately. To each flask 1 ml of Folin-Ciocalteu reagent and 1 ml of sodium carbonate was added. The mixture was vortexed for 15sec and allowed to stand for 15min for color development. The absorbance was measured at absorption maxima 765 nm using a spectrophotometer. The total phenolic content was determined by using calibration curve (10-50µg/ml).Three readings were taken for each and every solution for checking the reproducibility and to get accurate result. The total phenolic content, expressed as mg Gallic acid equivalents per mg/100 mg dry weight of sample.

Estimation of TFC by spectrophotometer

Determination of total flavonoid content was based on aluminium chloride method[34]. Aqueous and ethanolic extracts that have been adjusted to come under the linearity range i.e. (1mg/ml). Different dilution of standard solution of Quercetin (5- 25µg/ml) and extract were added to 10ml volumetric flask containing 1 ml of 2% $AlCl_3$ methanolic solution and allowed to stand for 15 min at room temperature; then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 420nm. Total flavonoid content of the extracts was expressed as percentage of Quercetin equivalent per mg/100 mg dry weight of sample.

RESULT AND DISCUSSION

Result of percentage yield of different extract

The crude extracts so obtained after the maceration extraction process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using ethanol and aqueous as solvents is depicted in the Table 1.

Table 1 Result of percentage yield of *Tephrosia purpurea* L.

S. No.	Parameter	Ethanolic			Aqueous		
		Leaves	Stem	Root	Leaves	Stem	Root
1.	(%) Yield	4.6	2.3	3.1	3.2	2.5	3.3

Result of present study showed that leaves of *Tephrosia purpurea* L. has highest ethanolic extractive percentage compare to aqueous extract.

Result of phytochemical screening

Phytochemical analysis of aqueous extracts of leaf, stem and root sample of *Tephrosia purpurea* L. showed the presence of flavonoid, amino acid, protein, saponins and diterpines while, alkaloid glycoside, and carbohydrate were not detected. From leaf, stem and root sample of *Tephrosia purpurea* L. the ethanolic extract which exhibited the presence of flavonoids while alkaloids, glycosides, phenol, amino acid, protein, and saponin were reported to be absent. Diterpine and flavonoid present in leaves and stem parts of ethanolic extract of *Tephrosia purpurea* L.

The phytochemical analysis of the ethanolic extract in the leaf and stem sample of *Tephrosia purpurea* showed the presence of phytochemical constituents such as diterpines and carbohydrate but alkaloids and glycosides, phenol, amino acid, protein, and saponin were absent (Table 2). The phytochemical analysis of root extract showed the presence of flavonoids while alkaloids, glycosides were reported to be absent [35.] There are certain phytochemical constituents such as flavonoid and diterpines which are similar in aqueous and ethanolic extract of *Tephrosia purpurea*. Roots of TP are rich in flavonoids like rutin and quercetin [36]. In the plant polyphenols are also strongly support the contribution of to the prevention of cardiovascular diseases, cancer, osteoporosis, neurodegenerative diseases, and diabetes mellitus, and suggest a role in the prevention of peptic ulcer. Some flavonoidal compounds, tephrosin, pongaglabol, and semiglabrin present in the *Tephrosia purpurea* [29]. Generally flavanoid in this plant enable it to prevent hepatotoxicity and have antioxidant activity[37].

Table 2 Result of phytochemical screening of *tephrosia purpurea*

S. No.	Constituents	Ethanolic			Aqueous		
		Leaves	Stem	Root	Leaves	Stem	Root
1.	Alkaloids	-	-	-	-	-	-
2.	Glycosides	-	-	-	-	-	-
3.	Flavonoids	+	+	+	+	+	+
4.	Phenolics	-	-	-	+	-	-
5.	Amino Acids	-	-	-	+	+	+
6.	Carbohydrate	+	-	-	-	-	-
7.	Proteins	-	-	-	+	+	+
8.	Saponins	-	-	-	+	+	+
9.	Diterpines	+	+	-	+	+	+

(+): Indicates the presence of phytochemicals (-): Indicates the absence of phytochemicals

Effect of TPC content

The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of aqueous extract of *Tephrosia purpurea* leaves showed the content values of 3.17 but aqueous and ethanolic extracts of root, stem have no phenolic content and ethanolic extract of leaves also have no phenolic content. Results are provided in (Table 3 and Fig. 1).

Table 3 Total phenolic content of *Tephrosia purpurea* L. extract

Estimation	Ethanolic extract			Aqueous extract		
	Leaves	Stem	Root	Leaves	Stem	Root
Total Phenolic (mg/ 100mg)	-	-	-	3.17	-	-

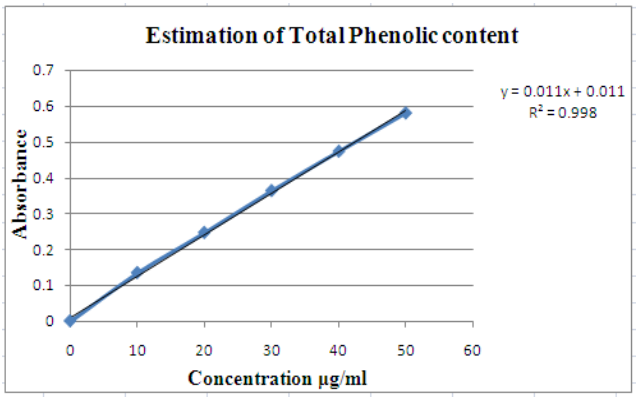

Figure 1: Graph of absorbance against concentration for total phenolic content

Effect of Flavonoid content

The total flavonoid content of the extracts was expressed as percentage of Quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of ethanolic and aqueous extracts of leaves, stem and root of *Tephrosia purpurea* showed the content values of 3.30, 2.05, 1.78 and 2.06, 1.75, 1.55 respectively. The above results showed that aqueous extract contain less flavonoid content than the alcoholic extract. It may due to the solubility of principle contents presence be higher in case of alcoholic solvent, thus it has been accepted that it is a universal solvent for the extraction of plant constituents. Results are provided in (Table 4 and Fig. 2).

Table 4 Total flavonoid content of *Tephrosia purpurea* L. extract

Estimation	Ethanolic extract			Aqueous extract		
	Leaves	Stem	Root	Leaves	Stem	Root
Total Flavonoids (mg/ 100mg)	3.30	2.05	1.78	2.06	1.75	1.55

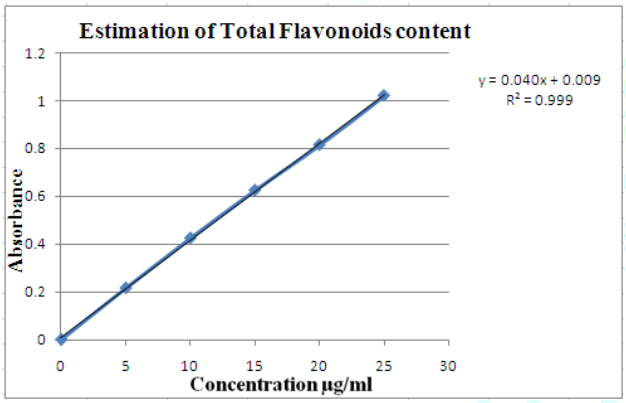

Figure 2: Graph of absorbance against concentration for total flavonoids content

CONCLUSION

Medicinal plants play a vital role in preventing various diseases. The hepatoprotective, antidiabetic, anti-inflammatory, antianalgesic, anticancer, antiviral, antimalarial, antibacterial and antifungal activities of the medicinal plants are due to the presence of the secondary metabolites. The different parts of selected plant also possesses the secondary metabolites i.e. flavonoids and phenol, saponins. So this study will help to identify the parts of the plants from which higher quantities of the phytochemical can be derived and for the development of new drugs medicinal plants are very helpful. Thus we hope that the important phytochemical identified by our study potentially serve as drugs and also provide newer leads and clues for new drug discoveries. Further study is needed for isolation, identification & characterization of the active compound from plant.

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