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Pharmacognostic and Phytochemical screening of the aerial parts of *Tephorsia purpurea* (Linn.) Pers (Fabaceae)

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Abstract

Tephorsia Purpurea commonly known in Sanskrit as Sharapunkha, a highly branched, sub erect and herbaceous perennial herb. Herb is found in tropical regions and bear white to purplish flowers. According to Ayurveda, the name of "Sarwa wranvishapaka" means, property to heal all type of wounds. Whole plant and its various parts are useful as ayurvedic medicines. Medicinal uses of drug are tonic, Laxative, diuretic, bronchitis, bilious febrile attack, boils, pimples, diarrhea, gonorrhea, rheumatisim, and cures disease of heart, spleen and blood. The plant has not been explored scientifically for its pharmacological or for pharmacognostical details. Therefore, the study of morpho-anatomical characters and phytochemical analysis of *Tephorsia purpurea* was undertaken to establish the pharmacognostic and phytochemical details about the plant. Various parameters such as morphology, microscopy and phytochemical profiles of the aerial parts of the plant were studied and the salient diagnostic features are documented.

Key-Words: *Tephorsia purpurea*, Standardization, Pharmacognostical screening, Phytochemical screening, Traditional uses

Introduction

There are various pharmacological works has been undertaken in *Tephorsia purpurea* but little pharmacognostic work has been done on *Tephorsia purpurea* with this background the present work was undertaken and was subjected to the pharmacognostic profile of *Tephorsia purpurea* using its Aerial parts. *Tephorsia purpurea* is a pan tropical coastal shrub that grows up to 1m in height[1]. It is also known as ahuhu, auhola or hola[2]. It is a species of flowering plant in the pea family Fabaceae. The genus *Tephorsia*, a pan tropical with about four hundred species distributed throughout the world[3]. Twenty four species of *Tephorsia* were recorded in India [4,5]and most of the *Tephorsia* species are herbs to under shrub and are grown as weeds. It is cultivated throughout India. Previous phyto chemical investigation on plant have shown presence of glycosides such as rutin and quercetin, retenoids like deguelin, elliptone, rotenone and tephrosin, flavonoids like purpurin, purpurenone and purpuritenin and sterols such as sitosterol[6], isoflavone, 7, 4-dihydroxy- 3,5-dimethoxyisoflavone and chalcone, (+)- tephropurpurin, are also reported to be present in *Tephorsia purpurea*[7]

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The major constituents in TP are Rutin, quercetin, retenoids deguelin, elliptone, rotenone, tephrosin and lupeol[8] and minor are flavanones, lanceolatin A,B & C, isolonchocarpin, and purpurin from root and from entire plant is pongamol[9]. An isoflavone 7, 4-dimethoxy -3, 5-dimethoxy isoflavone; a chalcone (+)-tephropurpurin, (+) -purpurin, pongamol, lanceolatin-B and *Tephorsia purpurea*. Traditionally used to cure several types of external wounds[10]and gastro-duodenal disorders[11] the plant has also been claimed to cure kidney, liver spleen, heart and blood related disorders [12, 13]. The dried herbs are effective as tonic laxative, diuretics, deobstruent and used in the treatment of bronchitis, bilious febrile attack, boils, pimples and bleeding piles[13]. An extract of pods is effective as analgesic, anti-inflammatory, and their decoction is used in vomiting like symptoms[14]. Ethanolic extract of plant has been reported as anticancer activity against in-vitro KB-cells culture[15]. The aqueous extract of seeds has significant *in-vivo* anti diabetic activity and ethanolic extracts is possessed as potential antibacterial activity[16]. Moreover, flavonoids have been found as antimicrobial activity[17]. Roots are given indyspepsia and chronic diarrhea[9] and it is an important component of some preparation such as Tephroli and Yakrifit, used for liver disorders[18]. In IndianAyurvedic system of medicine various parts of this plant are used as remedy

for impotency, asthma, diarrhea, gonorrhea, rheumatism, ulcer, urinary disorders and whole plant has been used to cure tumors, ulcers, leprosy, allergic and inflammatory condition such as rheumatism asthma and bronchitis[19].The present investigation was undertaken with the aim to determine morphological, microscopical, physicochemical parameters such as foreign matter, loss on drying, total ash, acid insoluble ash, water and alcohol insoluble extractive along with phytochemical studies that would serve as few of the basic protocols for standardization of medicinal plants and it can also be helpful in preparation of monograph of the plant.

Material and Methods

Aerial parts of the *Tephorsia purpurea* (Figure 1) were collected from the surrounding area of Bhopal (MP) in the month of April-May. The plants were identified and authenticated Dept of Botany, Safia College Bhopal (MP) and voucher specimen 321/bot.1/saf/12deposit in the college. The aerial part of *Tephorsia purpurea* was shade dried, coarsely powdered and sieved through 40 mesh size and plant material was stored in a well closed container.

Extraction

The dried coarsely powdered plant material (50 g) was successively extracted with the solvents of increasing order of polarity (petroleum ether, chloroform, acetone, and ethanol) by the hot extraction process using a Soxhlet apparatus for a period of 18-22 hours. After each extraction tests were performed to see whether the drug had been completely exhausted or not. The completion of extraction was confirmed by evaporating a few drops of the extract. The extracts obtained were concentrated to dryness in evaporated disc at 40^0C and stored the dried extract at 4^0C in the refrigerator until further use.

Pharmacognostic studies

Morphology

Macroscopical evaluation was carried out by using dissecting microscope. The shape, apex, base, margin, taste and odor of leaves were determined as per the reported methods.

Microscopy

Histological evaluation was done by preparing thin hand sections of midrib of leaves. Cleaning of sections were done with chloral hydrate solution, treated with phloroglucinol and hydrochloric acid, and mounted with glycerin and as per the standard procedures. To identify the starch grains a separate section was prepared by staining with iodine solution. The results obtained were recorded by taking photos with Olympus digital microscope assisted with 1/3" CCD Sony camera.

Determination of physical parameters

Physical standards are to be determined, wherever possible. These are rarely constant for crude drugs, but may help in evaluation, specifically with reference to moisture content, density, foreign organic matter, crude fiber content, ash value, extractive value etc.

Physical standards are to be determined, wherever possible. These are rarely constant for crude drugs, but may help in evaluation, specifically with reference to moisture content, density, foreign organic matter, crude fiber content, ash value, extractive value etc[20].

Moisture content

Procedure

Approximately 5 gm accurately weighed powdered sample was kept in IR moisture balance. The loss in wt. was recorded as percentage (%) moisture with respect to air-dried sample of crude drug[21].

Total Ash value

Procedure

Empty aluminium foil dish was ignited, cooled and then weighed. Approximately 2 gram of accurately weighed powdered sample was placed in the dish. The dish was placed in Muffle furnace and its temperature was maintained at around 450-500^{0}C. The ash was collected and weighed. The percentage of total ash was then determined[21].

Acid insoluble ash value

The ash collected from the above procedure. The ash was transferred in to a beaker and washed with 25 ml. dilute HCl and then boiled, filtered this ash by ash less filter paper and residue was washed twice with water. The collected residue and filter paper taken in a empty dish and put it in to Muffle furnace and its temperature was maintained at around 450-500 ^{0}C. The ash was collected and weighed. The percentage of acid insoluble ash was 0.93% determined.

Determination of water soluble ash

The ash was boiled with 25ml of water for 5 min. and the insoluble matter was collected on ash-less filter paper and washed with hot water, ignited for 15min. maintaining temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash and this represents the water soluble ash. Percentage of water soluble ash was determined with reference to the air dried sample.

Extractive values

The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of content of drugs, various solvents are used for determination of extractives. The solvent used for extraction should be

in a position to dissolve appreciable quantities of desired substances.

Water soluble extractive value

This method is applied to those crude drugs, which contain water soluble active constituents such as tannins, sugars, plant acids, mucilage, glycosides etc.

Procedure

Approximate 5 gram accurately weighed powdered sample was taken in a conical flask and about 100ml. solvent (Water) was added to it. The flask was cooled and set aside for 24 hrs with frequent shaking during first 6-8 hrs. After 24 hrs the content was filtered. The filtrate was collected and 25 ml filtrate was transferred to a porcelain dish. Filtrate was evaporated to dryness on a water bath and complete drying was done in oven at 105 ^{0}C. The dish was weighed after cooling and value in percentage was calculated[22]

Alcohol soluble extractive value

Alcohol is an ideal solvent for extraction of various chemicals tannins, alkaloids, resins, flavonoids etc. Generally, absolute alcohol (95% v/v ethyl alcohol) is used for determination of alcohol soluble extractive.

Procedure

Approximate 5 gram accurately weighed powdered sample was taken in a conical flask and about 100ml. Solvent (Ethanol) was added to it. The flask was cooled and set aside for 24 hrs with frequent shaking during first 6-8 hrs. After 24 hrs the content was filtered. The filtrate was collected and 25 ml filtrate was transferred to a porcelain dish. Filtrate was evaporated to dryness on a water bath and complete drying was done in oven at 105^{0}C. The dish was weighed after cooling and value in percentage was calculated[22].

Phytochemical screening of *Tephorsia Purpurea*

Qualitative analysis of extracts

The preliminary tests, for the detection of secondary metabolites, were carried out for the extracts of selected plant by adopting standard methods .The extracts obtained by successive solvent extraction were subjected to various qualitative tests to detect the presence of plant constituents like alkaloids, glycosides, carbohydrates, phytosterols, saponins, tannins, phenolic compound, proteins, free amino acids and flavonoids[23,24].

Preparation of test solution

500mg of each extract was dissolved in 100 ml of respective solvent and filtered through Wat man filter paper No.1.The filtrates thus obtained were used as test solutions for the following preliminary screening test

Test for alkaloids

A small portion from the respective extracts was shaken with about a 3 ml of 1.5%v/v hydrochloric acid and filtered. The filtrate was tested with the alkaloidal reagents like Mayer's reagent produce White precipitate or orange red precipitate, Dragendroff's reagent produce Orange on orange red precipitate Wagner's reagent produce Brown or reddish- brown precipitate and Saturated picric acid solution produce Crystalline yellow precipitate.

Test for glycosides

Keller – Killiani test

Small portion from the respective extracts was shaken with 1 ml Glacial acetic acid containing a trace of ferric chloride. 1 ml of concentrated sulfuric acid was added carefully by the sides of the test tube. A blue color in the acetic acid layer and red color at the junction of the two liquids indicate the presence of glycosides.

Legal test

A little fraction of respective extracts was taken in water and made alkaline. To alkaline solution few drops of sodium nitropruside solution was added. A blue color indicates the presence of glycosides.

Bontrager test

A small fraction from various extracts was dissolved in 1 ml of benzene and then 0.5 ml of dilute solution of ammonia was added to the benzene solution. A rose pink to red color was indicates the presence of glycosides.

Test for carbohydrates

The alcoholic extracts of each plant were tested with molisch's reagent, fehling's solution, Benedicts solution and tollen's reagents. The positive reaction indicates the presence of reducing sugar

Molisch's test

A small fraction from the respective extracts was taken in ethanol separately and a few drops of 20%, w/v solution of a-napthol in ethanol (90%) were added to it. After shaking well, about 1 ml of concentrated sulphuric acid was allowed to flow carefully by the side of the test tube. A reddish violet ring at the junction of the two layers indicated the presence of carbohydrates.

Fehling's test

A small fraction from various extracts was taken with about 1 ml of distilled water separately and filtered. Te filtrates were taken in test tubes separately and 1 ml of Fehling's solution (A and B mixed together) was added to each test tube. The Contents were placed in a boiling water bath for 2 minutes.Appearance of brick red precipitate of cuprous oxide indicated the presence of reducing sugars.

Tollen's test

A little portion from each extracts was taken separately with a small portion of the distilled water and filtered.

A few drops of the ammonical silver nitrate solution (Tollen's reagent) was added to each filtrate and kept in boiling water both for 5 minutes. Appearance of a silver mirror along the sides of the test tubes indicated the presence of reducing sugars.

Barfoed test

A little portion from each extracts was taken separately with 2ml of distilled water and filtered. Then a small volume of Barfoed reagent was added to each test tube and kept in a boiling water bath for 2 min. Appearance of red precipitate indicated the presence of monosaccharides.

Test for phytosterol

The petroleum ether extract and the benzene extract were separately refluxed with a solution of alcoholic potassium, hydroxide. After complete specification (one hour) the test mixture was diluted with water and extracted with three portions of 10 ml quantities of ether. The combined ether extracts were tested for the presence of phytosterols.

Hesse"s reaction

A little fraction from each extract was taken with a few drops of chloroform and an equal volume of concentrated sulfuric acid was added to it the sides of the test tube. Appearance of a blood red color indicated the presence of sterol.

Test for saponin

The presence of saponins is usually indicated in the alcoholic and water extract of the drug. 1 ml of both the extracts were diluted to 20 ml by the respective liquids and shaken well. The presence of saponin is indicated by the formation of sense foam. However, other extracts were also tested for the presence of saponins.

(a) Little fraction from the various extracts were boiled with about 1 ml of distilled water and shaken. Appearance of a characteristic foam formation indicated the presence of saponins.

(b) A Little fraction from various extracts was taken with about 2 ml of distilled water. A small quantity of sodium carbonate was added to each and shaken. The characteristic foam formation indicated the presence of saponins.

(c) Haemolysis test: Various extracts were diluted with normal saline so what it became nearly about 5 times diluted. In series of 5 test tubes, doses of 0.2, 0.4, 0.6, and 0.8, were added and volume was made in each test tube, up to 1 ml with normal saline. 1 ml of the diluted blood 10.5 ml of rabbit's blood was diluted to 25 ml with normal saline and added to each test tube. A drop from each test tube was viewed under microscope confirm the presence of haemolysis.

Test for tannins and phenolic compounds

A small fraction of the residue from each extracts was dissolved in about 2 ml of distilled water separately and filtered. The filtrate was tested with the following reagents.

Ferric chloride solution

Appearance of a blue to bluish green or bluish black color indicated presence of tannins and phenolic compounds.

Lead acetate solution

Appearance of brownish yellow precipitate indicated the presence of tannins.

Potassium chromatic solution

Appearance of orange yellow precipitate indicated the presence of tannins.

Gelatin test

The test was performed by adding 1% solution of gelatin containing 10% NaCl.

Test for proteins

On concentrating the aqueous extract to a small bulk a translucent mass was separated, which did not re-dissolve. This mass gave positive test for proteins by following reaction

Millon's reaction

A small fraction from each extracts was taken in water and filtered. To about 2ml of the filtrate, 5-6 drops of Millon's reagent were added yellowish- red precipitate was indicative the presence of proteins.

Xanthoproteic reaction

A small fraction form each extracts was taken in water. To this concentrated solution nitric acid was added. Appearance of yellow color was indicative of the presence of proteins.

Test for free amino acids

A little friction from each extract was taken in water and filtered. The filtrate was used as such and also after removing tannins by lead acetate methods. Then the spots were applied on chromatographic paper the spots were dried and the paper were then sprayed with ninhydrin reagent. The paper was then allowed to dry and then heated in an oven at 115^0C for 5 min. Appearance of violet colored spots indicated the presence of free amino acids

Test for Flavonoids

Sinoda test

Plant extract is treated with magnesium and concentrated HCl, usually in ethanol solution. The test is carried out by adding the acid drop wise to an alcohol solution containing fragments of magnesium ribbon. Characterized color produced with in a minute or two and the subsequent addition of more acid often causes modification of the color in a manner characteristic of the compound being examined.

The test is positive with production of pink, scarlet, crimson or occasionally green or blue color for flavonoids, flavanones and flavanonols.

Ferric chloride solution

5% Ferric chloride solution (freshly prepared) was added to the alcoholic extract produced green purple or brown color which indicates presence of flavonoids.

Results and Discussion

Morphological characters of the plant material are based on size, shape, colour, surface characteristics and texture. For the microscopical studies, free hand sections of the leaves of the selected medicinal plants were taken and stained with phloroglucinol and hydrochloric acid (1:1). Slides were observed (Figure 2) under microscope. Morphological investigation of aerial part of *Tephorsia purpurea* showed that Sub shrub to 1mm; branch lets pubescent – villous. Leaves to 7 cm; leaflets 4 -9 pairs, ovate, 0.8 – 2 × 0.3 – 0.7 cm, pubescent, base cuneate, margin entire, apex obtuse, mucronate; petiole to 1 cm; petiolule 1mm; stipules, lanceolate, 5mm Pseudo racemes leaf-opposed, to 8 cm; bract to 2mm, pubescent; lobes lanceolate; upper lobes 2.5 mm, equal to lower one. Corolla bluish-pink to purple; standard orbicular, 8.5 × 8mm, sericeous; wings 7.5 × 3 mm; keels 6.5 × 2.5 mm; staminal sheath 5 mm; filaments 2 mm. Ovary 5 mm, apprised – pubescent; style 3 mm, glabrous. Pod 4 × 0.4 cm, downy – puberulous, continuous within, slightly falcate; seeds ca. 7, ovoid, 3.5 mm, strophiole in the middle of seedTransverse section of the leaflet shows isobilateral nature of the leaf. The lamina portion consist upper epidermis, mesophyll tissue and lower epidermis. Upper epidermis of lamina consists of a single layer of cells which is tangentially elongated, covered with cuticle. Upper palisade has 3 to 5 layers of palisade parenchyma while the lower palisade has 2 to 3 layers. Spongy parenchyma lies in between the upper and lower palisade and composed of loosely arranged parenchyma cells with vascular elements and prisms of calcium oxalate crystals. The lower epidermis shows the presence of bi cellular, uniseriate covering trichomes. The lower epidermal portion of midrib is more convex than the upper epidermis. The midrib portion shows the presence of large, collateral type of vascular bundle surrounded by spongy parenchyma cells. The vascular bundle is arc shaped showing the presence of well-developed spiral xylem vessels. The physiochemical parameter like moisture content, total ash, acid insoluble ash, water soluble ash and foreign organic matter were 8.65%, 7.15%, 0.93%,2.58%, 0.31% respectively and shown in table 02. Alcohol soluble extractive value and water soluble extractive value was found to be 13.89% and 19.1% which is standard for the drug and shown in Table 3. Quantative analysis of the extract for the identification of secondary metabolities present in the extract were shown in Table 4 which shows that aerial part of *Tephorsia purpurea* contain cabohydrates, alkaloids, flavonoids, tannins, protein, and amino acid. Phytocontituents like steroids, glycosides and saponins showed negative result with extract which also shown in Table 4.Morphplogical and microscopical evaluation is indispensable in the initial identification of herbs as well as in detection of Adulterants and identifying the plant by characteristic features. The significane of performing physiological parameter was to identify the organic impurities in the material.Standardization is the prime need of time. These help in the establishment of quality and identidentify profile that can be used for the purpose of safety monitoring and overall quality assurance.Hence it is very essential to establish pharmacognostical standardization. The results obtained in the present study are encouraging and can be used as an effective reference data for the standardization of *Tephorsia purpurea.*

Concluion

Ayurvedic plant based medicines have their advantages over the allopathic treatment.*Tephrosia purpurea* has its traditional as well as folk medicine. Various preclinical investigations have been carried. The present investigation adds to the existing knowledge of *Tephrosia purpurea*and will bequite useful to pharmaceutical industries for quality control ensuring batch to batch consistency of raw drug and in the field ofmedical, pharmacological evaluation and development of aformulation for treating various ailments.Thus, findings of this study could beuseful for the compilation of a monograph in a suitablepharmacopoeia for its proper identification and quality control which can be used globally.

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Fig. 1:Aerial parts of *Tephorsia purpurea*

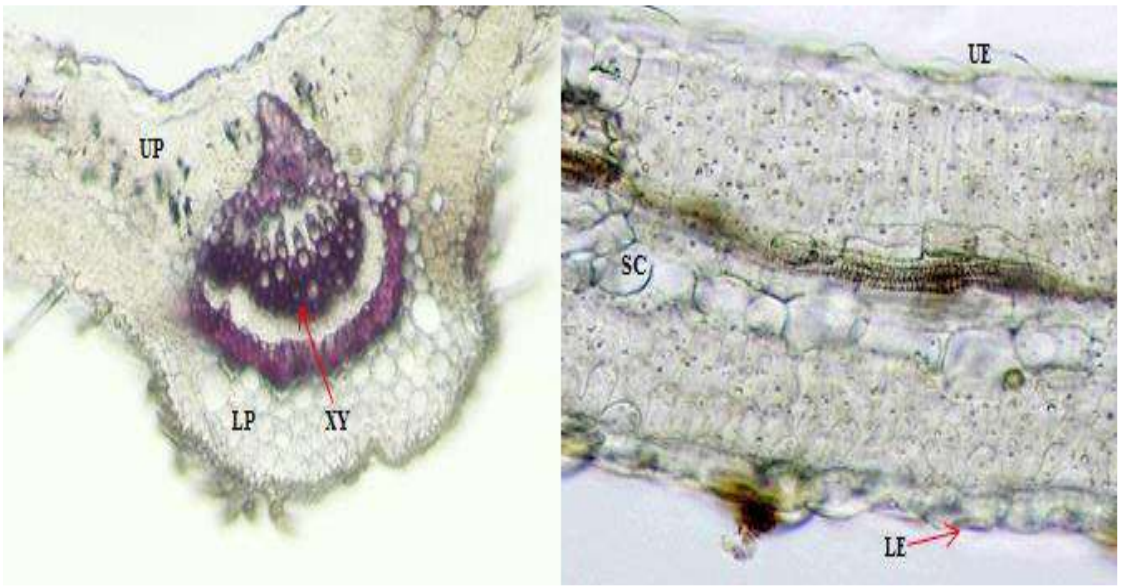

Fig. 2: TS of leaf of *T. purpurea* UP: Upper palisade; LP: Lower palisade; XY: Xylem; UE: Upper epidermis; LE: Lower epidermis; SC: Sclerenchyma

Table 1: Physiological parameters of the aerial parts of *Tephorsia purpurea*

S/No.	Parameters	*Tephorsia purpurea* (%)
1.	Moisture Content	8.65
2.	Total Ash	7.15
3.	Acid Insoluble Ash	0.93
4.	Water soluble ash	2.58
5.	Foreign organic matter	0.31

Table 2: Extractive values of *Tephorsia purpurea*

S/No.	Extractive values	*Tephorsia purpurea*
1.	Alcohol Soluble	13.89
2.	Water Soluble	19.1

Table3: Phytochemical screening of *Tephorsia purpurea* extract

S/No.	Test	*Tephorsia purpurea* extract
1	**Steroids**	
	Salkowaski Test	_
2	**Glycosides**	
	Bontrager	_
	Kellar killiani	_
	Legals	_
3	**saponin**	
	Foam test	_
	Haemolysis Test	_
4	**Cabohydrates**	
	Molisch Test	+
	Barfirds Test	+
	Fehling Test	+
5	**Alkaloids**	
	Mayer Test	+
	Wahners Test	+
	Dragondroff Test	+
	Haggers Test	+
6	**Flavonoids**	
	Shinoda Test	+
	Lead acetate Test	+
	Pew's Test	+
	NAOH Test	+
7	**Tannins**	
	Ferric choloride	+
	Gelatin Test	+
8	**Protein**	
	Precipitation test	+
	Xanthoproteic	+
9	**Amino acid**	
	Ninhydrine	+

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