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RESEARCH ARTICLE

Detailed Pharmacognostical Standardisation and Phytochemical Analysis of Whole Plant of *Tephrosia barber* — An Endemic Novel Medicinal Plant

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Abstract

Background: *Tephrosia barberi* J. R. Drumm. is a perennial herb belonging to the Fabaceae family. **Aim**: The present research work is focused on carrying out the pharmacognostic standardisation and primary phytochemical analysis of the whole plant of *T. barberi.* **Methods**: The morphology, microscopy, powder microscopy, quantitative analysis of leaf constants, fluorescence analysis and physio-chemical parameters were included in the pharmacognostical work for the whole plant followed WHO prescribed quality control methods. The primary phytochemical analysis is carried out for powder with different solvent extracts using standard procedure. **Results:** The study reveals that the microscopical character of the whole plant of *T. barberi* includes the presence of cork, phloem fibres, xylem vessels, starch grains, tracheids, unicellular warty trichomes, anamocytic type of stomata and prismatic type calcium oxalate crystal sheath. The total ash, acid insoluble ash, water soluble ash value, sulphated ash, and loss on drying were quantified as physiochemical parameters, which were observed by 3%, 1%, 2%, 5% and 8%, respectively, using *T. barberi* dry powder. Under daylight, long and short UV, extracts of the whole plant and with various reagents were seen as various fluorescent reflection shades, which showed satisfactory results. Additionally, the primary phytochemical analysis of different extracts of *T. barberi* showed the presence of alkaloids, flavonoids, steroidal glycosides, polyphenols and terpenoids. **Conclusion:** All these findings ensured that the whole plant profile for *T. barberi* could be accomplished, which may provide proper identification and probable to minimise the adulteration of other plant species. Furthermore, the primary phytochemical screening study would be useful to carry out the isolation of phytoconstituents and their therapeutic potency in future.

Keywords: Fluorescence, Macroscopy, Microscopy, Physiochemical, Phytochemical, *Tephrosia barberi*

1. Introduction

All over the world, there is a demand for herbal medicines for the health care system. Due to numerous advantages, plant and plant-derived products are gaining worldwide popularity. Over six thousand years ago, the ancient Chinese used the natural flora as medicine at first[1]. They anticipate the improvement of many modern medications as well. In self-help mode, millions of rural households used medicinal plants. There is currently a global evolution for evaluating plant resources, which have significant commercial and therapeutic importance[2]. The majority of people appear to appreciate the drugs' efficacy, safety, potency, and purity. It is therefore necessary to standardize and oversee the quality of herbal medicines, herbal products, and raw materials. Researchers are primarily

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concentrating on ethnobotany and ethnomedicinal analysis due to the rising demand for herbal goods[3].

The work on *Tephrosia* species indicates anti-oxidant[4], anti diabetic[5], anti inflammatory[6], hepato protective[7,8], anti-nociceptive[9], wound healing[10], anti ulcer[11], anti- hyperlipidemic[12], purgative[13], anti microbial[14,15], antifungal[16] and larvicidal activities[17,18]. Taxonomists have shown a great deal of interest in the *Tephrosia* species. Additionally, several research projects have been carried out to analyse the phytochemical components of the numerous species in the *Tephrosia* genus. The large amount of isolated as well as recognized phytocomponents in the *Tephrosia* was discovered to be flavonoids. Terpenoids, rotenoids, sterols, essential oils, and fixed oils make up the other major classes of compounds[19].

Tephrosia barberi is a novel medicinal plant belonging to the family of fabaceae under the subfamily Papilionaceae which grows along the coastal tracts of Thirunelveli and Tuticorin districts. They are perennial under shrubs with reddish flexuous branch leaves and deep roots. It is preferred the sandy clones are known as "*Teri*"[20]. Traditionally, the entire plant is used for cough relief, dyspnea, abdominal disorders, chronic ulcers, fever, bowel complaints vatham-based diseases and liver disorders. The seed is used in skin diseases[21]. Accurate verification of herbal material is vitally essential to the safety and usefulness of herbal medicine. Due to carelessness adulteration may be the chance, while collecting the plant. It may lead to the deterioration of the crude drug. To avoid this problem, macroscopic, microscopic evaluation is necessary. Hence, the present study first time focused to study the well-established pharmacognostic and phytochemical study for the whole plant of *T. barberi*.

2. Methods and Materials

2.1 Collection of Plant Material

In March 2021, the perennial herb of *T. barberi* (Family Fabaceae, subfamily Papilionaceae) was collected from Tirunelveli district, Tamil Nadu, India. At Central Siddha Medical and Research Institute, Chennai, Tamil Nadu, India, the herb was identified and authenticated taxonomically. A voucher specimen T30072101B/9/2021 was kept in the Central Siddha Institutional Herbarium for future reference. The fresh whole plant material collected was thoroughly cleaned with water and air-dried. It was then, ground into a fine powder and stored in an air-tight container for further studies.

2.2 Plant Profile

Kingdom	:	Plantae
Phylum	:	Spermatophyta
Class	:	Equisetopsida
SubClass	:	Magnoliidae
Order	:	Fabales
Subfamily	:	Papilionaceae
Family	:	Fabaceae
Genus	:	*Tephrosia*
Species	:	*Tephrosia barberi*

2.3 Pharmacognostical Studies

2.3.1 Macro, Micro and Powder Microscopic Study of T. barberi

The organoleptic characteristics of *T. barberi* whole plant were observed by the naked eye. The macroscopic observation of the whole plant of *T. barberi* was documented using a Nikon D-5600 Digital SLR camera[22]. By using a sharp blade, the fresh whole plant (root, stem, leaf each separately) was cut into thin transverse sections and stained with safranin and phloroglucinol-HCL. The microscopical characters were observed according to Fahn's and Sass methods[23,24]. The powder microscopy was done after clearing with 0.1% chloral hydrate, Jeffrey reagent and potassium iodide, a small amount of *T. barberi* powdered form was kept in a microscopic slide with a drop of 50% glycerol. The powder microscopical characters of *T. barberi* were observed as per IIyengar and Johansen methods[25].

2.3.2 Quantitative Microscopy

Tephrosia barberi fresh leaves were heated in chloral hydrate solution (0.1%), and slides were prepared. By using camera lucida, leaf constants (stomatal number, stomatal index, palisade ratio, vein islets, and vein termination) were drawn and quantified as per Wallis and mentioned in Siddha Pharmacopoeia[26, 27].

2.4 Physio-Chemical Research

According to WHO recommendations, physico-chemical parameters were established. According to standard procedure total ash value, water soluble ash, acid insoluble ash, alcohol soluble extractive value, water soluble extractive value, sulphated ash and loss on drying were quantified[28, 29].

2.4.1 Loss on Drying

A single evaporating dish was filled with 5 g of precisely weighed plant material. This was weighed after drying for an hour at 105°C. We continued drying and weighing at 1h intervals until we reached the desired weight. When there was less than a 0.1 g change between two weights after drying for 30 mins and cooling for 30 mins in a desiccator, the weight was considered constant.

2.4.2 Extractive Value

A known amount of plant material was taken in a five-conical flask. Then it was extracted successively with petroleum ether, ethyl acetate, chloroform, ethyl acetate, methanol, ethanol and water by maceration process. Then they were filtered. The filtered different extracts were dried on a dried basis. Finally, air-dried extracts were weighed quantitatively and the percentage concerning the weight of the plant material taken was calculated.

2.4.3 Total Ash Value

A muffle furnace or crucible made of platinum-coated silicon was used to burn about 1-2 g of ground plant material until it was carbon-free. After cooling, it was weighed. Calculations were made to determine the ash percentage of the air-dried plant material.

2.4.4 Acid Insoluble Ash Value

To the crucible containing total ash, 25 mL of dilute Hydrochloric acid was added. The insoluble matter was collected on an ashless filter paper (Whatman number 41) and washed with hot water until the filtrate was neutral. The filter paper containing insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 min. and weighed without delay. The content of the insoluble ash was calculated concerning the air-dried plant material.

2.4.5 Water Soluble Ash Value

The ash was boiled with 25 ml of water for 5 min. The insoluble matter was collected in a Gooch crucible or on an ashless filter paper, washed with hot water, and ignited at a temperature not exceeding 450°C. The difference in the weight of the insoluble matter and the weight of ash represented the water-soluble ash. The percentage of water-soluble ash was calculated concerning the air-dried plant material.

2.4.6 Sulfated Ash Value

A platinum/silica crucible was heated for 10 min until it turned red, then it cooled in a desiccator before being weighed. The plant material was accurately weighed at 1-2 g, added to the crucible, and gently ignited at first until completely charred. The residue was then chilled, wet with 1 mL of H_2SO_4, slowly heated until it no longer emitted white vapours, and then burned at 800°C ± 25°C until it no longer emitted any black particles. An area that was shielded from air currents was used for the igniting. The crucible was allowed to cool; a few drops of H_2SO_4 were added and the crucible was heated. Then it was ignited as before, allowed to cool, and weighed. The procedure was repeated until two successive weighs did not differ by more than 0.5 mg.

2.4 Fluorescence Analysis

T. barberi powder was individually mixed with various reagents like 1N NaOH, alcoholic NaOH, concentrated HCl, 1N HCl, concentrated H_2SO_4, 50% H_2SO_4, concentrated HNO_3, 50% HNO_3, CH_3COOH, $Fecl_3$ and ammonia with nitric acid. The colour change was observed under short UV light (254 nm) and long UV light (365 nm) to detect the emission of fluorescence. Additionally, about 5 g of whole plant powder of *T. barberi* was individually extracted with solvents like petroleum ether, acetone, chloroform, methanol, ethanol and water by maceration process. The crude extracts obtained were subjected to fluorescence under long UV light (365 nm), short UV light (254 nm), and the light of day[30].

2.5 Phyto-chemical Analysis

2.5.1 Preparation of the Extract

About 5 grams of *T. barberi* powder was extracted with different solvents like petroleum ether, chloroform,

ethyl acetate, ethanol and water by the maceration process. Then it was filtered and the filtrate was collected. The collected extracts were stored in the refrigerator (25°C) separately.

2.5.2 Preliminary Phytochemical Screening

Using a standard procedure, the above-prepared extracts were used to perform primary phytochemical screening[31].

3. Results and Discussion

3.1 Pharmacognostic Studies

3.1.1 Macro, Micro and Powder Microscopy of *T. barberi*

The macroscopic, microscopic and powder microscopical characteristics of *T. barberi* whole plant parts have been evaluated. The macroscopical observation of *T. barberi* leaves showed compound, Imparipinnate, rachis up to 6 cm long; leaflets 05 to 1.5 cm long cuneate, obovate with short petiolule, base acute, apex retuse, glabrous, stipules 2 to 4 mm long (Figure 1b); aromatic odour and very bitter taste. The morphological characters of the young stem of *T. barberi* showed yellowish brown and the mature stem greyish brown. The cut pieces varied in size, cylindrical, cut surface yellowish brown with a central pith, and fractured short (Figure 1a), The root of *T. barberi* has branched, cylindrical, fracture hard and fibrous, externally rough, exfoliating cork, which was reddish brown with longitudinal fissures (Figure1a). The flowers of *T. barberi* were reddish and about 1 cm long. It has five campanulate calyxes; the teeth are subequal or the lower two are frequently slightly connate. The flower petals were clawed; the standard petal was sub-orbicular, the stamens were ten and diadelphous, and the axillary filament was uniform (Figure1b). The fruit pod of *T.*

(a)

(b) **(c)**

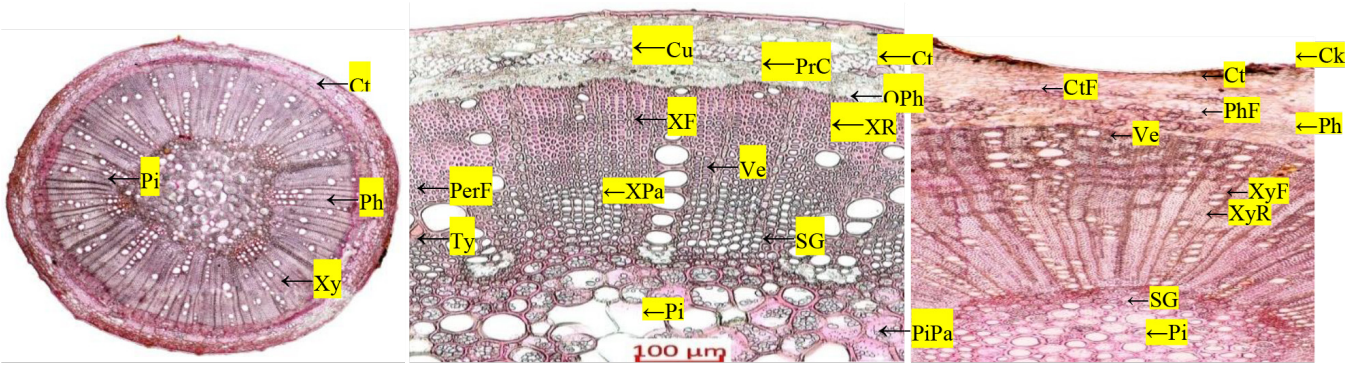
Figure 1. **(a).** *T. barberi* dried whole plant; **(b).** Habitat; **(c).** Fruits.

barberi was linear, flattened, papery, usually 2-3 cm long, rarely a little longer. A fruit contains 4-5 seeds which are kidney-shaped, elliptic ovoid, and glabrous (Figure 1c).

The diagrammatic Transverse Section (TS) of the stem of *T. barberi* was nearly circular in outline, with a narrow epidermis, cork, and phloem surrounded by a wide zone of xylem (Figure 2a). A cellular TS of a young stem revealed a single layer of epidermis covered by a thick cuticle, continued by thick and thin-walled cortex tissue with starch grains, chlorophyll content, and a few prismatic crystals of calcium oxalate; a discontinuous group of pericyclic fibres present between cortex and phloem tissue towards the inner side of the xylem. Xylem rays are uni- to tri-seriate and rarely multi-seriate; the central portion of the section is occupied with lignified, pitted thick-walled parenchyma cells embedded with abundant starch grains. The Mature stem has up to 15 rows of nonlignified, exfoliating cork cells with a reddish brownish content then, a narrow zone of thin-walled compressed parenchymatous cortex embedded with cortical fibre. Then, a wide zone of phloem transversed by groups of fibre and phloem rays running almost parallel to each other toward the periphery. The cortex and phloem tissue contain starch grains, resin masses and a few prism types of calcium oxalate crystals. Then, a pitted, thick-walled parenchymatous pith encircled by a wide zone xylem consisted of usual elements (Figure 2c).

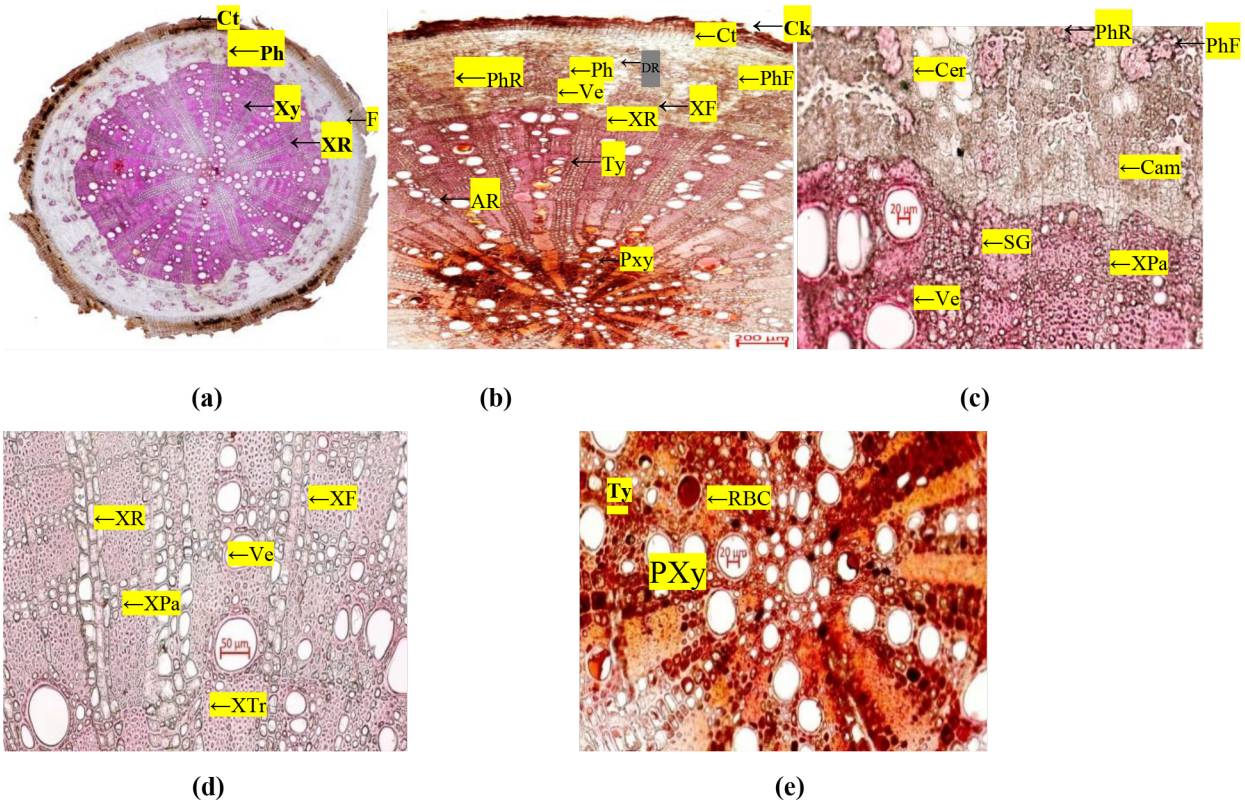
The diagrammatic TS of *T. barberi* root showed cork, cortex, phloem (narrow region) and central core xylem with multi-seriate xylem rays and radial rows of vessels (wide region) (Figure 3a). The detailed transverse section showed tangentially elongated, rectangular-shaped, up to 25 layered, non-lignified, exfoliating cork cells with reddish-brownish content. It is continued as a narrow sector of thin-walled, compressed parenchymatous cortex, interrupted by a wide zone of phloem tissue, which contains starch grains, resin masses, and a few prism types of crystals of calcium oxalate associated with fibre groups. The vessels are arranged in radial groups and few are isolated. Toward the inner vessels, cystoliths and xylem parenchyma are embedded in reddish brown content. Xylem rays were multi-seriate embedded with starch grains, and the central pith was occupied by a protoxylem (Figures 3(b-e)).

Diagrammatic TS of *T. barberi* rachis showed a heart-shaped outline embedded with two trace bundles on the upper side and a central parenchymatous pith encircled by a discontinuous ring of vascular bundles, each bundle capped by an arc of pericyclic fibres (Figure 4a). Detailed TS showed a distinct layered epidermis having unicellular covering trichomes and a few glandular trichomes covered with thick cuticles. After the epidermis, 5-7 layers of chlorenchymatous hypodermis, interrupted by a group of collenchymatous tissue near each tracheal bundle and the lower side of the vascular bundle, followed by 2 to 5 cell rows of parenchymatous cortex were observed. Ground tissue possessed a central prominent pith encircled by groups of discontinuous patches of vascular bundles. The vascular bundles are open, and collateral, and each



Ck - cork; **Ct** - cortex;**Cu**- cuticle; **E**- epidermis; **IPh**- inner phloem; **OPh**- outer phloem; **Pa**- parenchyma; **Per** - pericycle; **PerF**-pericyclicfibre; **Ph**- phloem; **PhF**- phloem fibre; **Pi** - pith; **PiPa**- pitted parenchyma; **PrC** - prismatic crystal; **SG** - starch grain; **Ty** - tylosis; **Ve**- vessel; **XPa**- xylem parenchyma; **XR** - xylem ray; **Xy** - xylem; **XF**–xylemfibre

Figure 2. **(a).** TS of *T. barberi* stem; **(b).** Detailed TS of the young stem; **(c).** Ts of the mature stem.



AR- annual ring; **Cam**-cambium; **Cer**-ceretenchyma; **Ck** - cork; **Ct** - cortex; **DR** - dilated ray; **ExCk**- exfoliating cork; **Ph** - phloem; **PhF**- phloem fibre; **PhR**- phloem ray; **PrC**-prismatic crystals of calcium oxalate; **PXy**-protoxylem; **RBC**- reddish brown content; **RC**-resin content;**SG**-starch grains;**Ty**-tylosis;**Ve** - vessel; **XF** - xylem fibre; **XPa**-xylem parenchyma; **XR** – Xylem ray; **XTr**- xylem tracheid; **Xy**- xylem

Figure 3. **(a).** TS of *T. barberi* root; **(b).** Detailed TS of root; **(c).** Middle vascular region of root; **(d).** Middle xylem region enlarged view; **(e).** Center xylem region enlarged view.

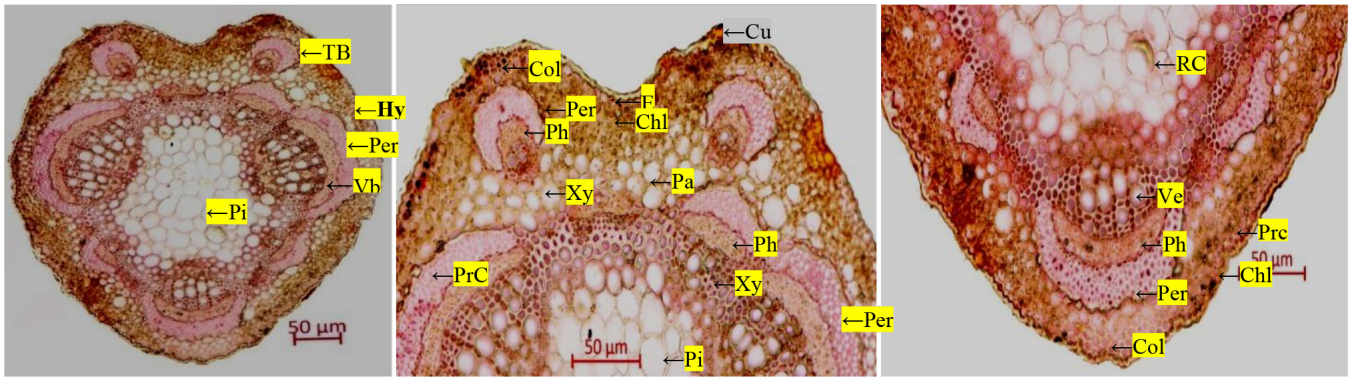
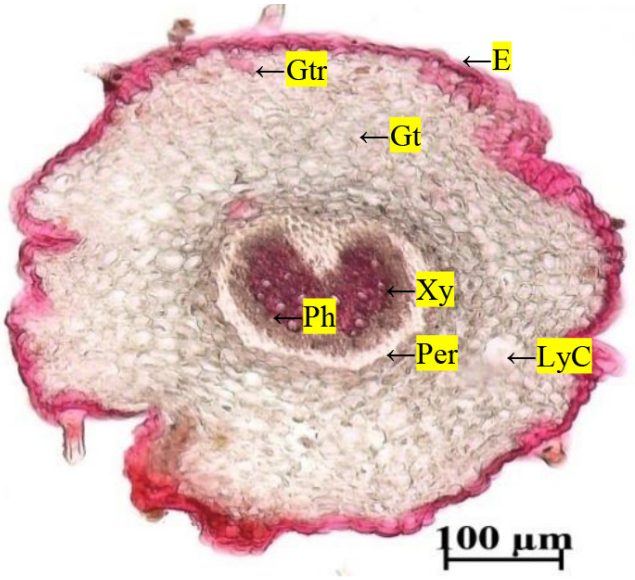


Figure 4. **(a).** TS of *T. barberi* rachis; **(b).** TS of rachis upper portion; **(c).** TS of rachis lower portion.

bundle is capped by an arc of 3 to 6 layers of thick-walled pericyclic fibre. Xylem and phloem consisted of usual elements. Throughout the section, a few parenchymatous cells surrounded with resin-like content and prism type of calcium oxalate crystals were seen (Figures 4(b, c)). The transverse section of the *T. barberi* petiole was circular with a wavy outline. The single-layered epidermis was covered by cuticle with a few covering and glandular trichomes; it was followed by a wide zone of thick-walled parenchymatous ground



Chl - Chlorenchyma; **Col** - collenchyma; **Cu**- Cuticle; **E** - epidermis; **Gt**-ground tissue; **Gtr** - glandular trichome; **Hy** - hypodermis; **LyC** - lysigenouscavity; **Pa** - parenchyma; **Per** - pericycle; **Ph**- Phloem; **pi** - pith; **PrC**- Prismatic crystals; **RC** - Resin content; **T**- Trichome; **TB** - trace bundle; **UE**- Upper Epidermis; **Ve**-Vessel; **Xy**– xylem

Figure 5. T. S. of *T. barberi* petiole.

tissue embedded with a few lysogenous cavities, starch grains, and an arc of conjoint, collateral vascular bundle at the centre encircled by thick-walled pericyclic cells (Figure 5).

The leaflet of *T. barberi* has isobilateral and amphistomatic. A single layer of upper and lower epidermis covered by thin cuticle with a few glandular and unicellular warty covering trichomes with sharp ends; an arc of conjoint, collateral vascular bundle covered by an arc of pericyclic fibre at the lower side and as a group on the upper side (Figures 6(a, b)). TS of the isobilateral lamina of *T. barberi* showed a single layer of upper and lower epidermis covered by thin cuticle with a few trichomes, followed by 2 to 4-celled palisade parenchyma and centrally located thin-walled spongy parenchyma tissue which embedded with chlorophyll content, vascular strands, mucilaginous cavities, reddish brown content, resin-like content, monoclinic styloid cystolith and prismatic crystals of calcium oxalate (Figures 7 (a, b)).

Additionally, the whole plant of *T. barberi* powder character was observed by the naked eye and under a microscope. The observation of *T. barberi* powder had

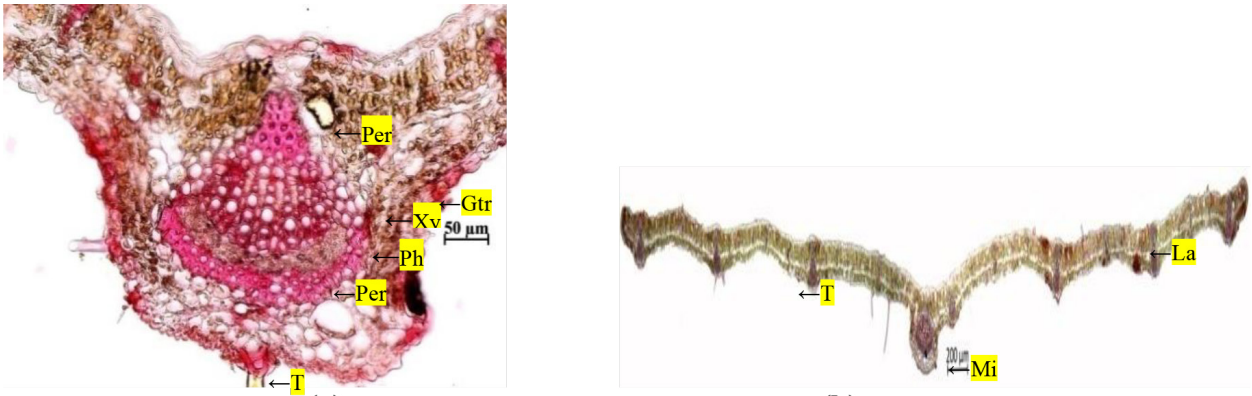


Figure 6. **(a).** TS of leaflet passing through midrib; **(b).** TS of leaflet.

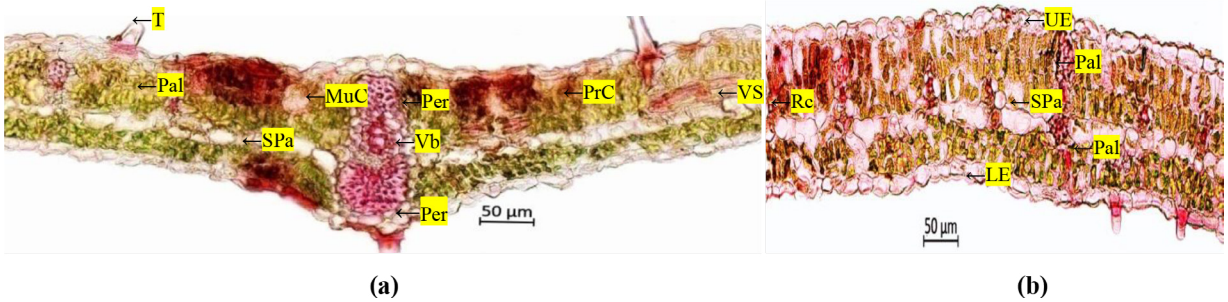


Figure 7. **(a).** TS of leaflet passing through lamina; **(b).** TS of lamina enlarged view.

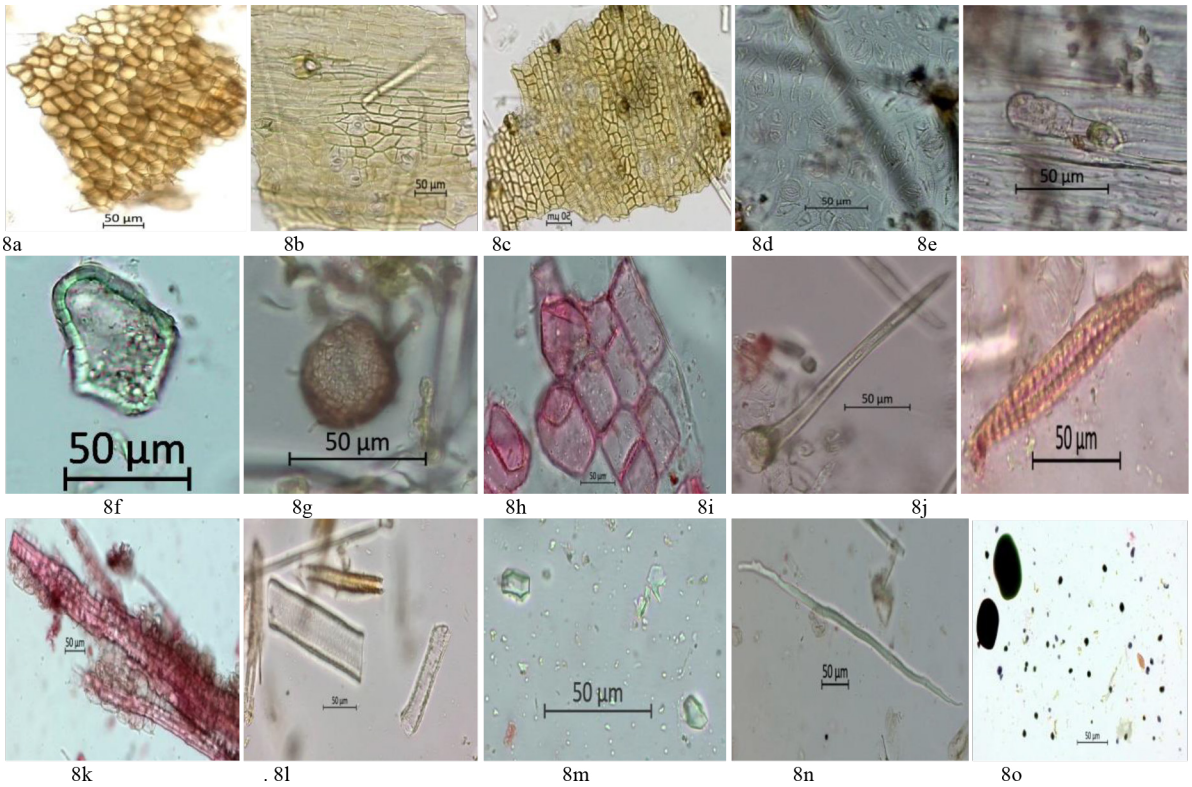


Figure 8. Pictorial representation of powder microscopy of the whole plant of *T. barberi*. **(a).** Root cork cell; **(b).** Rachis epidermal cells; **(c).** stem epidermal cells; **(d).** stomata; **(e).** glandular trichome; **(f).** stone cells; **(g).** resin content; **(h).** pitted parenchyma; **(i).** unicellular warty Trichome; **(j).** Tracheids; **(k).** crystal fibre; **(l).** pitted vessels; **(m**). Ca oxalate crystals; **(n).** fibre; **(o).** starch grains.

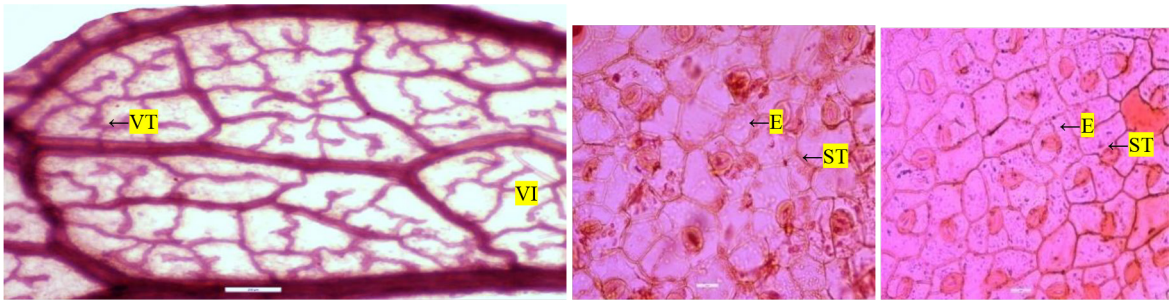


Figure 9. **(a).** Veinislet and veintermination of leaf; **(b).** Upper epidermis; **(c).** Lower epidermis.

reddish brown and contained cork cells from the root, epidermal cells from the stem and rachis, glandular and warty trichomes, crystal fibres, anomocytic and anisocytic type of stomata, mesophyll cells, stone cells, prismatic crystals of calcium oxalate, tracheids, pitted paren parenchyma, fibre bundle, pitted vessels, starch grains, resin-like content and reddish brown content cells (Figures 8 (a-o)).

The microscopical features of the entire plant of *T. barberi* exposed the existence of cork cells, epidermal cells, stomata, glandular trichome, stone cells, resin content, pitted parenchyma, unicellular warty Trichome, Tracheids, crystal fibre, pitted vessels, Calcium oxalate crystals, cystoliths fibre and starch grains which were supportive to recognize the adulteration of this perennial herb when it is also presented as powder form[32].

Table 1. Leaf constant values of *T. barberi*

Parameters	Upper epidermis	Lower epidermis
Epidermal number	740-790	820-1100
Stomatal number	150-172	220-262
Stomatal index	17.4	
Vein islet number	132-140	
Vein termination number	160-170	
Palisade ratio	35-57	

Table 2. Extractive values of the whole plant of *T. barberi*

Solvent	Weight of extract (g)	Weight of extract (%)
Petroleum ether	0.08	1.6
chloroform	0.21	4.2
Ethyl acetate	0.28	4.8
Methanol	0.52	10.4
Ethanol	0.35	7.0
Aqueous (water)	0.13	2.6

Table 3. Physiochemical standardization of the whole plant of *T. barberi*

Parameters	Weight (g)	Value in(%)
Total ash	0.03±0.12	3
Acid insoluble ash	0.01±0.21	1
Water soluble ash	0.01±0.08	1
Sulphated ash	0.05±0.16	5
Loss on drying	0.08±0.14	8

Table 4. Fluorescence analysis of various extracts of the whole plant of *T. barberi*

Extract	Daylight	UV @254 nm	UV@ 365 nm
Petroleum ether	Pale green	Green	Yellowish green
Chloroform	Brown	Light yellow	Green
Acetone	Brown	Pale green	Cream
Methanol	Green	Pale green	Yellowish green
Ethanol	Greenish brown	Green	Brown
Aqueous (water)	Yellowish brown	Pale brown	Greenish brown

Table 5. Fluorescence analysis of various reagents powder of *T. barberi*

Powder with reagents	Daylight	UV @254nm	UV@ 365 nm
Powder+ 1N NaOH	Yellowish brown	Green	Greenish brown
Powder+ 1N Alcoholic NaOH	Greenish brown	Green	Straw
Powder+ 1N HCl	Light brown	Light green	Cream
Powder+ CON.HCl	Dark brown	Yellowish green	Brownish-yellow
Powder+ CON H2SO4	Black	Dark green	Greenish black
Powder+ 50% H2SO4	Dark brown	Green	Brown
Powder+ CON HNO3	Reddish orange	Green	Dark green
Powder+ 50% HNO3	Brownish orange	Light green	Greenish yellow
Powder+ HNO3+ NH3	Pale orange	Light green	Orange
Powder+ Ammonia	Greenish brown	Green	Brown
Powder+ CH_3 COOH	Blackish brown	Light green	Green
Powder+ $Fecl_3$	Black	black	Dark green

3.1.2 Quantitative Microscopy of T. barberi

The leaf constant parameters like vein termination no, vein islet no, stomatal number, stomatal index and palisade ratio were quantified during microscopic observation of the epidermal husk of the leaf (Figure 9a) which was recorded in Table 1. The anomocytic stomata and anisocytic stomata were observed on both surfaces of leaves (Figure 9 (b, c)).

3.1.3 Physiochemical Parameters and Fluorescence Analysis

The measurement of physico-chemical parameters is crucial in assessing the efficacy and safety of herbal medications. Diverse solvents are employed to determine extractive values to accommodate the variety in the chemical structure and composition of medications 33. In the present investigation, the methanol extract of *T. barberi* exhibited the highest extraction value, followed by the ethanol extract. The

Table 6. The preliminary Phytochemical analysis of different extracts using *T. barberi*

Phytoconstituents	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
Alkaloids	---	--	+	+	+
Steroidal glycosides		+	+	+	+
Flavonoids	--	+	+	+	+
Polyphenols	--	+	+	+	+
Saponins	--	+	--	--	--
Tannins	--	--	--	---	--
Carbohydrates	--	--	---	--	--
Proteins	--	--	--	--	---
Resins	+	--	--	--	--

(+) Indicates Presence and (-) Indicates Absence

ash values of herbal medicines indicate the amount of earthy particles, inorganic substances, and other contaminants that are present. The loss-on-drying test procedure has been widely used to estimate the humidity content of a powdered material. When being stored, drugs should be kept at a low moisture level to stop the growth of bacteria, yeast, or fungi. *T. barberi* produced good results for all of the physiochemical parameters that were observed[34]. The observed physiochemical parameters in the whole plant of *T. barberi* are presented in Tables 2 and 3. Fluorescence analysis is a crucial ethnomedicinal factor. In daylight, some substances exhibited a visible range of glowing. There are numerous naturally occurring compounds that don't have remarkable luminescence during the day, but UV light causes fluorescence. If a product is not luminous by itself, it is frequently possible to change it into a fluorescent derivative or a degradation product by using specific reagents[35]. Fluorescence analysis is therefore an essential technique for determining the chemical components of herbal products[36]. The obtained results on the fluorescence characters of whole plant powder with different solvent extracts and with various reagents are shown (Tables 4 and 5) in varying colours (daylight, short and long UV light). Studying the fluorescence responses of *T. barberi* under various conditions, a chemical contained in the entire plant was shown to be correlated. Coumarin becomes yellowish-green in alkaline conditions when exposed to short-term UV rays. Under alkaline conditions, flavones appear yellow; but, when exposed to UV radiation, they appear brilliant yellow. Under brief UV light, terpenoids appeared yellowish green. Low fluorescence is exhibited by fats and fixed oils. Quinine and berberine (alkaloids) exhibit distinct fluorescence colors[37]. The observation of fluorescence analysis of the ethanol extract of *T. barberi* produced fluorescence colors also the same as those previously reported. Hence, it was found that coumarins, terpenoids, flavones, alkaloids, and steroids may be present in the *T. barberi* plant. The observation of all quality control parameters, physiochemical analysis, and fluorescence analysis using the whole plant of *T. barberi* showed satisfactory results.

3.1.4 Phytochemical Analysis

The primary phytochemical analysis of different extracts of *T. barberi* revealed the presence of steroidal glycosides, flavonoids, polyphenols, alkaloids, resins, and terpenoids (Table 6). These constituents are responsible for the drug's therapeutic efficacy and the ability to develop plant-based formulations for a variety of diseases[38].

4. Conclusion

Tephrosia barberi whole plant quality control parameters can be developed based on macro and microscopic features, physicochemical standards, fluorescence, and phytochemical analysis. Macroscopically, *T. barberi* leaves are 6 cm long, and leaflets are 05 to 1.5 cm cuneate. The flowers of *T. barberi* were reddish and about 1 cm long. The five eampanulate calyxes; flower petals were clawed, and a fruit contained 4-5 seeds which are kidney-shaped, elliptic ovoid which are characteristic features distinct from other *Tephrosia*

species. Microscopically both surfaces of the leaves of *T. barberi* showed anamocytic and anisocytic types of stomata, which is a specific characteristic of this species. The powder microscopy of *T. barberi* observed by cork cells from the root, epidermal cells from the stem and rachis, glandular and warty trichomes, crystal fibres, anomocytic and anisocytic types of stomata, mesophyll cells, stone cells, prismatic crystals of calcium oxalate, tracheids, pitted paren parenchyma, fibre bundles, pitted vessels, and starch grains, which were supportive of recognizing the adulteration of this perennial herb from related species. The methanol extract of *T. barberi* exhibited the highest extractive value, followed by the ethanol extract. The ash values of herbal medicines indicate the amount of earthy particles, inorganic substances, and other contaminants that are present. Fluorescence analysis is another useful technique for identifying the components of herbal medications and understanding their chemical composition. Various fluorescences produced from *T. barberi* treatment with different reagents confirmed the presence of numerous chemical compounds. Additionally, the primary phytochemical analysis of different extracts of *T. barberi* showed the presence of alkaloids, flavonoids, steroidal glycosides, polyphenols, and terpenoids, which would help in the investigation of its possible therapeutic actions. By observing the morphological, powder microscopical, and physiochemical characteristics of the crude drug, the results confirmed the whole plant of *T. barberi*. Consequently, in light of the current study on *T. barberi*, it can be considered a dependable tool for enhancing knowledge about its identification and authentication between related species. To the best of my knowledge, this is the first study on this species of that kind. Hence, this study is of great value for the correct identification of *T. barberi* and prevents adulteration from other species, which would be useful for future research on the isolation of phytoconstituents and therapeutic potency.

5. References

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