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Research Article

Quality Control and Comparative Study of Ayurvedic Plant *Tecomella undulata* (sm.) Seem. with its Adulterant *Aphanamixis polystachya* (Wall.) Parker

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ABSTRACT

Present study aims to carry out the quality standards and safety profile of stem bark of *Tecomella undulata* as per WHO guidelines and comparative evaluation with its adulterant *Aphanamixis polystachya*. Quality standards and safety profile parameters were performed as per WHO guidelines and comparative investigation is based on morphology, powder microscopy and HPTLC (High performance thin layer chromatography) fingerprinting using betulinic acid as an analytical marker. All the quality standards and safety profile parameters (Heavy metals, aflatoxins, microbial load and pesticidal residues) were found within the limit. Physiochemical parameters such as total ash value were 6.5 and extractive value was found highest in chloroform and lowest in hexane in cold and hot extraction. Preliminary phytochemical screening showed presence of glycosides, naphthoquinone, triterpenic acids and phenolic compounds. Comparative study of stem bark showed upper surface of *T. undulata* was like a crocodile skin with easily detachable fibres from the bark while *A. polystachya* have comparatively smooth upper surface and strongly adhered fibres with stem bark morphologically. Microscopically *T. undulata* contain prismatic calcium oxalate crystals with rare presence of sclereids and absence of stone cells as compared to powder of *A. polystachya* which showed rosette shaped calcium oxalate crystals with abundant sclereids and stone cells. HPTLC fingerprinting profile with betulinic acid indicated its presence in *T. undulata* and absence in *A. polystachya*. This study will guide in establishment of quality standards and identification of *T. undulata* with its commercial adulterant *A. polystachya*.

Key words: Quality standards, *Tecomella undulata*, betulinic acid, HPTLC, *Aphanamixis polystachya*, Safety profile.

INTRODUCTION

Tecomella undulata seem. (Bignoniaceae) is a deciduous medium sized tree, commonly known as Rohitaka, Rohira and Rakta-Rohida in India[1]. It was called as *Bignonia undulata* in past. In Indian system of medicine it has been used in the treatment of various liver diseases[2,3]. It contains chemically naphthoquinones, glycosides, phenolic compounds, betulinic acid and ferulic esters[3,4]. Its commercial adulterant is *A. Polystachya* which is commonly available in the market. Quality control and standardization are the important parameters to assure the identification and authentication of the herbal drugs. According to Handa, majority of herbal drugs used by industries and local communities come from wild collection and occasionally they are adulterated[5]. The increase demand of herbal drugs is the main cause of adulteration and it plays an important role in the decline of use of herbal drugs[6]. Adulteration also affects badly the promotion of herbal medicines and products[7]. Presence of adulteration in herbal drug has also been found to cause adverse events in most of the cases[8]. It is very difficult to identify adulterants without microscopic techniques and chemical analysis since commercial suppliers' use very high quality scientific processes to adulterate the drugs[9]. Among all quality standard parameters powder microscopy and HPTLC fingerprinting are mandatory to perform which is also mentioned in all official monographs. In past, simple and compound microscopes were used for microscopical identification of herbal drugs and its adulterants. The advancement in computer assisted microscopes produced more accuracy and authenticity in

Figure 1: Outer and inner surface of stem bark of *T.undulata*

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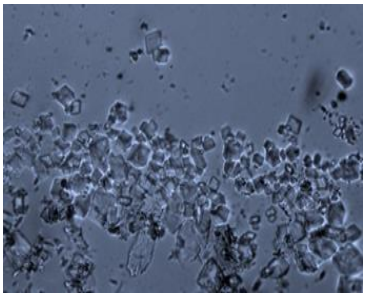
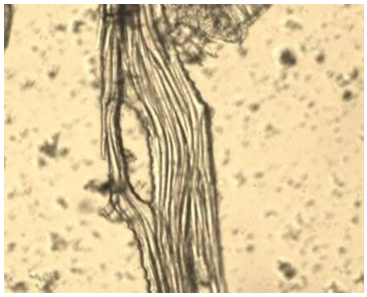
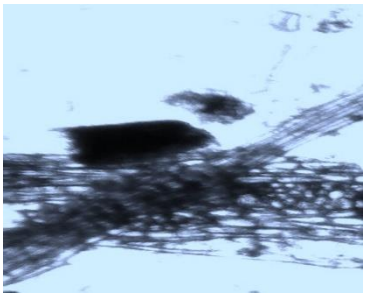


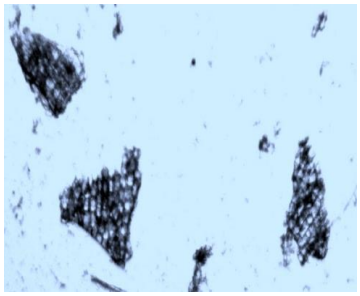
(a) Prismatic calcium oxalate crystals 10X



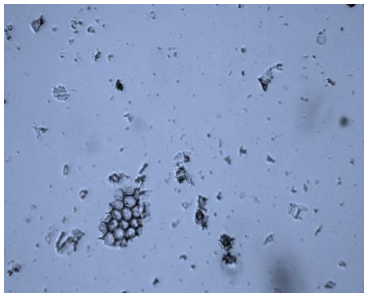
(b) Medullary rays with hollow pits 20X



(c) Medullary rays with filled pits 20X



(d) Ceratenchymatous cells 10X



(e) Cork cells 10X

Fig 2: Powder microscopy of stem bark of *Tecomella undulata*

evaluation of crude drugs. In recently published monographs, safety parameters are also included as these are very important parameters in respect to health issues related to herbal products. The underlying work deals with standardization (physico-chemical parameters), safety profile (determination of microbial growth, aflatoxins, pesticidal residues and heavy metal analysis) as per WHO guidelines. The comparative study of stem bark of *T. undulata* with its commercial adulterant *A.ploystachya* [1] is based on morphology, powder microscopy and HPTLC fingerprinting profile with betulinic acid as an analytical marker.

MATERIAL AND METHODS

Reagents and materials

Stem bark of *T. undulata* was collected from Lodhi garden, New Delhi and sample of *A. polystachya* was procured from Global Herbs, Chadini Chowk (Khari Bawli), New Delhi . The samples were authenticated by National Institute of Science Communication and Information Resources, New Delhi, India with ref no. NISCAIR/RHMD/Consult/2014/2472-51. A voucher specimen has been deposited for further reference. Betulinic acid was purchased from Sigma Aldrich and all the solvents of analytical grade from SD fine chemicals. Silica gel $60F_{254}$ HPTLC pre-coated plates were purchased from Merck.

Standardization of stem bark of Tecomella undulata (seem.)

Morphology

Visual examination of the untreated sample of stem barks of *T. undulata* was carried out under an artificial source of light similar to day light[10].

Powder microscopy

Powderd drug was cleared with the help of chloral hydrate solution and stained with phlouroglucinol and Conc. HCl.

Table 1: Foreign matter, Ash value, pH and Loss on drying

Tecomella undulata	% mean
Foreign matter	Nil
Ash value (w/w)	
Total ash %	6.5
Water soluble ash %	1.0
Acid insoluble %	0.8
pH	
1% solution	7.2
10% solution	7
Loss on drying	4.46

Powder was mounted with glycerine and observed under microscope for identification of lignification in cellular structures[11]; for stone cells and sclereides it was treated with dil. HNO3 and heated till boiled followed by a pinch of potassium chlorate[12]; for starch grains, powdered drug was treated with iodine water solution and calcium oxalate crystals were identified by suspending the powder in water and observed under motic microscope of 3.0 MP moticam (AE 2000)[11].

Physiochemical parameters

Extractive and ash value of the drug was carried out according to WHO guidelines[10] and compared with the limits mentioned in the monograph, ICMR[1]. Qualitative phytochemical tests were carried out by available standard methods[13, 11, 14]; pH and loss on drying was carried out according to Indian Pharmacopoeial Methods[15].

Safety profile of Tecomella undulata

Microbial load determination, aflatoxins, heavy metal analysis and pesticidal residues were determined according to methods prescribed in Indian Pharmacopoiea[16].

Comparative study of T. undulata with A. polystachya

Comparative evaluation of the stem bark of *T. undulata* with its adulterant *A. polystachya* was performed with the

Table 2: Extractive value of *Tecomella undulata*

Extraction (10gm *TU*)	Hexane	Chloroform	Ethyl acetate	n-Butanol	Aqueous
Hot (Soxhlet)	0.54 mg	2.546gm	2.41 gm	2.86 gm	1.67gm
Cold (Maceration)	0.16 mg	2.07 gm	1.87 gm	2.24 gm	1.42 gm

Table 3: Fluorescence analysis of *Tecomella undulata*

S.No	Treatment	Day light	UV short wave length (254 nm)	UV long wave length (366 nm)
1.	Powder as such	Dark brown	Greenish black	Purplish black
2.	Powder treated with distilled water	Brown solution	Greenish black	Purplish black
3.	Powder treated with H_2SO_4.	Black solution	Black	Black
4.	Powder treated with 1N aq. NaOH.	Brownish red	Blue fluorescence	Purple fluorescence
5.	Powder treated with $FeCl_3$	Dark reddish brown	Dark greenish fluorescence	White fluorescence

Table 4: Phytochemical tests of *Tecomella undulata*

Phytoconstituents present in the extracts	Result
glycosides	++
Phenolic acid	++
flavonoids	++
Carbohydrate	++
Naphthoquinones	++
Tannin	++

Table 5: Determination of heavy metals By ICP-OES Method

S.No	Heavy metals tested	Result	MDL
1.	Cadmium	Not detected	1ppm
2.	Lead	Not detected	1ppm
3.	Arsenic	Not detected	1ppm
4.	Mercury	Not detected	1ppm

Table 6: Aflatoxin determination By LCMS-MS

S.No.	Aflatoxins	Result	MDL
1.	B1	Not detected	1.0 µg/kg
2.	B2	Not detected	1.0 µg/kg
3.	G1	Not detected	1.0 µg/kg
4.	G2	Not detected	1.0 µg/kg

help of morphologyy[10], powder microscopy[11,12] with the help of computer assisted microscope and HPTLC finger printing with betulinic acid as an analytical marker.

HPTLC fingerprinting using betulinic acid as an analytical marker

Preparation of betulinic acid standard solution

Stock solution was prepared by dissolving 1mg of betulinic acid in 1 ml of HPLC grade methanol and stored at 2-8°C. Before the first use, the solution was filtered by 0.2 µm syringe filter (Axiva).

Sample Preparation

The powdered drugs, 10 gm each was extracted in methanol by using soxhlet apparatus to prepare methanolic extract of 10 mg/ml. The extract was filtered with 0.2 µm syringe filter (Axiva) and clear filtrate was taken and 6 µl was used for HPTLC analysis.

Chromatographic conditions

HPTLC analysis of methanolic extracts of *T. undulata* and *A. polystachya* was performed on HPTLC system (Shimadju, Japan) with Linomat 5 sample applicator and Linomat scanner III. The plate was developed in solvent system: toluene: ethyl acetate: glacial acetic acid (8.5: 1.5: 0.02). Sample was applied on precoated TLC plate in duplicate with the sample applicator and the plate was developed in twin trough chamber which was saturated with solvent system for 30 min, up to height of 8 cm. The plate was removed from the chamber and air dried for 30 minutes (because of toluene in solvent system) and was derivatized with anisaldehyde sulphuric acid reagent and scanned for betulinic acid at absorbance 510 nm.

RESULTS

Standardization of stem bark: Tecomella undulata

Morphology

The stem barks of *T. undulata* were slightly curved pieces. Upper surface was brown and rough due to presence of transverse cracks, rhytidoma and longitudinal furrows. Inner surface was dark brown and smooth. It was odourless and bitter in taste. Fibres detached easily from the main bark. Fracture was splintery and fibrous on inner side of the bark (Fig.1).

Powder microscopy

Brownish powder of stem bark of *T. undulata* contained hexagonal shaped cork cells single as well as in groups, cluster of prismatic calcium oxalate crystals and compound starch grains were found scattered throughout the slide. Wavy medullary rays with hollow pits filled with tannins and ceratenchymatous cells were distinctly observed and compared with ICMR monograph (Fig-2).

Physio-chemical parameters

All the phytochemical standards were established according to procedure laid down in WHO guidelines, Indian Pharmacopoiea and campared with ICMR monograph (Table-1).

The hot and cold extractive value was found to be highest

Table 7: Microbial load determination

S.No	Dilution of stock solution	No. of colonies		Colonies characteristics
		TU	Control	
1.	1:1	00	Nil	Not appeared
2.	1:10	00	Nil	Not appeared
3.	1:100	00	Nil	Not appeared

Table 8: Pesticidal Residue determination by GC-MS

S.No	Pesticide	Result	MDL
1.	Total DDE	Not detected	0.01 mg/kg
2.	Total DDT	Not detected	0.01 mg/kg
3.	Total DDD	Not detected	0.01 mg/kg
4.	2,4-D	Not detected	0.01 mg/kg
5.	Parathion	Not detected	0.01 mg/kg
6.	Malathion	Not detected	0.01 mg/kg
7.	Dieldrin	Not detected	0.01 mg/kg
8.	Aldrin	Not detected	0.01 mg/kg
9.	α- BHC	Not detected	0.01 mg/kg
10.	β- BHC	Not detected	0.01 mg/kg
11.	γ- BHC	Not detected	0.01 mg/kg

(a) Outer surface of stem bark

(b) Inner surface of stem bark

Fig 3: Macroscopical characters of stem bark of *Aphanamixis polystachya*

2.546 gm/10 gm in chloroform extract and lowest 0.54 mg/10 gm in hexane extract of the drug (Table-2). The powdered form of the drug was inspected in the visible light as well as in ultra violet light (254 and 366 nm) by treating with different reagents for fluorescence analysis (Table-3).

Phytochemical testing of the extract indicated the presence of naphthoquinones, ferulic acid esters, glycosides, triterpenic acids and phenolic compounds (Table-4).

Safety profile

Drug showed absence of heavy metals (Table-5) as well as aflatoxins (Table-6) and microbial load determination (Table-7). Pesticidal residues were found under the limit prescribed by the WHO guidelines and FAO and perfomed to check organo-phosphorous and organo-chlorine compounds and more harmful pesticides like total DDD, total DDE, total DDT, 2,4-D, Parathione, Malathione, Dieldrine, Aldrin, α, β and γ- BHC were not detectable in the sample of *Tecomella undulata* (Table-8).

Comparative evaluation of Tecomella undulata from Aphanmixis polystachya: an adulterant

Morphological comparison

Upper surface of stem bark of *T. undulata* (Fig-2) gave the appearance of a crocodile like skin and dark brown while in *A. polystachya* upper surface was comparatively smooth and greyish brown. *A. polystachya* was tasteless while *T. undulata* possessed bitter taste. *T. undulata* fibres were easily detachable from the main bark with splintery fracture while *A. polystachya* fibres were hard to detach from bark and tough to break (Fig-3).

Comparative study of powder microscopy

T. undulate powder showed prismatic calcium oxalate crystals with rare sclereids and absence of stone cells as compared to powder of *A. polystachya with* rosette shaped calcium oxalate crystals and abundant sclereids and stone cells; *T. undulata* powder showed hexagonal shaped cork cells as compared to pentagonal shaped in *A.polystachya* (Fig-4). Dark brown pigmentation of tannin was observed in medullary rays of powder of *T. undulata* (Fig-2).

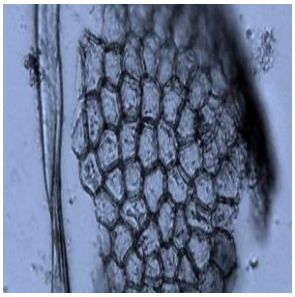
HPTLC fingerprinting profile comparison using betulinic acid

HPTLC of methanolic extracts of the stem bark of *T. undulata* and *A. polystachya* using toluene: ethyl acetate: glacial acetic acid (8.5: 1.5: 0.02) solvent system (fig-5). Plate clearly showed that the constituents of both drugs are different on the basis of *Rf* value, colour and number of spots in long wavelength (366 nm), short wavelength (254 nm) and after derivatization with anisaldehyde sulphuric acid reagent. Betulinic acid was visible as a purple colour spot, *Rf* (0.65) after derivatization in *T. undulata* but absent in *A. polystachya*. Betulinic acid being UV insensitive compound was visible on derivatization.

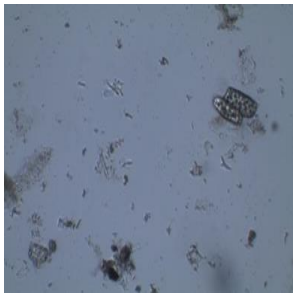
DISCUSSION

Establishment of quality control parameters, safety evaluation and authentication are the first and foremost steps in the research of any herbal drug or the preparation of formulation. At large, herbal plants are used without standardization due to which sometimes, its adulterants or spurious drugs are used in treatment of various diseases and produce harmful effect and hence ineffective. In the present study, foreign matter was absent due to self collection of stem bark of plant. Ash value has been carried out to determine the inorganic content which is associated with herbs such as dust, silica and stones but in our sample it was in lower amount as compared to ICMR monograph due to absence of dusty particles. Morphology of the crude drugs was observed by naked eye and the microscopy of powdered drug was performed to differentiate its cellular contents from its adulterants available in market. In this study, first time real photographical cellular structures have been presented. *T. undulata* can be easily differentiated from *A. polystachya* which is a commercial adulterant and mostly available in market in the place of *T. undulata*. The photographical presentation of the cellular contents of these plants will be helpful for the researchers to identify the genuineness of the plant. Extractive value is helpful for the determination of nature of compounds present in drugs such as in present study chloroform extract has highest extractive value which indicates the presence of medium polar compounds in the stem bark of *T. undulata.* Loss on drying was not more that 5%. In this study all the parameters are within the limit

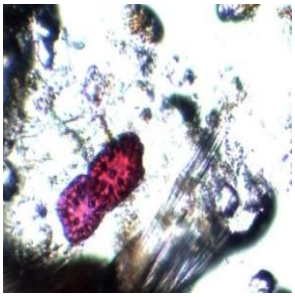
Comparative evaluation of stem bark of *T. undulata* from its adulterant *A. polystachya* will be helpful in authenticity of genuine drug and the parameters used for comparison are morphology, powder microscopy and HPTLC



(a) Cork cells along with fibre 20X

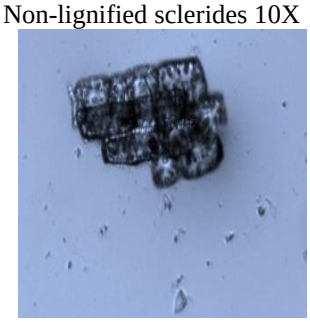


(b) Non-lignified sclerides 10X

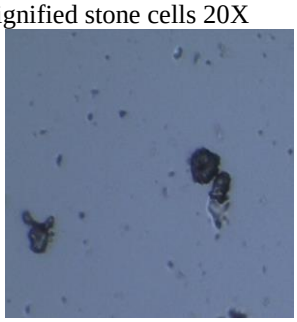


(c) Lignified stone cells 20X

(d) Lignified scleride cells 20X

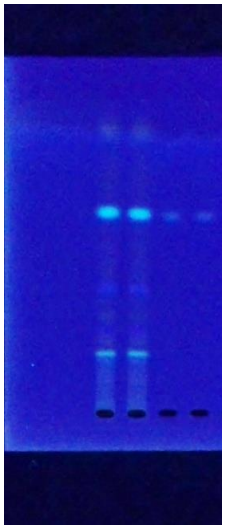


(e) Stone cells and sclerides in group 20X



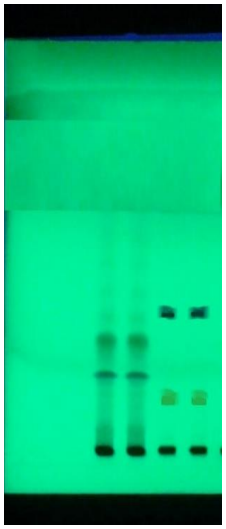
(f) Rossett calcium oxalate crystals 20X

Figure 4: powder microscopy of stem bark of *Aphanamixis Polystachya*



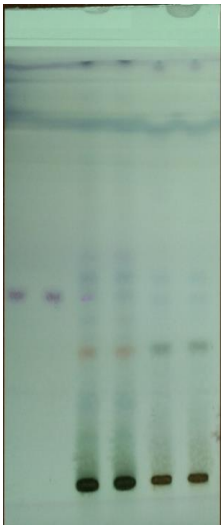
BA TU AP

(a) TLC fingerprinting of *TU*and *AP* with Betulinic acid at 366 nm



BA TU AP

(b) TLC fingerprinting of *TU* and *AP* with Betulinic acid at 254 nm



BA TU AP

(c) TLC fingerprinting of *TU* and *AP* with Betulinic acid after derivatization

TU: *Tecomella undulata*, *AP*: *Aphanamixis polystachya*, BA: Betulinic acid

Figure 5: HPTLC fingerprinting of *TU* and *AP* with Betulinic acid as marker

fingerprinting with Betulinic acid as analytical marker.

These are simple to perform and economic for the identification of *T. undulata*. Morphologically, upper surface of *T. undulata* was like a crocodile skin with easily detachable fibres from the bark while *A. polystachya* have comparatively smooth upper surface and strongly adhered fibres with stem bark. Microscopically *T. undulata* contain prismatic calcium oxalate crystals with rare presence of sclereids and absence of stone cells as compared to powder of *A. polystachya* which contain rosette shaped calcium oxalate crystals with abundant sclereides and stone cells. HPTLC fingerprinting profile with respect to betulinic acid indicated its presence in *T. undulata* and absence in *A. polystachya*.

CONCLUSION

The present study will be helpful in determination of purity and safety of the drug with the help of quality standards and safety profile parameters. Betulinic acid may be used as an analytical marker for *T. undulata* which is not present in *A. polystachya* and it will be helpful in authenticity of genuine drug. As per our information, real powdered microscopical photography of cellular contents of these

two plants with the help of computer assisted microscope has been presented for the first time.

ACKNOWLEDGEMENTS
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CONFLICT OF INTEREST
The authors have no conflict of interest.

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