Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2010; 2(2); 64-69

ISSN: 0975-4873

Research Article

Pharmacognostical Evaluation of *Tylophora indica* (Burm. F.) Merill. by Quality Control Parameters.

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ABSTRACT
For pharmaceutical purposes, the quality of medicinal plant material must be high as that of other medicinal preparations. Standardization problems arise from the complex composition of drugs which are used in the form of whole plant, parts of the plants and of plant extract. The present study involves the quality control study of parameters of leaves of *Tylophora indica* (Brum. F.) Merill., according to WHO guidelines and Pharmacopieal guidelines given for standardization of botanicals. Botanical evaluation- Morphological, Micrscopical, Physicochemical evaluation- Ash values and Extractive values, Moisture content, PH determination, Phytochemical screening, Powder drug reactions, Fluorescent analysis, Total microbial load present in the drug and TLC/HPTLC fingerprinting profile.

Keywords: Pharmacognostical evaluation, Standardization, *Tylophora indica*, Antioxidant, Antimicrobial.

INTRODUCTION

The herbal remedies are inherently safer then the potent synthetic drugs, which often produce undesirable side effects. Medicinal plants have been playing a significant role in the treatment of various ailments in India. A traditional medicine is fundamentally preventive, protective nutritive and curative. Therefore, traditional medicines are safe, sure and harmless which treat the patients without side effects. In spite of phenomenon progress in the area of development of new drugs from synthetic sources and appearance of antibiotic as major therapeutic agents, plants continue to provide basic raw material for some of the most important drugs [1].

Tylophora indica (Burm f.) Merill. (Family: Asclepidaceae) commonly known as Antmul is a twining perennial plant distributed throughout southern and eastern part of India in plains, forests, and hilly places [2]. The plant is found growing normally in Uttar Pradesh, Bengal, Assam, Orissa, Himalayas and sub Himalayas in India [3]. It is a branching climber or shrub that grows up to 1.5 meters, leaves are obvate-oblong to elliptic-oblong, 3-10cm long and 1.5-7cm wide [4]. Roots Long fleshy with longitudinally fissured light brown, corky bark. Flowers minute, 1-1.5 cm across, in 2-3 flowered fascicles in axillary umbellate cymes. Calyx divided nearly to the base , densely hairy outside; segments lanceolate, acute. Corolla greenish yellow or greenish purple; lobes oblong, acute. Fruit a follicle, up to 7 × 1cm, ovoid lanceolate, tapering at apex forming fine mucro, finally striate, glabrous, Seeds 0.6-0.8 × 0.3-0.4cm long [5]. The plant has been reported to contain 0.2-0.46% alkaloids viz. Tylophorine, tylophorinine, tylophorinidine, (+)septicine, isotylocrebrine, tylophorinicine, sterols, flavanoids, wax, resins, and tannins [6]. The plant has been traditionally used for the treatment of bronchial asthma, jaundice and inflammation [4, 7]. Its antitumor, immunomodulatory, antioxidant, antiasthmatic, smooth muscle relaxant, antihistaminic, hypotensive, antirehumatic activities are scientifically proven. In Ayurveda , the plant has been used in treatment of asthma , dermatitis and rheumatism [2, 8]. Although the leaf and root of this plant are widely used for treating jaundice in Northern Karnataka, there is a paucity of scientific evidence regarding its usage in liver disorder [4]. The other reported activities include immunomodulatory activity, anti-inflammatory activity, anticancer activity and antiamoebic activity [8, 9, 10, 11, and 12].

Fig 1 *Tylophora indica* (Burm f.) Merill.

MATERIALS AND METHOD

Collection of plant material: Fresh leaves of *Tylophora indica* is collected in the month of August-September (2009) from Herbal Garden of JAMIA HAMDARD, New

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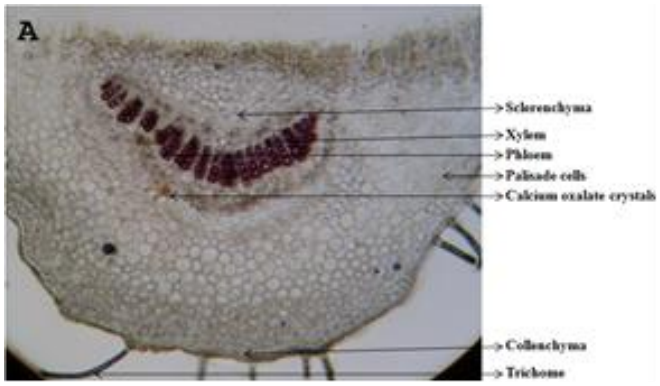

Fig 1 TS of Epidermal layer of *Tylophora indica* leaf

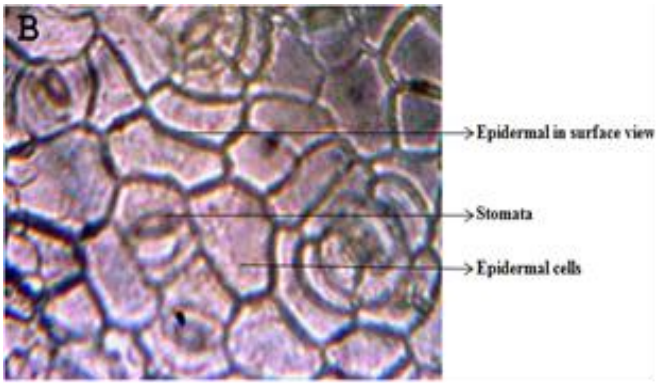

Fig 2 TS of Epidermal layer of *Tylophora indica* leaf

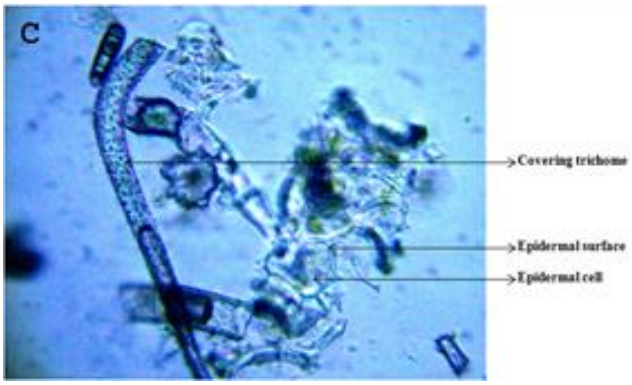

Fig 3 Powder microscopy of *Tylophora indica* Leaf

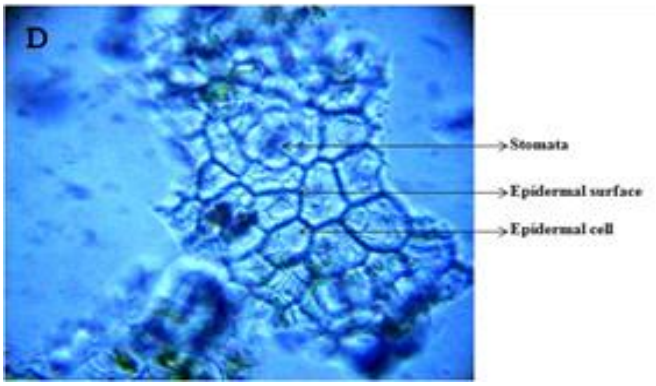

Fig 4 Powder microscopy of *Tylophora indica* Leaf

Table 1 Fluroscent analysis of leaves of *Tylophora indica*

S. No.	Solvent used	Ordinary light	UV light (254 nm)	UV light (366 nm)
1.	Methanol	Green	Blue	Yellow
2.	Hexane	Light yellow	Light green	Yellow
3.	Acetone	Yellowish green	Green	Orange
4.	Ethyl acetate	Yellowish green	Light green	Pink
5.	Chloroform	Yellowish green	Green	Orange
6.	Water	Light green	Green	Light yellow
7.	Conc HNO_3	Orange	Yellow	Yellowish brown
8.	Acetic acid	Green	Green	Orange
9.	Conc H_2SO_4	Greenish black	Dark green	Green

Delhi was identified and authenticated at Raw material, Herbarium and museum NISCAIR, CSIR, New Delhi, India and sample was submitted in museum for future reference. Ref. NISCAIR/RHMD/consult/-2009-10/1361/163.

Extraction of plant materials: 20gm course powdered of air dried leaves of *Tylophora indica* were packed in muslin cloth and subjected to soxhlet extractor for continuous successive hot extraction with petroleum ether, chloroform, acetone, methanol, and water respectively. Then each extracts were filtered and filtrate was evaporated to dryness. The percentage yield of Pet. Ether, chloroform, acetone, methanol and water is recorded. Individual cold & hot extractive values of powdered leaves is also determined and recorded according to the standard procedure [17]. Macroscopical and Microscopical Studies: Visual inspection is done on leaves to establish identity, purity and possibly quality of the sample. Parameters like color, odour, taste, size, shape, surface is observed. Macroscopy of the leaves is studied according to the standard procedure [17]. Once the leaves is examined and classified according to external characterstics, inspection by microscope can be carried out at the next step. The microscopical examination of leaves also in powder from carried out in accordance with the standard procedure described [17]. Photos were taken of the best slides observed during the microscopy examination with help of digital camera.

Physico-chemical Analysis: Physicochemical analysis i.e. Total ash, Acid- insoluble ash, water-soluble ash, foreign matter analysis, Fluorescent analysis, Powder drug reaction and moisture content is carried according to the WHO guidelines for standardization of botanicals [17]. For

Table 2 Powder drug reaction of *Tylophora inidca*

S. NO	REAGENTS	OBSERVATION
1.	Acetone	Yellowish green
2.	Water	Light green
3.	Methanol	Green
4.	Chloroform	Yellowish green
5.	Conc. HNO_3	Orange
6.	Conc. H_2SO_4	Greenish black
7.	Acetic acid	Green
8.	Hexane	Light yellow
9.	Ethyl acetate	Yellowish green

Table 3 Determination pH of the drug

S. No	% Drug	P_H
1.	1% filterd aq. Solution	6.7
2.	10% filterd aq. Solution	5.9

Table 4 Total Microbial Load of the drug

S.NO	Stock solution dilution	CFU(Colony forming units) g/ml
1.)	1:1	10^3
2.)	1:10	10^1
3.)	1:100	10^0

PH determination calibrated digital PH meter was used to measure the PH of 1 and 10% aqueous extracts.

Preliminary Phytochemical screening: Preliminary phytochemical screening for the detection of various chemical constituents is carried out by using standard test procedures described by Harborne [18] and khandelwal [19].

Total Microbial Load: One gram of drug was taken and suspended in 50ml of sterile distilled water. The suspension was shaken for sufficient period of time so as to allow maximum mixing. After this the suspension was filtered by using a disposable sterilized filter paper. The filtrate was used as stock solution. Series dilution (1:1, 1:10, 1:100) of this stock solution were made and 1ml of different diluted solution was separately inoculated (with spreading method) on a nutrient agar medium and incubated at 37°C for 24 hours. After 24 hours, the Petri plates with most clearly visible colonies were taken and number of colonies determined by using colony counter. The microbial load per gram of sample was then calculated by using dilution factor.

Composition of nutrient agar medium

a. Agar	15.0%
b. Peptic Digest of Animal Tissue	5.0%
c. Sodium Chloride	5.0%
d. Beef Extract	1.5%
e.Yeast Extract	1.5%
f. pH	7.4 ± 0.2 at 25°C
g. Distilled Water	1000ml

The medium was autoclaved at 15lbs per square inch pressure at 121°C.

TLC/HPTLC Profile: Developing solvent system- A number of solvent systems were tried, for extracts Methanolic extract, Chloroform extract, and Petroleum ether extract For chloroform extract-Chloroform(90): Methanol (5) : Ethyl acetate (5), Methanol Extract-Toluene(5): Chloroform(90), Ethyl acetate(5) and for Petroleum ether extract-Hexane(40) : Ethyl acetate (60) v/v.

Sample application: Application of bands of each extract was carried out (5mm in length and 2 µl in concentration) using spray technique. Sample were applied in duplicate on pre-coated silica gel $60F_{254}$ aluminium sheets (20x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software .

Development of chromatogram: After the application of spots, the chromatogram was developed in Twin trough glass chamber 20x 10 cm saturated with solvent ethyl acetate: acetone (4:1 v/v) for 20 min.

Detection of spots: The air-dried plates were viewed in ultraviolet radiation to mid day light. The chromatograms were scanned by densitometer at 366 nm. The R_f values and finger print data were recorded by WIN CATS software. The data obtained are summarized in Table (1, 2 &3) for where as the developed chromatogram can be seen at 366 nm.

RESULTS AND DISCUSSION

Macroscopic characters of the leaves: Leaf 5-10 cm long, 2.5-5.7 cm broad, ovate or epileptic-oblong, acute or acuminate, often apiculate, glabrous, more or less pubescent especially when young, petioles 6-13mm long.

Microscopic characteristics: TS of dorsiventral leaf is composed of an outermost layer of thin walled, single layered epidermal cells covered by thin cuticle. Mesophyll differentiated into 2-3 layered palisade and 6-8 layered spongy parenchyma, the latter containing rosettes of calcium oxalate (druses). Epidermal peeling exhibits characteristics covering trichomes, multicellular (3 to 8 celled), uniseriate, bent and tapering at the end. Paracytic stomata are seen only on the abaxial surface. In the midrib region, collenchyma is present below the upper epidermis and above the lower epidermis. In the centre, xylem elements are arranged in an arc and phloem occurs on both sides of it. Many idioblasts with crystals are found in the ground tissue.

Physicochemical parameters: The successive solvent extractive values of leaves of T*ylophora indica* with solvents: petroleum ether, chloroform, acetone, methanol and water extract was found 1.8, 1.0, 0.9, 15.5, 6.8 to be respectively in successive extraction. Extractive value are found to be maximum in methanol i.e. 15.5% w/w. The individual (Hot) solvent extractive value of petroleum ether, methanol and water extract was found to be 2.44, 5.67, 15.53 respectively in individual hot extraction. Extractive value are found to be maximum in water i.e. 15.53% w/w. The Individual (Cold) extractive value of petroleum ether, chloroform, acetone, methanol and water extract was found to be 2.53, 6.85, 13.44 respectively in cold extraction. Extractive value are found to be maximum in water i.e. 13.44% w/w. Total ash, acid insoluble and water soluble ash was found to be 10.19, 3.45, 5.34% w/w in leaves of *Tylophora indica.* The foreign matter in leaves of *Tylophora indica* was found to

Table 5 Phytochemical screening of leaves of *Tylophora indica*

S. No	Phytoconstituent	Tests	Water extract	Hydro-alcoholic Extract
1.	Alkaloid test	Dragandroff test	+ ve	+ ve
		Hager test	+ ve	+ ve
		Wagner test	+ ve	+ ve
2.	Test for glycosides	Borntrager test	+ ve	+ ve
		Keller- killiani test	+ ve	+ ve
		Legal test	+ ve	+ ve
3.	Test for Phenolics	Ferric chloride test	+ ve	+ ve
		Lead acetate test	+ ve	+ ve
4.	Tannins test	Gelatin test	+ ve	+ve
		Vanilllin test	+ ve	+ ve
		Matchstick test	+ ve	+ ve
5.	Protein & Amino acids test	Biuret test	+ ve	+ve
		Xanthoprotic reaction	+ ve	+ ve
		Millons reaction test	+ ve	+ ve
		Ninhydrin test	+ ve	+ ve
6.	Carbohydrate tests	Fehling test	+ ve	+ ve
		Molish test	+ ve	+ ve
7.	Sterol test	Salwoski reaction	- ve	- ve
		Libermaan buchard test	- ve	- ve
8.	Saponins test	Foam test	+ ve	+ ve
		Haemolysis test	+ ve	+ ve
9.	Tests for acid compounds	Sodium bicarbonate test	- ve	- ve
		Litmus paper test	- ve	- ve

HPTLC (High performance Thin Layer chromatography)
For Petroleum Ether:

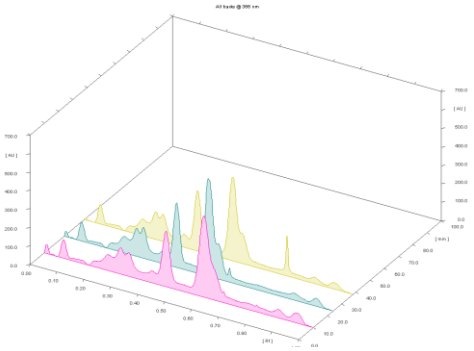

Fig 5 Pet. Ether Ext. 3-D View at 366nm.

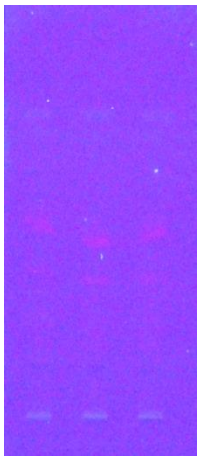

Fig 6 Developed TLC plate of Pet. Ether extract of *Tylophora indica* at 366nm.

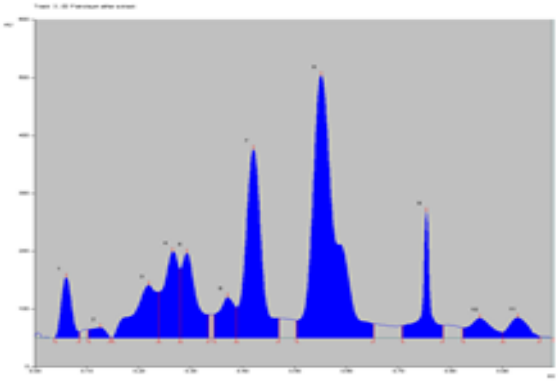

Fig 7 Pet. Ether Ext. peaks at 366nm.

For Methanolic extract:

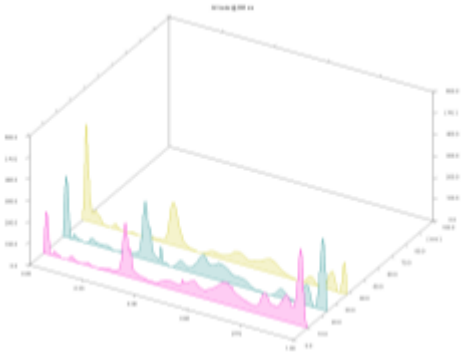

Fig 8 Methanolic Ext. 3-D View at 366nm.

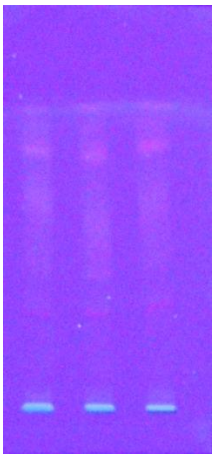

Fig 9 Developed TLC plate of methanolic extract of *Tylophora indica* at 366nm

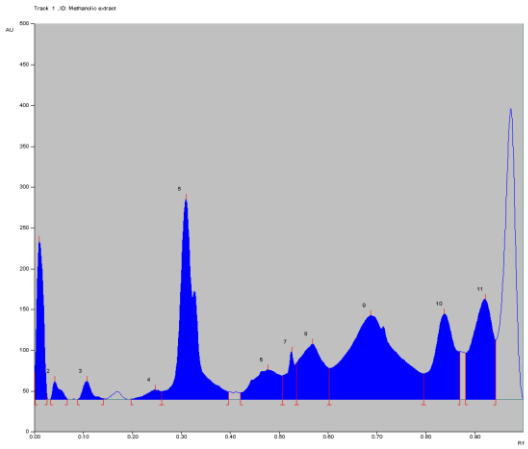

Fig 10 Methanolic Ext. peaks at 366nm.

Table 6 TLC of Leaves of *Tylophora indica*

Extract	Solvent system	No. Peaks/ Rf value
Chloroform	Chloroform (90): methanol (5) : ethyl acetate (5)	**(8)** 0.03, 0.52, 0.59, 0.66, 0.74, 0.78, 0.86, 0.95
Methanol	Toluene(5):Chloroform(90):ethyl acetate(5)	**(12)** 0.01, 0.04, 0.11, 0.17, 0.24, 0.31, 0.37, 0.45, 0.53, 0.60, 0.83, 0.92
Pet. Ether	Hexane(40):ethyl acetate(60)	**(12)** 0.01, 0.07, 0.13, 0.22, 0.27, 0.29, 0.37, 0.42, 0.53, 0.61, 0.85, 0.94

be 2.8 % w/w. The moisture content of *Tylophora indica* leaves powder was found to be 1.367% w/w.

Preliminary Phytochemical Screening: The phytochemical screening of leaf extract of Aq. & methanolic extract shows the presence of Alkaloids, Glycosides, Phenolics, Tannins, Proteins & Amino acids, Carbohydrates, and saponins compounds in them. Total Microbial Load: The microbial load in Aq. leaf extract of *Tylophora indica* was found to 10^3 CFU g/ml in 1:1 dilution, 10^1 CFU g/ml in 1:10 dilution and 10^0 CFU g/ml in 1:100 respectively.

CONCLUSION

Herbal drugs are derived from heterogeneous sources leading to variations. This makes the standardization of herbal medicines more important as erroneous results can cause variations in pharmacological studies. The pharmacognostic characters and physicochemical values in this paper could be used as a diagnostic tool for the standardization of this medicinal plant *Tylophora indica*. Presence of adulterants can be easily identified using these parameters. The microscopical feautures could help in laying down micro mophorphological standards as per WHO guidelines for authentification of the drug. After present investigation it can be concluded that the standardization, preliminary phytochemical screening, TLC/HPTLC fingerprinting profiling can serve as important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies. This study is an essential step and it further requires a long term study to evaluate therapeutic efficacy of leaf as the drug.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Sayeed Ahmad, Assistant Professor, Faculty of Pharmacy, Jamia Hamdard, New

For Chloroform Extract:

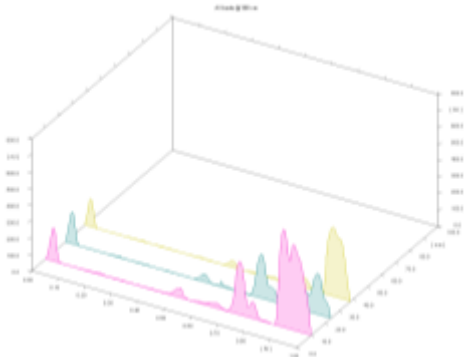

Fig 11 Chloroform extract 3-D view at 366nm.

Fig 12 Developed TLC plate of chloroform extract of *Tylophora indica* at 366nm

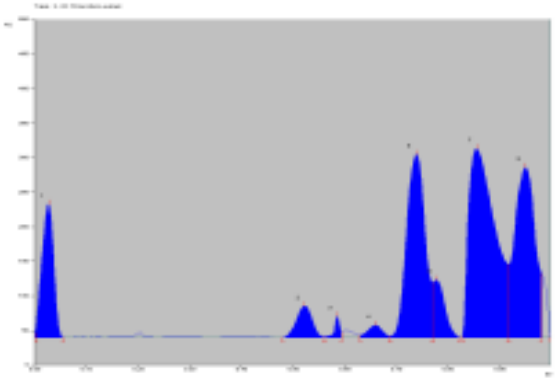

Fig 13 Chloroform Ext. peaks at 366nm

Delhi, for providing chemicals and reagents and providing laboratory facilities. We extend our sincere thanks to Dr. Hayat M. Mukhtar, Principal/Director, SBS College of pharmacy,Patti, Punjab, for his encouragement and critically reading manuscript and providing the valuable suggestions.

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