RESEARCH ARTICLE

Physicochemical, qualitative, and high profile thin-layer chromatography study of *Tylophora indica* (Burm. f) Merr. leaves and roots

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Abstract

Introduction: India is the land with near estimation of 6000–7000 medicinal floras. Comprehensive ethnobotanical studies of these natural healers highlight their multiple therapeutic applications. Moreover, different parts of a plant reveal different pharmacological activities due to the variation of phytoconstituents. Hence, standards should be available for all parts which are playing an important role in the pharmacological profile of the drug. The whole plant of *Tylophora indica* is used for the different therapeutic attributes, but standards of only leaves are available. Therefore, in the present study roots of *T. indica* were evaluated for establishing the standards of roots. **Materials and Methods:** *T. indica* leaves and roots were collected from the Ayushya Vatika, Lovely Professional University and subjected to physicochemical, qualitative, and high profile thin-layer chromatography (HPTLC) study. **Results:** Results of physicochemical analysis of leaves were complying with the standards and results of roots for loss on drying, total ash, water soluble ash, acid insoluble ash, methanolic extractive values, and water-soluble extractive were 11.3 ± 0.6%, 6.8 ± 1.32%, 4.16 ± 0.98%, 1 ± 1.0%, 30.4 ± 1.75%, and 20 ± 1.6%, respectively. Qualitative analysis revealed that leaves are devoid of steroids, terpenoids, and amino acids. In HPTLC analysis two different solvents were used for both the samples and different numbers of RFs were observed in different samples. X-ray powder diffraction study of root indicates the absence of heavy metals such including mercury, lead, and arsenic. **Conclusion:** The investigated *T. indica* leaf samples comply with the standards. The results of the study revealed entirely different physicochemical, qualitative, and HPTLC profiles of roots. Hence, standards should be developed for the individual plant part which is going to be used as a medicine.

Key words: High profile thin-layer chromatography, physicochemical, phytochemical, qualitative, *Tylophora indica*, X-ray powder diffraction

INTRODUCTION

Recent research has shown its inclination toward the field of pharmaceutics, nutraceuticals, and food industry with the herbal drugs. Nearly 80% of the total world population is still dependent on herbs as the primary health-care treatment.[1] India is one of the countries which possess about 15 agro-climatic zones and has the richest biodiversity in the world with approximately 1178 medicinal species.[2] Nearly 242 medicinal species have an annual consumption level above 100 metric tons which estimate the recognition of these herbs as medicines.[2] Ethnomedicine highlights the therapeutic value of different parts of a plant in different ailments. This has even been investigated with a scientific approach such as *Randia dumetorum* and *Azadirachta indica*. In case of *R. dumetorum*, only the fruits are emetic because of the presence of glycosides such as radianin and radioside A, which is not found in other parts of the plant such as root and bark.[3] The seed oil of *A.*

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indica possesses compounds such as nimbin and nimbidine which is responsible for its efficacy against leprosy. The compounds found in the bark are margolone, margolonone, and isomargolonone which are antibacterial in nature.[4] Therefore, the difference of bio-constituents in different parts is responsible for distinctive pharmacological actions. Due to this difference, there is a need for establishing standards for different parts of plants using standard parameters. Even in general practice, the standards of one part of a plant are established, and all other parts of the plant are also used. Therefore, such parts should also be standardized before their use to know the quality of part used. Hence, standards of each part of plants should be made available the to enhance the quality, safety, and efficacy of phytopharmaceuticals.

Tylophora indica (Burm. f.) Merr. is a climber of family Asclepiadaceae. In general, its leaves and roots are used since classical era. *T. indica* has a significant role in the management of asthma, diarrhea, and rheumatoid arthritis. *Bengal Pharmacopeia* has officially enlisted this knotted climber with the name of Antmool in 1884. The phytochemicals of this perennial climber are reported to the presence of alkaloid and non-alkaloid inclusions. The presence of different phenanthroindolizidine alkaloids has been reported including tylophorine, tylophorinine, and tyloindicines whereas non-alkaloid bioactive constituents are β-sitosterol, kaempferol, quercetin, wax, and pigments. Massive exploitation of *T. indica* for its use as the anti-asthmatic therapeutic agent has enlisted it in endangered species. Immense work has been reported for its propagation by micropropagation techniques for the maintenance of the species.[5]

In the present study, an effort was made to evaluate the different analysis (physicochemical, phytochemical, and elemental) of leaves and roots of an indigenous plant, *T. indica* (Burm. f.) Merr.

MATERIALS AND METHODS

Collection and Identification of the Plant Material

A whole plant of *T. indica* [Figure 1] was collected in the months of June 2017 from Ayushya Vatika of Lovely Professional University, Punjab, and authenticated from the Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab. The collected sample was washed thoroughly to remove foreign matters and shade dried. The dried leaves and roots were coarsely powdered and stored for the further analysis.

Figure 1: The figure represents the various parts of *Tylophora indica*. (a) *T. indica:* Whole plant. (b) *T. indica*: Leaves. (c) *T. indica*: Dried roots

Physicochemical Analysis

The coarse powders of leaves and roots of *T. indica* were subjected to the different physicochemical parameters such as loss on drying (LOD), ash values (total, acid insoluble, and water soluble), and extractive values (water and alcohol) as per the standard protocol.[6-10]

Preliminary Phytochemical Screening

The aqueous and methanolic extracts of *T. indica* were preserved in sterilized containers in the refrigerator and were utilized for qualitative analysis to determine the presence of chemical constituents of different classes as per the standard protocols.[6-10]

High Profile Thin-layer Chromatography (HPTLC) Profile

HPTLC studies were carried out from the Herbal Health Research Consortium, Amritsar, Punjab, India, with CAMAG Linomat 5 instrument. Two solvent systems were used to analyze the presence of different chemical components. Toluene, Ethyl acetate, and Diethylamine in the ratio of 14:2:2, respectively, were used as a first solvent system[11] whereas Toluene, Chloroform, and Diethylamine in the ratio of 5:90:5, respectively, were used as a second solvent system.[12] The plates were analyzed at 254 nm and 366 nm. The peaks and respective RF values were observed and recorded.

Elemental Analysis of *T. indica* Root

X-ray diffraction technique was used to analyze the presence of different elements and minerals present in roots of *T. indica*. The *T. indica* root powder was sieved (100 no.) and 5 g sample was provided for X-ray powder diffraction (XRD) at SAIF, Punjab University, Chandigarh, India.

RESULTS

Physicochemical Analysis of *T. indica*

The LOD, total ash, acid insoluble ash, water-insoluble ash, methanol soluble extractive (MSE), and water-soluble extractive (WSE) of *T. indica* leaves and roots were examined, and the results have been presented in Table 1. Six replicates were prepared and mean of the values with standard deviation has been reported.

Preliminary Qualitative Phytochemical Analysis

The phytochemical screening of leaves indicates the presence of alkaloids, carbohydrates, flavonoids, and glycosides. Saponin has been revealed in aqueous extract while not in methanolic extracts of both leaves and root. Amino acids, steroids, and terpenoids are not present in the leaf extracts. The root extracts demonstrated the presence of alkaloids, carbohydrates, flavonoids, glycosides, tannins, steroids, and amino acids [Table 2].

HPTLC Studies

The methanolic extracts of leaves and root were examined for their constituents by HPTLC with two different solvent systems [Figure 2-5]. The RF values of the conducted studies have been presented in Table 3.

Elemental Analysis of *T. indica* Roots

The fine root powder was tested for the presence of various inorganic elements by XRD analysis. The results have been presented in Table 4.

DISCUSSION

Crude drugs play a very important role in the treatment of various diseases as single or multidrug formulations. The active constituents present in them are responsible for characterizing them as medicine. The controlling authorities have elaborated the drug standardization parameters which help in recognizing the quality of the crude drugs. In the present study physicochemical analysis, phytochemical screening, HPTLC and XRD investigation of *T. indica* leaves and roots had been conducted.

Physicochemical analysis plays an important role in understanding quality and purity of crude drugs.[13] In the present study, the result of physicochemical analysis of dried leaves of *T. indica* was found within the suggested limits.[14] The LOD values of dried leaf and root were found to be 4.5 ± 0.5 % and 11.3 ± 0.6%, respectively. The proper LOD of crude drugs reduces the moisture and thereby inhibits the growth of microbes and fungi leading to reduced biological contamination.[15] This helps in long-term retention of active constituents in the crude drug by inhibiting their degradation by microbes. Ash values contribute significantly in determining the purity and authenticity and hence are an important tool for determining the quantitative standards of a crude drug. They may also help in understanding the extent of adulteration and impurities present in the raw material.[16] This can help the manufacturers in understanding the quality of raw material for product production. Total ash determined in leaves and root has been 11.08 ± 0.9% and 6.8 ± 1.32%, respectively. Acid-insoluble and water soluble ash were higher in dried leaves 12.8 ± 1.16% and 6.6 ±

Table 1: Values of physicochemical analysis of *T. indica*

Parameter	Observation (dried leaves) (%)	Observation (roots) (%)
LOD	4.5±0.5	11.3±0.6
Total ash	11.08±0.9	6.8±1.32
Acid insoluble ash	12.8±1.16	1±1.0
Water-soluble ash	6.6±0.4	4.16±0.98
MSE	29.0±2.35	30.4±1.75
WSE	38.9±2.4%	20±1.6

T. indica: Tylophora indica, LOD: Loss on drying, MSE: Methanol soluble extractive, WSE: Water soluble extractive

Table 2: Results of qualitative analysis of various phytochemicals in *T. indica*

Component analyzed	Leaf Extracts		Root extracts	
	Aqueous	Methanolic	Aqueous	Methanolic
Alkaloid	+	+	+	+
Carbohydrates	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Glycosides	+	+	+	+
Steroids and terpenoids	–	–	+	+
Saponins	+	–	+	–c
Amino acids	–	–	+	+

+: Present, –: Absent. *T. indica: Tylophora indica*

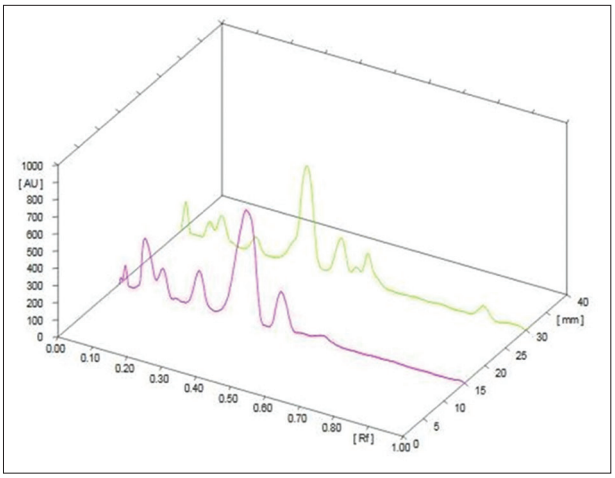

Figure 2: Three-dimensional view of methanolic extract of *Tylophora indica* with solvent system 1 at 254 nm

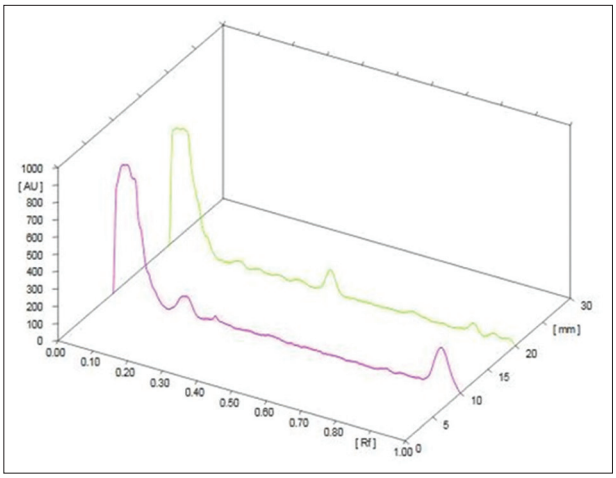

Figure 4: Three-dimensional view of methanolic extract of *Tylophora indica* with solvent system 2 at 254 nm

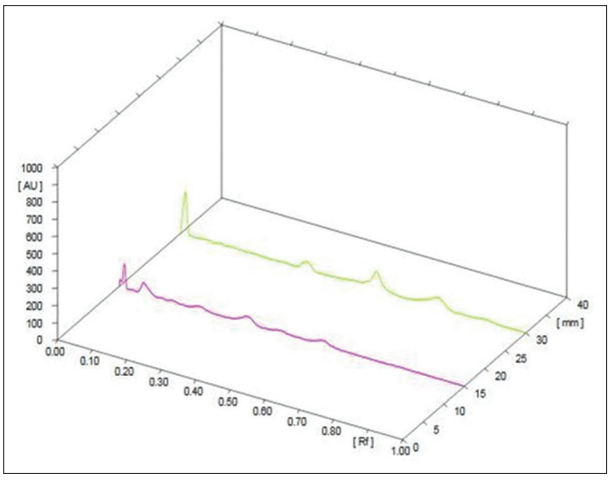

Figure 3: Three-dimensional view of methanolic extract of *Tylophora indica* with solvent system 1 at 366 nm

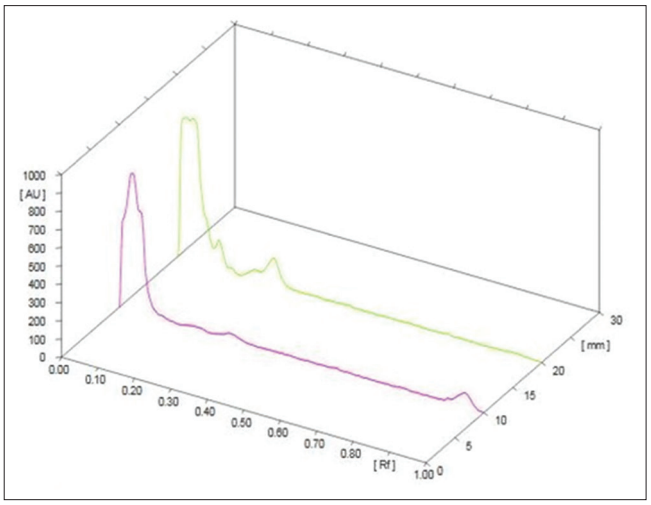

Figure 5: Three-dimensional view of methanolic extract of *Tylophora indica* with solvent system 2 at 366 nm

Table 3: Determined RF values of *T. indica*

Solvent system 1				Solvent system 2			
Root extract		Leaf extract		Root extract		Leaf extract	
254 nm	366 nm	254 nm	366 nm	254 nm	366 nm	254 nm	366 nm
0.02	0.02	0.04	0.04	0.04	0.04	0.03	0.03
0.07	0.07	0.21	0.21	0.21	0.21	0.04	0.04
0.13	0.24	0.30	0.22	0.30	0.22	0.20	0.11
0.23	0.37	0.45	0.30	0.45	0.30	0.26	0.15
0.47	0.47	0.46	0.95	0.46	0.95	0.33	0.21
0.59	0.59	0.79		0.79		0.37	0.27
		0.54		0.84		0.47	
		0.88				0.71	
		0.96				0.74	
						0.88	
						0.94	

T. indica: *Tylophora indica*

Table 4: The reported values of various elements detected in root sample of *T. indica*

Component detected	Value (%)	S. No.	Component detected	Value
$C_6H_{10}O_5$	95.69	13	Sr	0.01%
Ca	1.03	14	Ba	0.01%
K	0.89	15	Zn	75 ppm
Si	0.74	16	Mn	56 ppm
Mg	0.44	17	Cu	26 ppm
Al	0.32	18	Ni	15 ppm
Na	0.27	19	Ru	13 ppm
Fe	0.17	20	Cr	10 ppm
P	0.14	21	Mo	7 ppm
Cl	0.12	22	Rb	7 ppm
S	0.12	23	Zr	5 ppm
Ti	0.02	24	Sum	100%

T. indica: Tylophora indica

0.4%, respectively. The ash values are indicative of various inorganic matters and metallic salts such as carbonates, phosphates, silicates, sand, and so on which may be present in the crude samples and are soluble in different solvents like water and acids.[17]

The extractive values of a crude drug demonstrate the solubility of chemical constituents in the different solvents. The extractive values can be used as a reliable diagnostic tool for knowing the adulteration of different samples.[18] The MSE values of dried leaves and roots were comparable (29.0 ± 2.35% and 30.4 ± 1.75%) whereas WSE of leaves and roots were 6.6 ± 0.4% and 4.16 ± 0.98%. No reference has been found in the literature for physicochemical analysis of *T. indica* dried root. It has been reported first time in the present study.

Various chemical constituents play a significant role in establishing pharmacological properties of any drug. Literature has suggested that *T. indica* possess various alkaloid and non-alkaloid compounds. The solubility of active constituent is depended on its chemical nature and also a solvent. Hence, methanolic and aqueous extracts of leaf and roots were evaluated for the presence of different phytochemicals. The qualitative analysis of leaf revealed the presence of alkaloids, carbohydrates, glycosides, and tannins. However, amino acids, steroids, and terpenoids were not detected in any extracts of the leaf. The root extracts revealed the presence of alkaloids, carbohydrates, glycosides, tannins, steroids, and amino acids. However, saponin was present in the aqueous extracts of both, i.e. leaves and roots. This study indicates the presence of various secondary metabolites which are responsible for multiple pharmacological effects of the plant.[19]

Chromatographic techniques are other advancements which help to separate and analyze the various molecules of a drug due to their differences in composition and/or structure.[20] In this work, the methanolic extracts were analyzed by HPTLC. The different number of peaks has been observed in the chromatograms and presented as various RF values [Table 3] and they determine the presence of different compounds in the extracts. The RF of the tylophorine has been reported 0.59 in solvent system 1 at 254 nm.[11] One of the RF values of root extract corresponds expressing the presence of tylophorine.

XRD analysis is helpful in confirming and estimating the presence of various matters of mineral origin in the fine powder sample.[21] This technique plays a significant role in assessing the presence of heavy metals which are of high concern in health issues. This assay indicates the presence of 22 elements of metallic (calcium, potassium, silicon, magnesium, etc.) and non-metallic (chlorine, sulfur, phosphorus, etc.) origin. The heavy metals such as mercury, arsenic, lead, and cadmium have not been reported in the root sample.

CONCLUSION

One focus of good manufacturing practices include the quality and purity of raw materials. Use of substandard raw materials may lead to the poor quality of finished products which can affect the health of patients. Therefore, the collected leaves and roots of *T. indica* have been investigated for the assessment of their quality. The results of physicochemical analysis of *T. indica* leaves revealed that the drug collected for the analysis having the best quality because all parameters of leaves were within the prescribed limits of the standards. The results of root have been reported for the first time. The phytochemical screening conveys that various constituents are present in the samples and these products of metabolism recognize this plant to be a potent medicine such as anti-asthmatic, anti-inflammatory, hepatoprotective, antioxidant, and so on. This has further been confirmed by chromatographic studies with different RF values some of

which can be correlated with the reported studies. Moreover, the XRD studies indicate that heavy metals are not present in the samples. The data obtained in this investigation may serve as a key role in establishing the suitable standards to determine the quality of this plant for future investigations.

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