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PHYSICO-CHEMICAL AND CHROMATOGRAPHIC (HPTLC) STANDARDIZATION OF *HOLOPTELEA INTEGREFOLIA* (STEM BARK)

DANISH IQBAL[1]*, RAJU K. PAWAR[2], RAJEEV KR. SHARMA[3]

[1]Department of Biotechnology, Integral University, Lucknow-226026, [2-3] Pharmacopoeial Laboratory for Indian Medicine, Department of AYUSH, Kamla Nehru Nagar, Ghaziabad-201001, (U.P.).
E mail: *danishik786@gmail.com*

ABSTRACT

Background: *Holoptelea integrifolia* has several medicinal properties but adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal. The significant popularity of HPTLC in the analytical testing and standardization of pharmaceutical, bulk drugs and herbal drugs lends its fame to the attributes.
Objective: The present communication attempts to investigate physico-chemical analysis and chromatographic (HPTLC) profiles of *Holoptelea integrefolia* (stem bark) (Family: Ulmaceae).
Material and Method: The physico-chemical properties of aqueous extract values have been analyzed. HPTLC of Hexane extract of *Holoptelea integrefolia* stem bark were carried out in two lanes: - T1 – Hexane extract of Local Market Sample Ghaziabad, T2 – Hexane extract of Museum Sample of PLIM Ghaziabad, to lay down the fingerprint profile of drug by using Linomat V applicator of Camag HPTLC instrument. TLC of the drug on Silica Gel60 GF_{254} pre coated plate using Toluene: Ethyl acetate (9:1) V/V as mobile phase.
Results: Under UV Light 254 nm four spots appeared of T1 sample and seven spots appeared of T2 sample and under UV Light 366 nm three spots appeared of T1 sample and six spots appeared of T2 sample. After derivatization of TLC plate with Anisaldehyde-Sulphuric acid reagent followed by heating at 110 ^{0}C for 10 minutes; seven spots appeared. **Conclusion:** The study revealed specific identities for *Holoptelea integrefolia* (stem bark), R_f values and colour of bands under UV light 254nm and 366nm which may play a key role in identification of plant (stem bark).

KEY WORDS*; Physico-chemical, HPTLC fingerprint, Holoptelea integrefolia (stem bark), Hexane extract.*

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INTRODUCTION

Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments. [1] The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments. [2] More than 500 traditional communities use about 800 plant species for curing different diseases. [3] One major obstacle that might impair the potential use of traditional medicine as medicine of choice is the lack of standardization. Adulterations and substitutions are common in raw material trade of medicinal plants. Unintentional adulterations also exist in herbal raw material trade due to various reasons such as confusion in vernacular names between indigenous systems of medicine and local dialects, lack of knowledge about the authentic plant, non-availability of the authentic plant, similarity in morphology and/or aroma or careless collection. [4] Furthermore, adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal. [5] Modern techniques such as HPTLC, HPLC, and GC etc. can be used to develop the methods for the quantification of marker compounds in these types of multicomponent herbal formulations. [6] It becomes necessary to develop more effective, accurate, reliable and sensitive methods for the authentication of herbs. In the present study an effort has been made to establish physico-chemical parameters and HPTLC fingerprint

which could be helpful in identification of the authentic plant samples and differentiating it from adulterants. [7]

Chromatography is a separation technique whereby the components of a mixture may be separated by allowing the sample to be transported through packed bed of material by fluid mobile phase. [8], [9] Out of almost 700 pharmaceutical formulations that are documented in the USP almost 43% of the procedures are those documented by thin layer chromatography. The significant popularity of HPTLC in the analytical testing of pharmaceutical, bulk drugs and herbal lends its fame to the attributes. [10], [11]

Holoptelea integrifolia (Name- English: Indian Elm, Sanskrit: chirivilva, Hindi: chilbil, Family: Ulmaceae) is a large deciduous tree, growing up to 18 m tall, distributed throughout the greater part of India up to an altitude of 2,000 ft. It has grey bark, covered with blisters, peeling in corky scales on old trees (fig.1). The plant has several medicinal properties. In India, decoction of the bark of this plant is externally used in rheumatism. [12] Oral application of the bark is used to treat intestinal tumors. [13] Dried bark is useful as an oxytocic in pregnant ladies. [14] Decoction of the leaves is orally given to regulate fat metabolism. [15] Leaves along with garlic are externally used to treat ringworm eczema and cutaneous diseases. [16] Leaves of the plant *Holoptelea integrifolia*, Garlic (Allium sativum) and Black Pepper (Piper nigrum) are mixed and crushed to make tablet, one tablet per day can be given to the patient suffering from jaundice. [17] Paste of the stem bark is externally applied to treat the inflammation of lymph glands. *Holoptelea integrefolia* stem bark powder is externally applied on the forehead of the patient suffering from common fever. [18] Moreover; paste of the stem bark is externally applied in cases of ringworm and scabies. Stem bark acts as an anti-inflammatory agent specifically for eyes. [19] In Nepal, bark is externally used to relieve rheumatic swellings. [20] Bark and leaf paste of Holoptelea integrifolia plant are applied externally on the white patches or leucoderma. [21]

Identification of plants with botanical verifications is essential as adulteration due to misidentification of plant species or parts are common. Standardization of medicinal plant product is the prime need of the current time. HPTLC method is simple, accurate, precise, specific, highly sensitive, cost effective and less time consuming. Unlike other methods, HPTLC produces visible chromatograms: complex information about the entire sample is available at a glance. Multiple samples are seen simultaneously, so that reference and test samples can be compared for identification. [22] Therefore, HPTLC studies of hexane extract of *Holoptelea integrifolia* (Stem Bark) has been carried out to lay down the fingerprint profile of drug. The study revealed specific identities for *Holoptelea integrifolia* (Stem Bark), which may play a key role in identification of plant and can be useful in standardization of the herbal drugs.

MATERIAL AND METHODS

Plant Material-

The Sanskrit: chirivilva Stem Bark was procured from the Local Market, Ghaziabad (T1). It was identified and authenticated by the Botanists of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad. One genuine sample also taken from the Museum of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad (T2).

Equipments- Cammag Linomat V applicator, Cammag Twin Trough Chamber (size 20x10 cm) with SS lid, Cammag Dipping Chamber, TLC Aluminum pre coated plate with Silica gel 60 GF_{254} (10X10 cm^2; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India).

Chemicals- Analytical grade; Alcohol, Toluene, ethyl acetate, Formic acid, Chloroform, Methanol, Anisaldehyde, Sulphuric acid and n-Hexane were used; obtained from S.D. Fine Chem. Ltd. (Mumbai, India).

Experimental-

Sample preparation- 1g of coarsely powdered drug samples were extracted with 10 ml Hexane for 24 hours by cold extraction method. The extracts were filtered by Whatmann no. 42 filter paper and make up to 10 ml in a volumetric flask.

Chromatography-

TLC Aluminum pre coated plate with Silica gel60 GF_{254} (20x10 cm^2; 0.2 mm thick) was used with Toluene : Ethyl acetate (9:1) V/V as mobile phase. Hexane extract of samples applied on plate by using Linomat V applicator 9µl on each Track T1 and T2. Cammag Twin Trough Glass Chamber (20x10 cm^2) with SS lid was used for development of TLC plate. The Twin Trough

Sr. No.	Name of Physico-chemical constants	Local Market sample, Ghaziabad	Museum of PLIM, Ghaziabad
1.	Loss on Drying at 110^{0}c	9.97% w/w	9.03% w/w
2.	pH (of 5% aq. Solution)	6.03	6.17
3.	Total ash	10.04% w/w	9.33% w/w
4.	Acid in-soluble ash	0.71% w/w	0.78% w/w
5.	Water soluble ash	7.05% w/w	7.67% w/w
6.	Water soluble extractives	10.78% w/w	10.42% w/w
7.	Ethanol soluble extractives	2.34% w/w	2.44% w/w
8.	Chloroform soluble extractives	2.97% w/w	3.01% w/w
9.	Hexane soluble extractives	0.65% w/w	0.57% w/w

Glass Chamber was saturated with mobile phase for 30 minutes. TLC plate was developed to 8 cm distance above the position of the sample application. The plate was removed from the chamber and air dried at room temperature. This plate was sprayed (derivatized) with Anisaldehyde-Sulphuric Acid reagent followed by heating at 110 ^{0}C for 10 minutes and HPTLC finger print profile was snapped by Cammag Reprostar III, before deivatization under UV 254 nm, 366 nm and after derivatization.

Determination of Physico- chemical constants:

Like Loss on Drying at 110 ^{0}C, Total ash, Acid insoluble ash, Water soluble extractive, Water soluble ash, Ethanol soluble extractives, Chloroform soluble extractives, Hexane soluble extractives, and pH of 5 % w/v solution of aqueous extract values were carried out as per the Ayurvedic Pharmacopoeia of India guidelines. [23]

Results:

Physicochemical parameters

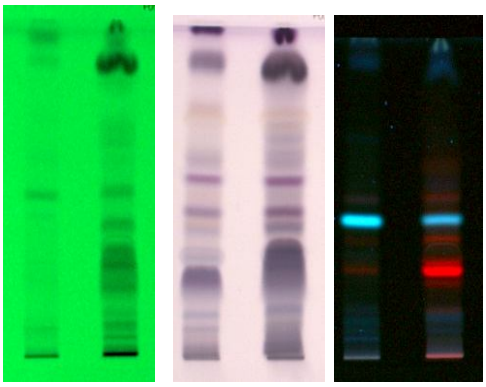
The percent of Loss on Drying at 110 ^{0}C, Total ash, Acid insoluble ash, Water soluble extractive, Water soluble ash, Ethanol soluble extractives, Chloroform soluble extractives, Hexane soluble extractives, and pH of 5 % w/v solution of aqueous extract values has been shown in Table 1.

Table No. 1: Physico-chemical constants of *Holoptelea integrifolia*

Table no. 2: R_f. values and colour of bands of *Holoptelea integrefolia* (stem bark)

Sr. No.	Detection/ visualization	Local Market Sample Ghaziabad (T1)		Museum Sample of PLIM Ghaziabad (T2)	
		R_f. values	**Colour of band**	**R_f. values**	**Colour of band**
1.	**Under UV 254 nm**	.10 0.49 0.87 0.93	dark grey dark grey dark grey dark grey	0.09 0.12 0.21 0.27 0.43 0.49 0.87	dark grey dark grey dark grey dark grey dark grey dark grey dark grey
2.	**Under UV 366 nm**	0.27 0.43 0.93	light blue blue light blue bright	0.12 0.27 0.37 0.43 0.49 0.93	light blue red light red blue light red light blue
3.	**After derivatization**	0.12 0.27 0.39 0.43 0.51 0.75 0.87	violet violet violet violet violet brown violet	0.16 0.27 0.30 0.39 0.43 0.51 0.75 0.87	Violet Violet Violet Violet Violet violet brown violet

***Holoptelea integrefolia* (D)**



T1 T2 T1 T2 T1 T2

UV 254nm UV 366nm After Derivatization
Fig.: 2- H.P.T.L.C. Finger print of *Holoptelea integrifolia* (Stem Bark)

T1 – Hexane extract of Local Market Sample Ghaziabad
T2 – Hexane extract of Museum Sample of PLIM Ghaziabad

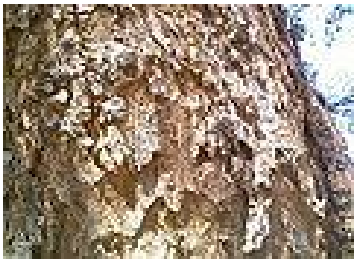


Fig.1: Stem (Bark) of *Holoptelea integrifolia*

HPTLC study
The Hexane extracts of T1 and T2 sample were used to carry out HPTLC. TLC of the drug on Silica Gel60 GF_{254} pre coated plate using Toluene : Ethyl acetate (9:1) V/V as mobile phase shows, under UV Light 254 nm four spots appeared at Rf. 0.10,0.49, 0.87, 0.93, (all dark grey) of T1 sample and seven spots appeared at Rf. 0.09, 0.12, 0.21, 0.27, 0.43, 0.49, 0.87 (all dark grey) of T2 sample and under UV Light 366 nm three spots appeared at Rf. 0.27 (light blue), 0.43 (blue), 0.93 (light blue bright) of T1 sample and six spots appeared at Rf. 0.12 (light blue), 0.27 (red), 0.37 (light red), 0.43 (blue), 0.49 (light red), 0.93 (light blue) of T2 sample. After derivatization of TLC plate with Anisaldehyde-Sulphuric Acid reagent followed by heating at 110^{0} C for 10 minutes; seven spots appeared at Rf. 0.12, 0.27, 0.39, 0.43, 0.51, 0.75, 0.87 (all violet), Data and HPTLC fingerprint also shown in Table 2 and Figure 2 respectively.

Discussion/Conclusion:
This study presents a set of diagnostic characters of *Holoptelea integrefolia* (stem bark) that will help to identify the drug in fragmentary condition as well as in whole form. The results of parameters for preliminary physiochemical screening, UV analysis and HPTLC studies can act as biomarkers for identification and authentification of raw drug samples and play an important role in quality control and prevention of adulteration. The proposed HPTLC method is simple, rapid, accurate, reproducible, selective and economic and can be used for routine quality control analysis of *Holoptelea integrifolia* (Stem Bark).

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