Original Research Article **Pharmacognosy**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PHYSICO-CHEMICAL PARAMETERS AND HPTLC FINGERPRINTING PROFILE OF *DILLENIA INDICA* MIQ. F. ELONGATA (MIQ.) AND *TECTONA GRANDIS* LINN. WITH REFERENCE TO BETULIN

PREET AMOL SINGH[1] AND VIDHU AERI[2]*

[1]M. Pharmacy, Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, Hamdard Nagar, New-Delhi.
[2]PhD, Associate Professor, Department of Pharmacognosy and Phytochemistry Jamia Hamdard, Hamdard Nagar, New Delhi, 110062, India.

ABSTRACT

The present study was carried out to evaluate physicochemical parameters and HPTLC fingerprinting profile of *Dillenia indica* Miq. f. elongata (Miq.) and *Tectona grandis* Linn. with reference to betulin. Physicochemical parameters such as ash value (total ash, acid insoluble ash), loss on drying, alcohol soluble extractive, water soluble extractive were evaluated using WHO guidelines. HPTLC fingerprinting profiling was performed using Camag Linomat applicator model V, Camag TLC visualizer. Samples were applied on aluminium HPTLC silica gel plates 60 F254 p. Solvent system i.e. n-Hexane-Ethyl Acetate (8:2) was developed for betulin separation. Camag TLC developing chamber was used for developing the profile of given samples. Finally developed TLCs were derivatized in chromatogram immersion device III with anisaldehyde sulphuric acid reagent. The physicochemical parameters of the plants were found to be in proper range as per WHO guidelines and available literature. The violet and fluorescent golden band of betulin was observed in white light and at 366nm respectively after derivatization with anisaldehyde sulphuric acid reagent. Physico-chemical properties can provide valuable information regarding plant identification and its authentication. Betulin, a known pentacyclic triterpenoid is known to exhibit diverse pharmacological properties. Hence, the stem barks of *Dillenia indica* Miq. f. elongata (Miq.) and *Tectona grandis* Linn. can be effectively utilized for the isolation of betulin.

KEY WORDS: ***Dillenia indica* Miq. f. elongate (Miq.), *Tectona grandis* Linn, Betulin, Physico-chemical parameters, HPTLC fingerprinting**

VIDHU AERI
PhD, Associate Professor, Department of Pharmacognosy and Phytochemistry Jamia Hamdard, Hamdard Nagar, New Delhi, 110062, India.

INTRODUCTION

Dillenia indica Miq. f. elongata (Miq.) evergreen forest plant is significant in the lupeol group of triterpenoids like betulinic acid, betulin and flavonoids that possess diverse pharmacological activities[1]. Leaf, bark (used for astringent properties), fruit (laxative properties and to treat fever)[2], alcoholic extract (show CNS depressant activity) and the mixed juice of leaf, bark and fruits are consumed orally for the treatment of cancer and diarrhea[3]. *Tectona grandis* Linn. (Verbenaceae), popularly known as teak, distributed in south and southeast Asia and is found in greater part of India. It is widely cultivated for the commercial value of its wood due to beautiful surface and its resistance to termite and fungal damage[4]. It is a tall, branched, deciduous tree with large leaves, possessing good pharmacological properties. Traditionally, the bark of this plant is used to treat headache, leprosy, bronchitis, leukoderma, constipation and dysentery[5]. Betulin, lup-20(29)-ene-3β,28-diol, is a pentacyclic triterpene alcohol with a lupane skeleton, is freely found in outer bark of white birch[6]. The preparations based on lupane series are used in treatment of many diseases and the expectations are linked to betulin which is used as such or transformed to betulinic acid (its biologically more active derivative)[7]. Betulin, which is the main constituent of the extract of birch bark, exhibit wound healing, choleretic, anti-inflammatory, liver-protecting, cholesterol lowering action and anti-HIV properties. Furthermore, plant extracts containing lupeol, betulin and betulinic acid are known to possess anti-tumour property[8]. The present study was undertaken to evaluate the physico-chemical parameters and to detect the presence of betulin in *Dillenia indica* Miq. f. elongata (Miq.) and *Tectona grandis* Linn. using HPTLC, so that these plants can find their way in pharmaceutical industries in a more constructive way. Furthermore, the relevance of the present study is the easy availability of these plants in India and a substantial amount of betulin present in these plants thus promoting their role in being the natural and rather good source of betulin.

MATERIALS AND METHODS

Standard Solutions and Reagents

The standard betulin was obtained from Sigma-Aldrich Chemical Corporation (USA). Distilled water was prepared with a Milli-Q academic water purification system (Millipore, Bedford, MA, USA). Ethyl acetate (S.D Fine chemicals India) and HPLC grade methanol (Merk Ltd, Mumbai, India) were used for the HPTLC analysis.

Plant Material

Bark of *Dillenia indica* Miq. f. elongata (Miq.) was collected from Yadvindra garden Pinjore, Haryana, India. Likewise, bark of *Tectona grandis* Linn. was collected from Environmental park, Patiala, Punjab, India. Bark of *Dillenia indica* Miq. f. elongata (Miq.) and *Tectona grandis* Linn. were authenticated with the help of taxonomist, Department of Botany, Punjabi University Patiala, India with Voucher no: PUN-59202 and PUN-59201 respectively.

Soxhlet's Extraction

Accurately weighed dried and powdered bark samples (100 g each) of *Dillenia indica* Miq. f. elongata (Miq.) and *Tectona grandis* Linn. were extracted with ethanol as solvent using Soxhlet extractor. These extracts were concentrated to dryness using a rotary vacuum evaporator, weighed and kept in refrigerator for further studies.

Physico-chemical Analysis

Physico-chemical parameters such as loss on drying, ash values (total ash and acid insoluble ash) and extractive values (water soluble and alcohol soluble extractives) were performed according to the prescribed official method and WHO guidelines on quality control methods for medical plants material [9-11].

Preparation of Standard solution

Standard betulin (1mg) was dissolved in HPLC grade methanol making the volume up to 10 ml using volumetric flask so as to make a final concentration of 0.1 mg/ml.

Preparation of Sample solution

Dried ethanol extracts of *Dillenia indica* Miq. f. elongata (Miq.) (51.18 mg) and *Tectona grandis* Linn. (51.89) respectively, were weighed and dissolved in methanol using sonicator to make the volume upto10 ml using a volumetric flask. These were syringe filtered and were put in use to study the HPTLC fingerprinting profile of samples with reference to betulin [12].

HPTLC instrumentation[13]

HPTLC fingerprinting profiling was performed using Camag Linomat applicator model V, Camag TLC visualizer. Samples were applied on aluminium TLC silica gel plates 60 F254 p. Camag TLC developing chamber was used for developing the profile of these plant samples. The plate was developed up to a height of 8 cm in TLC developing chamber saturated with 10 ml of n-Hexane-ethyl acetate mixture (8:2) mobile phase. Standard betulin and plant samples were loaded in a plate of 10 x 10 cm size with 3 tracks of 8 mm band width. Application volume of *Dillenia indica* Miq. f. elongata (Miq.), standard betulin and *Tectona grandis* Linn was 2.0µl, 10µl and 4µl respectively which were applied on the TLC plate using Linomat applicator. Finally developed TLCs were derivatized in chromatogram immersion device III using anisaldehyde sulphuric acid reagent. After proper drying, the developed plate was scanned and visualized at 530 and 366 nm wavelengths. Band of betulin was confirmed by comparing the R_f values and spectra of samples with the standard compound .The measurement table and instrument details at 530 nm are tabulated in Table 1 and 2 respectively.

Table 1
Measurement table at 530 nm using Camag HPTLC instrument

Wavelenght	530 nm
Lamp	Tungsten
Measurement type	Remission
Measurement mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	265 V

Table 2
Instrument details

Executed by	CAMAG TLC Scanner 3
Number of tracks	3
Position of first track X	15.0 mm
Distance between tracks	35.0 mm
Scan start position Y	5.0 mm
Scan end position Y	90.0 mm
Slit dimensions	6.00 × 0.45 mm, Micro
Optimize optical system	Light
Scanning speed	20 mm/s
Data resolution	100µm/step

RESULTS AND DISCUSSION

Physico-chemical parameters

The ash values of a drug generally give an idea about the earthy, inorganic and other impurities present in that particular drug. So it is useful for checking the genuity of the raw samples. Physico-chemical parameters were evaluated and were found to be in the desired range as per WHO guidelines and present literature which indicated that the samples were of better quality. The results are tabulated in Table 3.

Table 3
Represents the physico-chemical parameters of stem bark of Dillenia indica Miq. f. elongata (Miq.) and Tectona grandis Linn.

Physico-chemical parameters	*Dillenia indica* Miq. f. elongata (Miq.) (bark) %w/w*	*Tectona grandis* Linn. (bark) %w/w*
Total ash	16.19	1.80
Acid insoluble ash	4.94	1.23
Alcohol soluble extractive	8.90	5.91
Water soluble extractive	17.04	9.25
Loss on drying	9.87	5.77

Visualization and scanning of Tectona grandis (bark) and Dillenia indica Miq. f. elongata (Miq.) (bark) with reference to betulin using HPTLC

Today sophisticated analytical instruments are available for the determination of chemical constituents in a qualitative and quantitative way but the determination of a particular chemical constituent with the help of HPTLC is still one of the correct and the simplest method for the confirmation of that particular chemical constituent present in extract. Betulin is a pentacyclic triterpenoid hence; it is only visible after derivatizing with anisaldehyde and sulphuric acid reagent. Ethanol extracts of *Dillenia indica* Miq. f. elongata (Miq.) (bark) and *Tectona grandis* (bark) had shown clear spots of betulin when compared with the spot of standard betulin in HPTLC visualizer after derivatization with anisaldehyde and sulphuric acid reagent. Violet band of betulin in white light (530nm) and fluorescent golden band at 366 nm was observed. It is shown in fig.1(A) and (B). After visualization, the extracts were scanned for the peak of betulin with their 3-D spectrum with reference to standard betulin. Clear peak of betulin was observed in the plant extracts as shown in fig.2. Scanning of the individual peak of betulin was also carried out in plant extracts for stern confirmation of the presence of betulin as shown in fig.3(A),(B) and (C). Rf values of betulin were calculated in the plant extracts that were almost comparable to the standard betulin which further confirmed the presence of betulin. The Rf values and areas of the peak of betulin in plant extracts and standard betulin are shown in Table 4, confirming that it is beneficial in quantitative estimation of betulin in plant extracts with reference to standard betulin.

Figure 1

(A and B) Represents the HPTLC chromatographic profile at wavelength 530 and 366 nm respectively of the ethanol extract of (1) Dillenia indica Miq. f. elongata (Miq.) (bark) and (2) Tectona grandis (bark) with reference to (S) Standard betulin

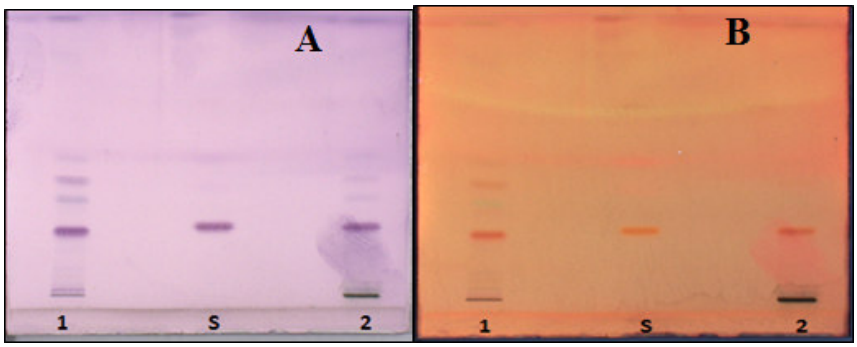

Figure 2

Represents the peak of betulin in (1) Dillenia indica Miq. f. elongata (Miq.) (bark), (2) standard betulin and (3) Tectona grandis Linn. with their 3-D spectrum.

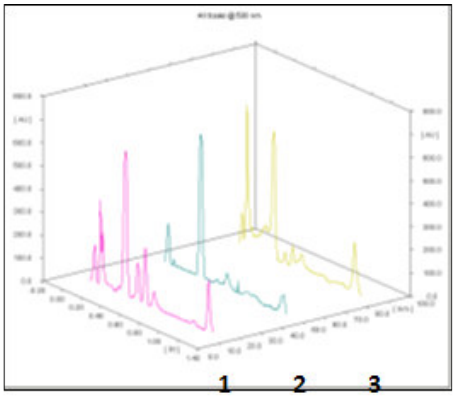

Figure 3

(A) Represents the individual peak of betulin in Dillenia indica Miq. f. elongata (Miq.) (B) represents individual peak of standard betulin (C) represents individual peak of betulin in Tectona grandis Linn.

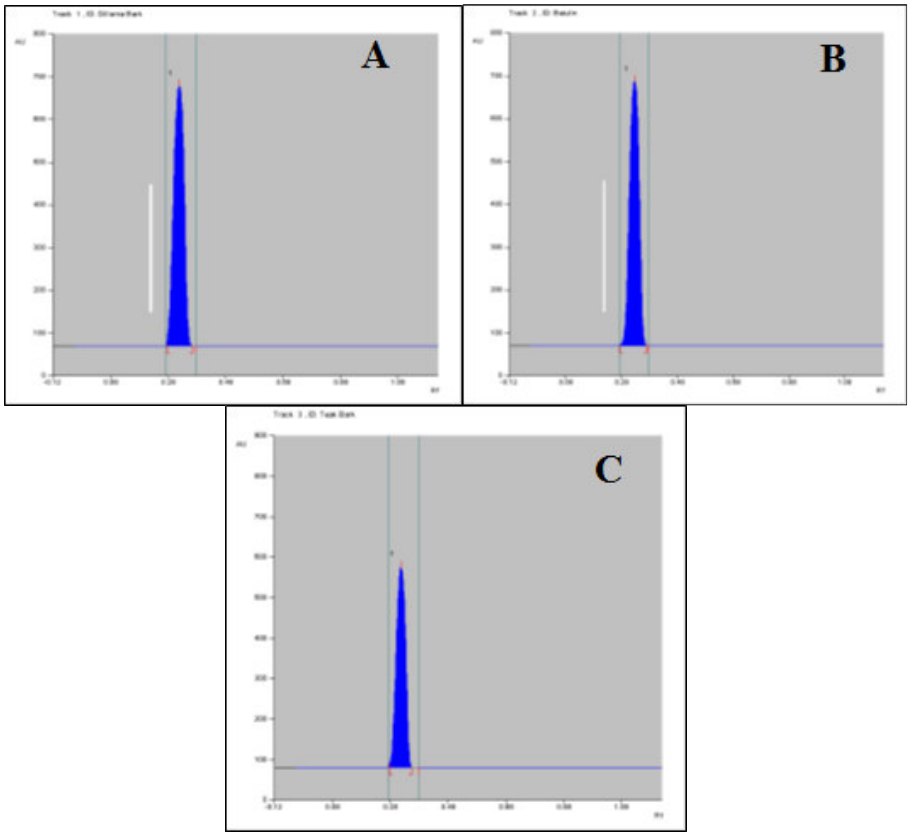

Table 4

Comparative area and Rf values of betulin in ethanol bark extracts of Dillenia indica Miq. f. elongata (Miq.) and Tectona grandis Linn. and standard solution of betulin using HPTLC

Peak	Start Rf	Start Height	Max. Rf	Max. Height	Max. %	End Rf	End Height	Area
Dillenia indica Miq. f. elongata (Miq.)	0.27	1.9	0.32	613.2	100.0	0.37	0.0	17024.9
Tectona grandis Linn.	0.27	1.4	0.32	500.7	100.00	0.36	0.7	12764.3
Standard betulin	0.27	0.2	0.33	617.2	100.00	0.37	0.3	17598.0

CONCLUSION

In the present study, physico-chemical parameters like ash and extractive values were studied and it can be easily concluded that *Dillenia indica* Miq. f. elongata (Miq.) and *Tectona grandis* Linn. raw samples were almost devoid of adulteration and this can be linked to the genuity of the HPTLC results. Efficient solvent system for the separation of betulin using HPTLC was established. To our best of knowledge and literature surveyed it was revealed that no systematic research on fingerprinting profile of *Tectona grandis* Linn. (bark) and *Dillenia indica* Miq. f. elongata (Miq.) (bark) has been carried out with reference to betulin using HPTLC. The method developed for the determination of betulin using HPTLC was accurate and reproducible hence it can be utilized further for the quantitative analysis of betulin in these plants using HPTLC. The study confirmed that *Tectona grandis* Linn. and *Dillenia indica* Miq. f. elongata (Miq.) can become the natural source of betulin and can be utilized in various pharmaceutical formulations.

ACKNOWLEGEMENT

Authors are thankful to Dr.N.B.Brindavanam, Dr.G.P.Kimothi, Mr.Mahesh Joshi and Dr.C.S.Rana, for their valuable help in completion of the work. Authors specially acknowledge the contribution of Dabur DRDC, Ghaziabad, India.

CONFLICT OF INTEREST

The authors report no conflict of interest related to manuscript.

REFERENCES

1. Kumar S., Kumar V., Prakash O. Microscopic evaluation and physico-chemical analysis of *Dillenia indica* leaf. Asian Pac J Trop Biomed, 337-40, (2011).
2. Alam M.B., Chowdhury N.S., Mazumder M.E.H., Haque M.E. Antimicrobial and toxicity study of different fractions of *Dillenia indica* Linn. bark extract. IJPSR, 2: 860-66, (2011).
3. Muhit M.A., Tareq S.M., Apu A.S., Basak D., Islam M.S. Isolation and identification of compounds from the leaf extract of *Dillenia indica* Linn. Bangladesh Pharmaceutical Journal, 13: 49-53, (2010).
4. Lacret R., Varela R.M., Molinillo J.M.G., Nogueiras C., Macías F.A. Tectonoelins, new norlignans from a bioactive extract of *Tectona grandis*. Phytochem Letters, 5:382–86, (2012).
5. Rana V., Das M.K., Gogoi S., Kumar V. Multifunctional properties of polysaccharides from *Dalbergiasissoo*,*Tectona grandis* and *Mimosa diplotricha*. Carbohydrate Polymers, 102:341– 50, (2014).
6. Zhao G., Yan W., Cao D. Simultaneous determination of betulin and betulinic acid in white birch bark using RP-HPLC. Journal of Pharmaceutical and Biomedical Analysis, 43:959–62, (2007).
7. Sami A., Taru M., Salme K., Jari Y.K. Pharmacological properties of the ubiquitous natural product betulin. European journal of pharmaceutical sciences, 29:1-13, (2006).
8. Tolstikov G.A., Flekhter O.B., Shultz E.E., Baltina L.A., Tolstikov A.G. Betulin and its deravatives. Chemistry and Biological activity. Chemistry for sustainable development, 13:1-29, (2005).
9. WHO. Quality control methods for medicinal plant material, Mondiale De La Sante: Geneva, 22-34, (1992).
10. The Ayurvedic Pharmacopoeia of India, Appendix, Ministry of Health and Family welfare, Department of AYUSH Government of India:India, 213-214, (2001)
11. Khandelwal K.R. Practical pharmacognosy, 18th Edn, Nirali Publication: Pune, 23.1-23.17, (2007).
12. Nesy E.A., Mathew L. HPTLC profiling on cardiac glycosides of *Thevetia peruviana* leaf extracts of three morphovariant plants grown in Kerala, India. Indian J Pharm Sci, 7:191-195, (2015).
13. Rakesh S.U.,Salunkhe V.R., Dhabale P.N., Burade K.B. HPTLC Method for Quantitative Determination of Gallic Acid in Hydroalcoholic Extract of Dried Flowers of *Nymphaea stellata* Willd. Asian J Research Chem 2: 131-134,(2009).