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Isolation of tannins from the leaves of *Nephelium lappaceum* L. (Sapindaceae) and the HPTLC of the isolated compound

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Abstract

Since disease, decay and death have always co-existed with life, the study of diseases and their treatment must also have been contemporaneous with the dawn of the human intellect. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. So, the preliminary phytochemical screening of the leaves of *Nephelium lappaceum* L. were conducted and it showed the presence of Tannins. Tannins are astringent plant bitter polyphenols that either bind and precipitate or shrink proteins. Tannins have also been reported to exert other physiological effects such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immune responses. Hence an attempt has been made to isolate the tannins and its HPTLC profile was compared with standard Tannic acid. The area under the curve was also determined.

Keywords: *Nephelium lappaceum* L., phytochemical screening, tannins, HPTLC, tannic acid

Introduction

Green plants synthesise and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Plants can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and or reduced toxicity. The isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. [1, 2]

The name 'tannin' is derived from the French word 'tanin' (tanning substance) and is used for a range of natural polyphenols. Since ancient times it is known that certain organic substances have tanning properties and are able to tan animal skins to form leather. The Phytochemical Screening of the leaves of *Nephelium lappaceum* L. revealed the presence of Tannins. So, an attempt has been made to isolate the tannin by a simple procedure. Thin layer chromatography studies are among the key identity tests in most Pharmacopoeial monographs. An extension of TLC is high performance thin layer chromatography (HPTLC) is robust, simplest, rapid and efficient tool in quantitative analysis of the compounds, offers better resolution and lower limit of detection [3, 4, 10]. Hence the HPTLC profile of the isolated compound was compared with that of the Standard Tannic acid and the area under the curve was determined.

Materials and Methods**Collection and Preparation of the leaves**

The leaves were collected from Vazhayila, Thiruvananthapuram District, Kerala. Then it was washed thoroughly under tap water, dried in shade and crushed in an electric blender.

Method of Isolation of Tannins [7, 8]

100g of the dried leaves were homogenised in 400ml of Methanol with a blender. The cell debris and insoluble matter were removed by filtration through a muslin cloth. The filtrate was centrifuged at 11,000rpm for ten minutes. A suitable amount of 2M Potassium Dihydrogen Phosphate was added drop by drop to the combined clear supernatant in an ice bath until white and wooly material precipitated out completely. The precipitate was collected by centrifugation at 1000rpm for ten minutes. The precipitate was washed with Methanol three times and 1.2 N Hydrochloric acid in an ice bath by stirring for a few hours. As quickly as possible neutralisation was performed by the addition of saturated Sodium Carbonate solution to the Tannin solution in an ice bath. The solution was adjusted to about pH 4.0. A dried tannin powder was obtained by lyophilisation.

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Conditions of Chromatography

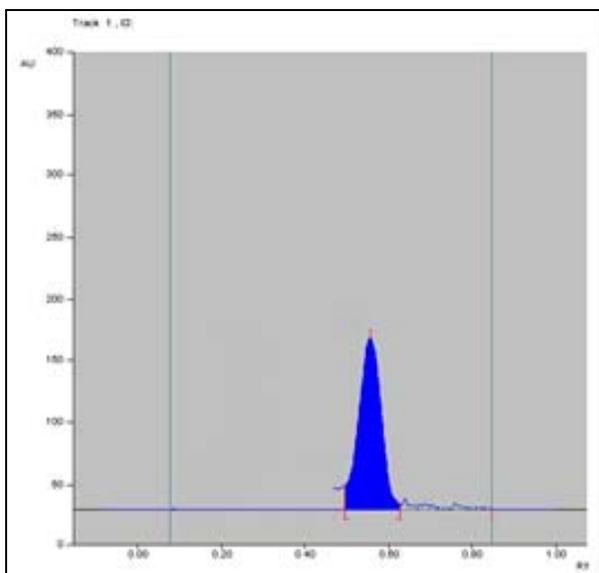
1. Test plate: HPTLC Silica gel 60F254 Aluminium sheets 20x20cm
2. Starting Position :10mm
3. Plate Width: 40mm
4. Bandwidth: 4mm
5. Space Width: 10mm
6. Rate: 4 μ l/sec
7. Volume: 2 μ l
8. Separation technique: Ascending
9. Development chamber: Twin-trough glass chamber (10X 10cm²)
10. Mobile phase: Toluene: Acetone: Acetic acid (5:5:0.3)
11. Scanner: CAMAG TLC SCANNER 3

Chemical Tests for the identification of the isolated Tannins [5, 6, 9]

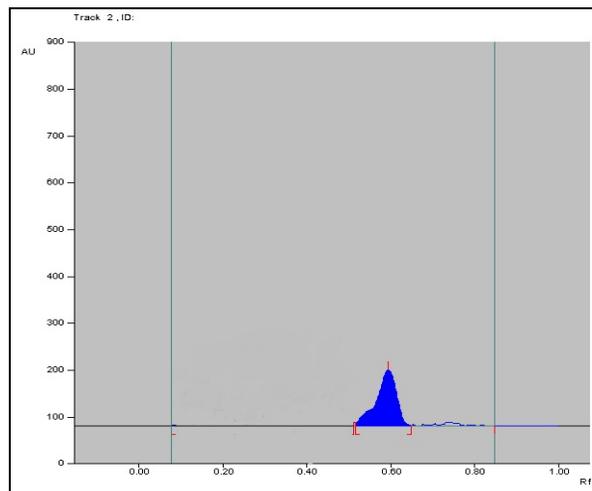
- (a) **Ferric Chloride Test:** 50 mg of the product obtained was treated with 3-4 drops of 5% Ferric chloride solution.
- (b) **Lead acetate test:** To 50mg the product 10% Lead acetate was added.
- (c) **Gelatin Test:** To 50mg of the product, 2ml of 1% gelatin solution containing 10% Sodium chloride was added.

Results and Discussion

Tannins were isolated from the leaves of *Nepheium lappaceum* and it was confirmed by chemical tests. The Physico-chemical parameters of the isolated tannins were determined. Both the Standard tannic acid and isolated Tannins were spotted, HPTLC was performed and compared. From the Area Under Curve the amount of Tannins present in the leaves of *Nepheium lappaceum* as well as the amount of Tannic acid present in the standard was calculated.



Graph 1: HPTLC of Tannic acid



Graph 2: HPTLC of the isolated tannin

Table 1: Chemical Tests for the identification of the isolated Tannins

Tests	Result
Ferric Chloride test	Positive
Lead acetate test	Positive
Gelatin salt test	Positive

Table 2: Physico-Chemical Evaluation of the isolated tannins

Parameters	Observation
Colour	Light brown
Odour	Characteristic
Taste	Characteristic

Table 3: Determination of HPTLC of standard tannic acid and isolated tannins

Sample	Spot no.	Rf	Area	Area%
Tannic Acid	1	0.63	5803.7 AU	49.60%
Isolated Tannin	2	0.65	4328.6 AU	20.59%

The amount of Tannic acid present in the Standard was found to be 0.020 mg/ml.

The amount of Tannic acid present in the leaves of *Nepheium lappaceum* L. was found to be 0.013 mg/ml.

Conclusion

Tannins were isolated from the leaves of *Nepheium lappaceum* and it was confirmed by chemical tests. The Physico-chemical parameters of the isolated tannins were determined. Both the Standard tannic acid and isolated Tannins were spotted, HPTLC was performed and compared. From the Area Under Curve the amount of Tannins present in the leaves of *Nepheium lappaceum* L. as well as the amount of Tannic acid present in the standard was calculated. Further spectral characterization of the isolated compound can yield promising drugs of future use.

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