COMPARATIVE HPTLC ANALYSIS OF STEM OF NOTHAPODYTES NIMMONIANA
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KEYWORDS:
Nothapodytes nimmoniana, physicochemical, maceration extraction, vortex extraction, HPTLC.

ABSTRACT
Medicinal plants play a vital role for the development of new drugs. Preliminary pharmacognostical screening was studied in Nothapodytes nimmoniana stem to establish authenticity and possible to help and distinguish the drug from other species. Nothapodytes nimmoniana contains camptothecin which is used in the treatment of colon, stomach, breast and bladder cancers. Analysis by HPTLC was done to determine percentage of camptothecin in various extracts obtained by maceration and vortex extraction. Result show that the yield of camptothecin was more in methanol and chloroform extracts.
INTRODUCTION:
Ayurveda, the science of life, prevention and longevity is believed to be the oldest and most holistic or comprehensive medical system available. Ayurveda is one of the most ancient systems of life, health and cure. Ayurveda is a highly evolved and codified system of life and health science based on its own unique and original concepts and fundamental principles. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal values of these plants are found in some chemical active substances that produce a definite physiological action on the human body. The Nothapodytes nimmoniana vernacular names are Ghanera, Durvasane mara, Kalgur, Kalagaura. The plant is distributed in the Western Ghats - South, Central and south Maharashtra Sahyadris, some parts of Assam, the Himalayan foothills, Ceylon, Burma and Thailand. The plant is a small tree, 3-8 m tall, with smooth, grey, wrinkled bark, about 5 mm thick. Branchlets are slightly angled, corky, with prominent leaf scars. Alternately arranged leaves are slightly leathery, broadly egg-shaped to elliptic-oblong, 1-25 cm long and 4-12 cm wide. Leaf base is often unequal; tip is pointed to long pointed. Leaves are crowded at the ends of branchlets. Leaf stalks are 3-6 cm long. Flowers are bisexual, creamy yellow, foul smelling, about 5 mm across, in flat-topped clusters at the end of branches. Petals are hairy inside. Fruits are oblong to ellipsoid, about 2 x 1 cm, smooth, purplish black when ripe, with a single seed. Camptothecin (CPT), a monoterpene indole alkaloid, is regarded as one of the most promising anticancer drug of the twenty first century. The cellular target of camptothecin is DNA topoisomerase I and numerous analogues have been synthesized as potential therapeutic agents. CPT inhibits the replication of Human Immuno Deficiency Virus (HIV) in vitro and is also shown to be effective in the complete remission of lung, breast, uterine and cervical cancer.

HPTLC is the most simple separation technique today available to the analyst. Thin layer chromatography (TLC) is a type of planar liquid chromatographic (LC) technique. A sample mixture (solute) is introduced into chromatography system by depositing it at a certain place on the layer. A solvent (mobile phase) passes through the system and the separation occurs based on relative solubility and adsorption. This is the basic principle of TLC. Several stationary phases are available, which include silica gel, alumina, kieselghur, cellulose, magnesia etc. These stationary phases can be coated on certain supports (glass/plastic/aluminum sheet). In the initial stages all the stationary phase’s material were coated by hand. However, with the availability of pre-coated plates, the use of handmade plates is on decline. The choice of the mobile phase for a given separation constitutes a very important stage in realizing a good separation in HPTLC.
Solvents used in HPTLC should follow the criteria such as ease of purification, cost, low viscosity, compatible with thin layer material, support plate and binder used. Sample mixtures to be separated should be soluble in these solvents.

**MATERIALS AND METHODS**

**Procurement of Plant Material:** Plant material of *N. nimmoniana* was collected from Mahabaleshwar region of Maharashtra, India, in the month of August. The herbarium was authenticated by Botanical Survey of India (BSI) and voucher specimen (NNASPI) was kept at departmental herbarium of BSI. The collected plant material was dried in shade and ground in the grinder. The dried powdered drug materials was extracted by 9 different solvents by cold maceration for 48 hrs at room temperature (pharmacognostical) and were also extracted (analytical) for analysis of camptothecin. The extracts were filtered and concentrated at 40°C. The residues were stored in a freezer until further tests.

**Techniques for the Extraction of *Nothapodytes nimmoniana***

**Extraction of plant material**
The plant material was stored under drying conditions; different parts of the plant were separated as leaves, stems and roots. The separated plant parts were then dried under shade and then stem was finely powdered with the help of a grinder. The powder of stem was then subjected to maceration and vortex mixer extraction processes.

**Different Methods of Extraction**

**Maceration**
Maceration was carried out on Mechanical shaker. Plant material was extracted with n-hexane, toluene, petroleum ether, chloroform, ethyl acetate, acetone, methanol, DMSO and water. Stem of *N. nimmoniana* (5 gm) was put into a 250 mL flask and added appropriate solvent (100 mL). The material was extracted continuously for 1 hr. After extraction the contents of the flasks were filtered through filter paper (Whatman No. 1).

**Vortex/ Cyclo mixer extraction**
Vortex mixer assisted extraction was performed in vortex mixer/ cyclo mixer. 5 gm of powdered stem was extracted with n-hexane, toluene, petroleum ether, chloroform, ethyl acetate, acetone, methanol, DMSO and water (100 mL), and kept for 30 min at room temperature. After extraction, the contents were filtered and evaporated to dryness.

**Phytochemical analysis**
The phytochemical analysis was carried out by HPTLC method.

**High performance thin layer chromatography (HPTLC)**

**HPTLC Apparatus**
The spotting device was Linomat IV Automatic Sampler (Camag), 100 μL syringe (Hamilton). The TLC chamber, glass twin trough chamber (20 × 10 × 4 cm; Camag), TLC Scanner 3 linked to
WINCATS software (Camag) as densitometer, the HPTLC plates of 20 × 10 cm, 0.2 mm thickness, precoated with silica gel 60 F254 (E. Merck Kga A, Cat. no. 1.05548, Darmstadt, Germany) were used. Reference standard of CPT (purity 95% w/w) was purchased from Hi Media (Mumbai, India), and Knowshine Pharmaceuticals, China.

**Extraction of drug material and preparation of sample solutions**

Plant material (stem) of *N. nimmoniana* were collected, washed and dried at 55°C in an air dryer for 48 hr. Dried materials were powdered with a Wiley mill (Model 4276-M, Thomas Scientific, USA) to pass a 20 mesh sieve and stored in sealed plastic bags. Dried powdered materials of stem (5 gm) were taken and extracted by maceration and vortex mixer extraction techniques. The dried extracts were transferred to petriplates and dissolved in 5 mL of respective solvents and applied.

**Preparation of standard solution of camptothecin**

A stock solution of CPT was prepared by dissolving 2 mg of accurately weighed CPT in chloroform: methanol mixture (3:1) and making up the volume to 10 mL with methanol. From this stock solution, standard solutions of 10 μg/mL to 50 μg/mL were prepared.

**Calibration curve for camptothecin**

10 μL of each of the standard solutions of CPT was applied in triplicate on a TLC plate. The plate was developed in a solvent system chloroform: ethyl acetate: methanol (4:5:0.5 v/v) at 25 ± 2°C temperature and 40% relative humidity up to a distance of 8 cm. After development, the plate was dried in air and scanned at 360 nm. The peak areas were recorded. Calibration curves of CPT were prepared by plotting peak area vs. concentration.  

**Estimation of marker compounds**

The plant extracts and the standards were spotted on one plate of size (20×10 cm) and were dipped in the solvent system (chloroform: ethyl acetate: methanol = 4:5:0.5) for 30 min. The plate was then dried under fan and was viewed under UV lamp at 360 nm for camptothecin. Then the plate was scanned under Linomat V, Wincat software.

**RESULT AND DISCUSSION**

The data for concentration and mean peak areas for preparation of calibration curve of camptothecin was calculated. Standard CPT was run simultaneously with various extract of stem in one solvent system which showed the resolution factor at 0.49 respectively. The equation obtained by the calibration curve was $y = 42.230x + 860$ and correlation coefficient was $R^2 = 0.997$ for camptothecin (Figure 1).

Out of the various stem extracts of various solvents prepared by different techniques (maceration, vortex mixer) used in phytochemical study of *N. nimmoniana*, methanol extracts prepared by maceration and vortex mixer showed highest concentration (0.513% and 0.592% respectively).
whereas second highest concentration was shown by chloroform extracts prepared by maceration and vortex mixer (0.442% and 0.506% respectively), least concentration of camptothecin was found in toluene and DMSO extracts (Table 1). Calibration curve of camptothecin was determined (Figure 1). Camptothecin was detected in all extracts of stem except in water, n-Hexane and petroleum ether extracts (Figure 2).

**Figure 1: Calibration curve of camptothecin**

**Table 1: Comparative yield of camptothecin in various extracts prepared by different extraction technique**

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Solvents</th>
<th>Maceration (% Yield)</th>
<th>Vortex Mixer (% Yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chloroform</td>
<td>0.442</td>
<td>0.506</td>
</tr>
<tr>
<td>2</td>
<td>Methanol</td>
<td>0.513</td>
<td>0.592</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl Acetate</td>
<td>0.176</td>
<td>0.204</td>
</tr>
<tr>
<td>4</td>
<td>Toluene</td>
<td>0.083</td>
<td>0.116</td>
</tr>
<tr>
<td>5</td>
<td>Acetone</td>
<td>0.126</td>
<td>0.1304</td>
</tr>
<tr>
<td>6</td>
<td>DMSO</td>
<td>0.064</td>
<td>0.097</td>
</tr>
</tbody>
</table>

**Figure 2: Comparative HPTLC of various extracts of stem of *N. nimmoniana***
CONCLUSION: Preliminary qualitative phytochemical studies of plants are an integral part of pharmacognosy. The objectives of qualitative evaluation of phytodrugs are twofold. It gives a preliminary insight into various compounds present in a plant, based on which a researcher can proceed further towards the biological activities of the compounds. Secondly, the study yields information on the purity of the drug as well as the genuineness of the drug. Camptothecin is regarded as one of the most promising anticancer drug of the twenty first century. The cellular target of camptothecin is DNA topoisomerase I and numerous analogues have been synthesized as potential therapeutic agents. In the present study the analytical investigation was done to detect the presence of camptothecin in stem extracts of *Nothapodytes nimmoniana*, extracted in different solvents by two main extraction techniques mainly maceration and vortex mixing., and maximum amount of camptothecin was found in methanol and chloroform extract.

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REFERENCES
