PHARMACOGNOSTICAL AND PHYTOCHEMICAL ANALYSIS OF NOTHAPODYTES NIMMONIANA STEM

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Received: 04 July 2012, Revised and Accepted: 11 Aug 2012

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. Different physicochemical parameters such as percentage yield, extractive value, chemical evaluation were carried out as per WHO recommended physicochemical determinations and authentic phytochemical procedures. Also analysis by HPLC was done to determine percentage of camptothecin in various extracts obtained by soxhlet extraction. Preliminary qualitative chemical tests for the extract shows the presence of alkaloids, carbohydrates, saponins, steroids, terpenoids, phenolics, coumarins and fixed oil.

Keywords: Nothapodytes nimmoniana, Physicochemical, Phytochemical, Soxhlet extraction, HPLC.

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.

Plant based drugs provide outstanding contribution to modern therapeutics; for example Vinblastine isolated from the Catharanthus roseus is used for the treatment of Hodgkin's, non-Hodgkin lymphomas, leukemia in children, testicular and neck cancer. Vinristine is recommended for acute lymphocytic leukemia in childhood, advanced stages of Hodgkin's lymphosarcoma, small cell lung, cervical and breast cancers. Podophyllotoxin is a constituent of Podophyllum eunodi currently used against testicular, small cell lung cancer and lymphomas. Indian indigenous tree of Nothapodytes nimmoniana earlier known as Nothapodytes foetida or Mappia foetida are mostly used for the treatment of cervical cancer. Plant derived drugs are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer. Medicinal plants play an important role in the development of potent therapeutic agents. Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine.

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 grounder. 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 by soxhlet extraction (analytical) method for analysis of camptothecin. The extracts were filtered and concentrated at 40°C. The residues were stored in a freezer until further tests.

Pharmacognostic Studies

Extractive Values

For calculation of extractive value 5 gm air dried powder of stem was taken, coarsely powdered and macerated with 100 ml of respective solvents in a closed flask for 24 hr, shaken frequently during 6 hr and allowed to stand for 18 hr, filter, evaporate and finally weighed to calculate extractive value.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out by using procedures described by Kokate (1991) and Harborne (1973). It is obvious that any study in pharmacognosy must embrace a through consideration of both primary and secondary metabolites derived as a result of biosynthetic pathway. Therefore, the plant material was subjected to preliminary phytochemical screening in order to detect plant constituents. As per procedure the drug was first subjected to extraction with organic solvents in the increasing order of their polarity. Taking the last drop from thimble on a watch glass and observing residue formation which ensures complete extraction by
each solvent. It is also ensured that powdered material is completely
dried and freed from traces of previous solvents. After which the
extracts were subjected to qualitative chemical tests\(^\text{5}^\text{9}\).

**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 soxhlet extraction processes.

**Chemicals:** Solvents viz. n-hexane, toluene, petroleum ether,
chloroform, ethylacetate, acetone, methanol, DMSO and water.

**Soxhlet extraction**
For chemical analysis of camptothecin, soxhlet extraction was
performed in which 5 gm of dried powdered stem of *N. nimmoniana*
was put into 200 mL Soxhlet thimble. The apparatus was fitted with
250 mL round bottom flask containing 100 mL of n-hexane, toluene,
petroleum ether, chloroform, ethylacetate, acetone, methanol,
DMSO and water. The extraction temperature was controlled at 70°C
with a regulator. The flask was heated for 1 hr. After extraction, the
contents were filtered and evaporated to dryness.

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

**High performance liquid chromatography (HPLC)**
Isocratic analytical HPLC assay was performed on a Jasco 900
instrument and 20 μL of supernatant extracts was loaded onto ODS
(5 μm; Hypersil) column (250×4.6 mm) along with guard column.
Acetonitrile: water (45:55) was used as mobile phase at a flow rate
(1 mL/min and camptothecin was detected at 360 nm by UV
instrument and 20 μL of supernatant extracts was loaded onto ODS
(5 μm; Hypersil) column (250×4.6 mm) along with guard column.

**Preparation of standard solution of Camptothecin**
A stock solution of camptothecin was prepared by dissolving 2 mg of
standard 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 by
transferring aliquots (0.1 to 0.5 mL) of stock solution to 10 mL
volumetric flasks and adjusting the volume with methanol\(^{20}\).

**Calibration curve for camptothecin**
20 μL of standard solutions of camptothecin was injected in triplicate
in column. The peaks were detected at 360 nm. Calibration curves of
camptothecin were prepared by plotting peak area vs. concentration.

**Sample preparation for soxhlet extracts of stem of Nothapodytes nimmoniana**
For determination of camptothecin content, the concentrate of all
different extracts were dissolved in 5 mL of respective solvents and
1 mL was taken in appendorf tubes. Then 100 μL is taken and
diluted with 900 μL of respective solvents (n-hexane, toluene,
petroleum ether, chloroform, ethyl acetate, acetone, methanol,
DMSO and water). Again 100 μL was taken from above solution and
diluted with 900 μL of respective solvents.

**Estimation of marker compounds**
A standard solution of CPT was introduced into the column using the
same mobile phase. Extracts were also introduced into the column
individually after been filtered with syringe filter, when the extracts
were run guard column was used. The extracts containing the
compounds resolved at same retention time as that was the time for
the given standard solution of CPT. Wavelength was adjusted at 360
nm under UV detector. Flow rate was 1 mL/min.

**RESULT AND DISCUSSION**

**Extractive Values**
The stems of *N. nimmoniana* gave different range of yields in various
solvents. The drug was extracted with various solvents with the help
of maceration process. Percentage of aqueous extract, DMSO and
alcoholic extract was higher than other extracts. Extractives values of
different extracts are shown in Table 1.

| Table 1: Extractive value of different extracts |
|-----------------|-----------------|-----------------|
| **S. No.** | **Solvent** | **Extractive value** |
| 1. | n-hexane | 0.01 |
| 2. | Dimethyl sulphoxide | 0.0664 |
| 3. | Petroleum ether | 0.01 |
| 4. | Chloroform | 0.0474 |
| 5. | Water | 0.0704 |
| 6. | Toluene | 0.0345 |
| 7. | Acetone | 0.03 |
| 8. | Methanol | 0.0567 |
| 9. | Ethylacetate | 0.04 |

**Phytochemical Screening**
Characteristic phytochemical tests showed the presence of alkaloids,
carbohydrates, saponins, steroids, terpenoids and phenolics. Hence,
through the phytochemical screening showed the presence of
various classes of chemical compounds in the stem extracts of *N.
immoniana* (Table 2).

**HPLC RESULTS**
Linear regression revealed good relationship between the
concentration of standard solutions and the peak response within
the concentration range of 10 to 50 μg/mL with a correlation
coefficient \((r^2)\) of 0.998 ± 0.02 (Y= 94861 X+ 42713) for
camptothecin (Figure 1). The chromatogram of standard CPT,
methanol extract and chloroform extract showed retention time at
4.2 min for CPT (Figure 2,3,4). In the various stem extracts used in
the study, the highest concentration of camptothecin was found in
methanol and chloroform extracts \((1.12%, 0.98\% \text{ respectively})\)
where at least concentration of camptothecin was found in DMSO
extract \((0.18\%)\), camptothecin was absent in water, n-hexane and
petroleum ether extracts.
Table 2: Preliminary Phytochemical Analysis of various extracts of *Nothapodytes nimmoniana*

<table>
<thead>
<tr>
<th>Chemical Test</th>
<th>Pet. ether extract</th>
<th>Alcoholic extract</th>
<th>Aqueous Extract</th>
<th>Chloroform extract</th>
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<td><strong>Alkaloids</strong></td>
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<td>Tannic acid</td>
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<tr>
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<td>Keller Kiliani</td>
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<tr>
<td>Foam</td>
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<tr>
<td>Lieberman’s</td>
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</table>

Fig. 2: Estimation of standard camptothecin

Fig. 3: Estimation of camptothecin in methanolic extract of *N. nimmoniana*
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 phytochemical investigation was done to detect the presence of camptothecin in stem extracts of *Nothapodytes nimmoniana*, extracted by different solvents.

**ACKNOWLEDGEMENT**

The author is in debt to Invertis Institute of Pharmacy, Invertis University for their advice and encouragement and also to Knowshine Pharmaceuticals, China for providing pure drug sample of camptothecin.

**REFERENCES**


