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Research Article

# PHARMACOGNOSTICAL AND PHYTOCHEMICAL ANALYSIS OF *NOTHAPODYTES NIMMONIANA* STEM

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**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[1]. 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[2]. The medicinal values of these plants are found in some chemical active substances that produce a definite physiological action on the human body[3].

Plant based drugs provide outstanding contribution to modern therapeutics; for example Vinblastine isolated from the *Catharanthus rosesus*[4] is used for the treatment of Hodgkins, non-Hodgkins lymphomas, leukemia in children, testicular and neck cancer. Vincristine is recommended for acute lymphocytic leukemia in childhood, advanced stages of Hodgkins, lymophosarcoma, small cell lung, cervical and breast cancer[5]. Podophyllotoxin is a constituent of *Podophyllum emodi* 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.

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[6]. 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[7].

*Nothapodytes nimmoniana* contains camptothecin as its active constituent which is used in the treatment of cancer. Camptothecin (CPT), a monoterpene indole alkaloid, is regarded as one of the most promising anticancer drug of the twenty first century[8,9].The cellular target of camptothecin is DNA topoisomerase I and numerous analogues have been synthesized as potential therapeutic agents[10]. 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[11,12,13]. Several water soluble derivatives of camptothecin (topotecan and irinotecan) are currently being used for the treatment of colorectal and ovarian cancer[14,15,16]. The molecular and cytotoxic effect of camptothecin on *Plasmodium falciparum* proves that CPT is an interesting target for new anti-malarial drug development[17]. CPT was first discovered in the Chinese deciduous tree, *Camptotheca acuminata*[18]

## 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 (NNASP1) 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$^{o}$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[19].

**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, ethyl acetate, 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, ethyl acetate, acetone, methanol, DMSO and water. The extraction temperature was controlled at $70^0$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 of 1 mL/min and camptothecin was detected at 360 nm by UV detector (UV-975, Jasco). The peak areas corresponding to camptothecin were integrated by comparison with external standard calibration curves. The results of the five injections from the same samples at the five concentrations (10-50 µg/mL) showed similar retention time.

**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. | Dimethylsulphoxide | 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. | Ethyl acetate | 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. nimmoniana* (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% respectively) where as least concentration of camptothecin was found in DMSO extract (0.18%), camptothecin was absent in water, n-hexane and petroleum ether extracts.

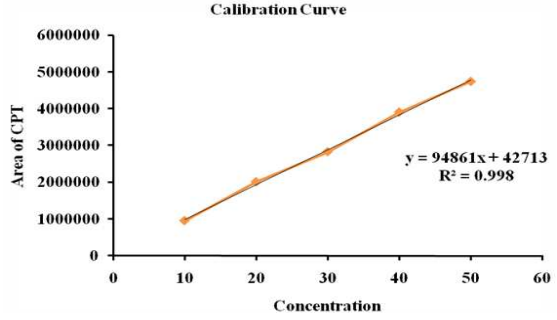


**Fig. 1: Calibration curve for camptothecin**

**Table 2: Preliminary Phytochemical Analysis of various extracts of *Nothapodytes nimmoniana***

| Chemical Test | Pet. ether extract | Alcoholic extract | Aqueous Extract | Chloroform extract |
|---|---|---|---|---|
| **Alkaloids** | | | | |
| Dragendorff's reagent | - | + | - | + |
| Mayer's reagent | - | + | - | + |
| Hager's reagent | - | + | - | + |
| Wagner's reagent | - | + | - | + |
| Tannic acid | - | + | - | + |
| **Glycosides** | | | | |
| Fehling's | - | - | + | - |
| Legal | - | - | + | - |
| Keller Kiliani | - | - | - | - |
| **Tannins** | | | | |
| Lead acetate | - | + | + | + |
| Gelatin | - | + | + | + |
| **Carbohydrates** | | | | |
| Molisch's | - | - | + | - |
| Fehling's | - | - | + | - |
| Phloroglucinol HCl | - | - | + | - |
| **Amino acids** | | | | |
| Ninhydrin | - | + | - | + |
| Millon's | - | + | - | + |
| Biuret | - | + | - | + |
| Xanthoprotein | - | + | - | + |
| **Phenol** | | | | |
| Ferric chloride | - | + | - | + |
| **Saponin** | | | | |
| Foam | - | - | + | - |
| **Terpenoids** | | | | |
| Lieberman Burchard's | + | + | - | + |
| Salkowski's | + | + | - | + |
| **Steroids** | | | | |
| Lieberman's | + | + | - | + |

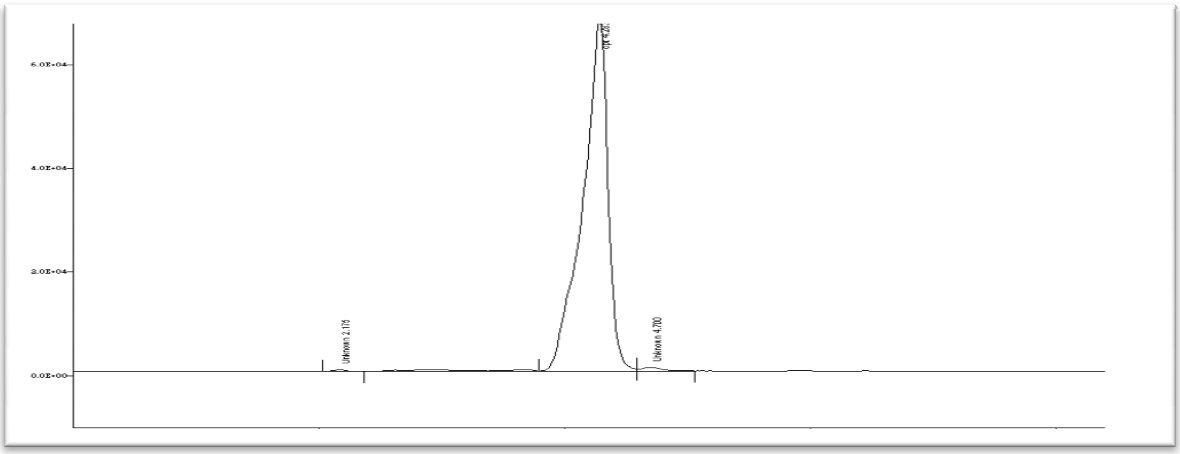


**Fig. 2: Estimation of standard camptothecin**

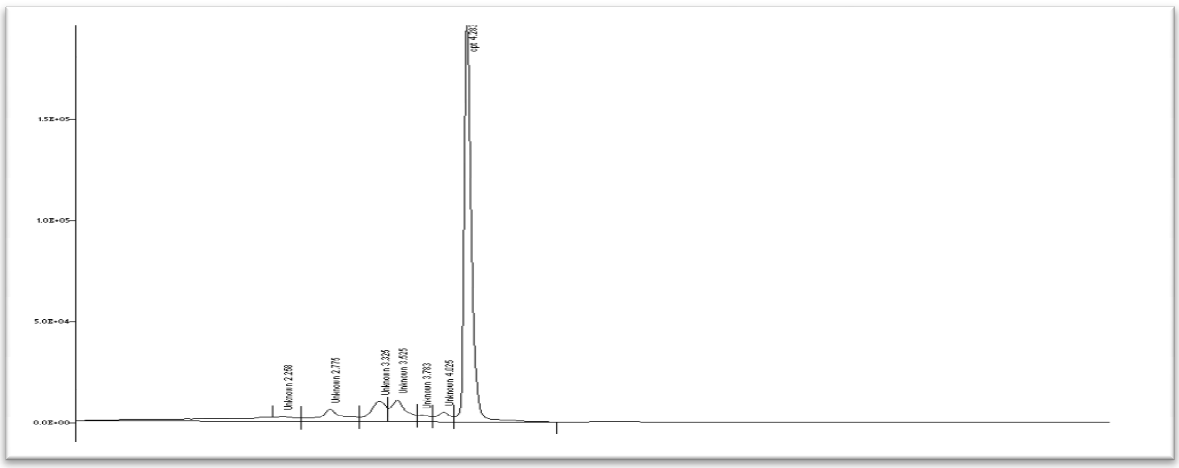


**Fig. 3: Estimation of camptothecin in methanolic extract of *N. nimmoniana***

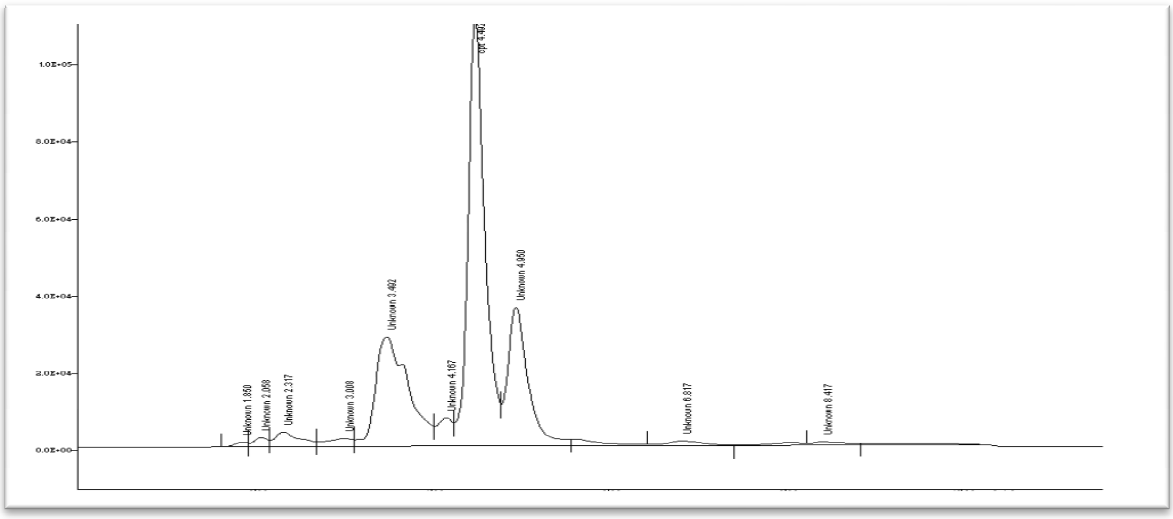


**Fig. 4: Estimation of camptothecin in chloroform extract of *N. nimmonian***

The amount of camptothecin extracted from each extacts is determined with the help of HPLC and represented graphically in figure 5, which clearly shows the amount of camptothecin is more in methanol and chloroform extract whereas absent in water, n-hexane and petroleum ether extract.

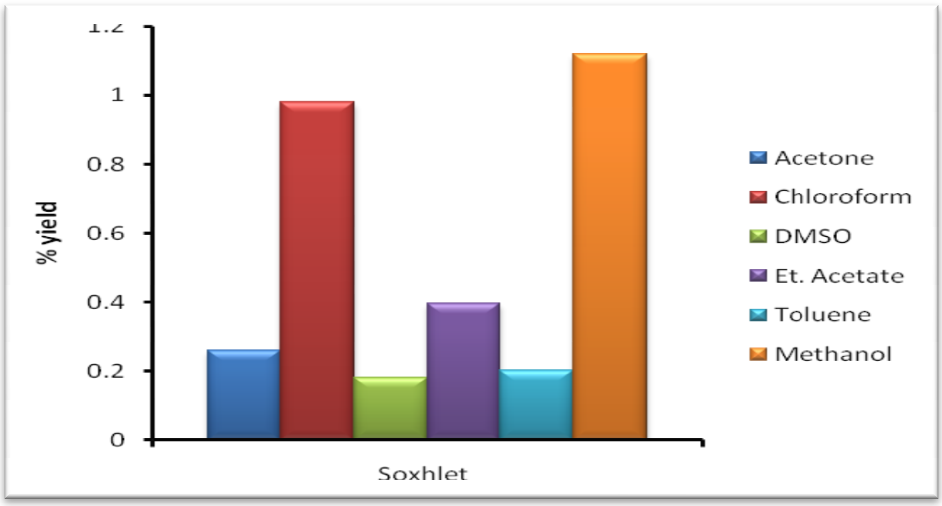


**Fig. 5: Graph of percentage yield of camptothecin in stem extracts by HPLC**

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.

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