WJPMR, 2025, 11(12), 119-128

Research Article

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Impact Factor: 6.842

ISSN (O): 2455-3301

ISSN (P): 3051-2557

Coden USA: WJPMBB

PHYTOCHEMICAL SCREENING, QUANTIFICATION, ANTIOXIDANT ACTIVITY AND EXTRACTION OF ACTIVE COMPOUND (CAMPTOTHECIN) FROM LEAF EXTRACT OF NOTHAPODYTES NIMMONIANA (J. GRAHAM) MABB

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DOI: https://doi.org/10.5281/zenodo.17746521

How to cite this Article: Sumathi Ethiraj*, Janarthanam Balasundaram, Divya Ravichandran and Kanaga Subramani. (2025). PHYTOCHEMICAL SCREENING, QUANTIFICATION, ANTIOXIDANT ACTIVITY AND EXTRACTION OF ACTIVE COMPOUND (CAMPTOTHECIN) FROM LEAF EXTRACT OF NOTHAPODYTES NIMMONIANA (J. GRAHAM) MABB. World Journal of Pharmaceutical and Medical Research, 11(12), 119–128.

Article Received on 02/11/2025 **Article Revised on 21/11/2025** **Article Published on 01/12/2025**

ABSTRACT

The aim of the current study was to analyse the phytochemical constituents, total phenol, flavonoid and alkaloid, anti-oxidant activity and high-performance liquid chromatography (HPLC) analysis of Camptothecin compound from the leaf extract of *Nothapodytes nimmoniana*. Preliminary screening involved the qualitative methods to detect the presence of terpenoids, flavonoids, phenols, tannins, steroids, quinones, saponins, cardiac glycosides and alkaloids in the leaf extracts of *Nothapodytes nimmoniana*. Total phenol, flavonoid and alkaloid contents were quantitatively estimated. Total phenolic content was estimated by Folin-Ciocalteau method and the total flavonoid content was determined by aluminium chloride colorimetric method. *In vitro* antioxidant activity of aqueous, ethanol, acetone, chloroform and petroleum ether extracts was evaluated by studying 1, 1-diphenyl-2-picrylhydrazyl free radical scavenging activity using the standard procedure. The leaf extract was screened for a major active compound namely Camptothecin compound using HPLC. Qualitative phytochemical screening of ethanolic leaf extract of *Nothapodytes nimmoniana* revealed the presence of significant secondary metabolites such as quinones, saponin, cardiac glycosides, alkaloids, terpenoids, flavonoids, steroids, phenols, tannins and glycosides. Phytochemical quantification of the leaf extracts showed that the highest total phenolic content of 39.13 ± 0.27mg Gallic Acid Equivalents (GAE)/g, total flavonoid content of 13.82 ± 0.19 mg Quercetin Equivalents (QE)/g and alkaloid content of 12.71 ± 0.38 mg/g. Five different solvent extracts of *Nothapodytes nimmoniana* leaf were evaluated for antioxidant activities by DPPH assay using Butylated Hydroxy Toluene (BHT) as standard. Among five different solvents used, maximum antioxidant activity was found in ethanol leaf extract (90.18 ± 0.32 %) followed by others. The results of HPLC analysis in the leaf extract of *Nothapodytes nimmoniana* revealed the presence of the active principle namely Camptothecin. The HPLC method is efficient, precise, accurate and sensitive to determining active compounds in plant extracts and it is recommended for efficient assays in routine work.

KEY WORDS: *Nothapodytes nimmoniana*, Phytochemical Screening, Antioxidant Activity, HPLC analysis, Camptothecin.

INTRODUCTION

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years. Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind (Momin and Kadam, 2011). Therapeutic benefits can be traced to specific plant compounds; many herbs contain dozens of active constituents that, together, combine to give the plant its therapeutic value (Helen *et al.*, 2013). A growing body of evidence indicates that secondary plant

metabolites play important roles in human health and may be nutritionally important (Jeeva *et al.*, 2012). Plant based chemicals have recently received a lot of interest due to their wide range of applications. Medicinal plants are a diverse set of species that have an extensive spectrum of active chemicals that may be used to cure a variety of human and animal disorders. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.*, 2008). Alkaloids, carbohydrates, terpenoids, steroids, flavonoids and tannins are only a few examples of phytochemicals found in medicinal plants that have a certain physiological impact on the human body and are useful for treating and curing human illnesses (Krishnaiah *et al.*, 2007). Phytochemicals have a major impact on various preparations like food, cosmetics, pharmaceutical, flavours and agrochemical etc. as antimicrobial agents as well as antioxidants. Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like Alkaloids, Flavonoids, Phenolic Compounds, Saponins, Steroids, Tannins, Terpenoids etc. (Akindele *et al.*, 2007).

Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and peroxynitrite) produced during aerobic metabolism in the body can cause oxidative damage of amino acids, lipids, proteins and DNA (Heim et al., 2002; Arika *et al.*, 2019). It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and Alzheimer's diseases (Devasagayam *et al.*, 2004; Iqbal and Bhanger, 2006). The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, either exogenous or endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders (Evans *et al.*, 1995; Tiwari, 2001).

In addition, phenolic compounds and flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. (Miller, 1996). The crude extracts of herbs, spices and other plant materials, rich in phenolics and flavonoids are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Chu *et al.*, 2000). Medicinal plants are abundant in natural antioxidants, the best known are tocopherols, carotenoids, vitamin C, flavonoids and different other phenolic compounds (Iqbal and Bhanger, 2006). Recently, among natural antioxidants, flavonoids have received increasing attention. As compared with Vitamin C and E, dietary flavonoids are considered to be more powerful antioxidants (Sultana and Anwar, 2008). Flavonoids and alkaloids are known to be highly effective antioxidants by scavenging oxygen radicals, by having interesting anti-cancer, hypolipidemic, anti-ageing and anti-inflammatory activities (Braca *et al.*, 2002).

Nothapodytes nimmoniana (Grah.) Mabb. belonging to the family Icacinaceae, is commonly known as Amruta and found in Western Ghats of Maharashtra, Goa, Tamil Nadu Kerala, Karnataka, Assam and Jammu and Kashmir. (Mythili *et al.*, 2013). *Nothapodytes nimmoniana* is a small tree acquiring a height of 4–10 meter, with a spreading crown. This shrubby small tree has broad dark green leaves and flowers. (Khan Nazeerullah *et al.*, 2012). This plant shows many pharmacological activities such as anti-cancer, anti-HIV, antimalarial, antibacterial, antioxidant and anti-inflammatory, antifungal activity. It is also useful in curing anaemia as well as sarcomas such as lungs, breast and uterine cervical cancers (Senthil Rajan Dharmalingam *et al.*, 2014). The major source of a potent alkaloid, namely camptothecin (CPT), which is used in various types of cancer namely breast cancer, cervical cancer, ovarian cancer, lung cancer has been reported (Sumitra *et al.*, 2018). Hence, the present study was performed to investigate the phytochemical constituents, total phenol, flavonoid and alkaloid, anti-oxidant activity and high-performance liquid chromatography (HPLC) analysis of Camptothecin from the leaf extract of *N. nimmoniana*.

MATERIALS AND METHODS

Collection of *Nothapodytes nimmoniana*

The healthy plants of *Nothapodytes nimmoniana* leaf (Fig.1a, b, c) were collected from different places of Tamil Nadu namely Kolli Hills, Tirunelveli and Kodaikanal and the plant was authenticated by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre, Sakthi Nagar, West Tambaram, Chennai- 600 045. The collected leaves were brought to the laboratory processed and shade dried. The shade dried plant samples were maintained at Department of Biotechnology, University of Madras, Guindy Campus, Chennai -600 025.

Preparation of the Plant Extract

Preparation of the extracts was done according to a combination of the methods as described by Pizzale *et al.*, 2002 and Lu and Foo, 2001. The dried leaf fine powder of *N. nimmoniana* plant material was extracted with pestle and mortar and extracted with 150 ml aqueous, ethanol, chloroform, acetone and petroleum ether for 1 minute using an Ultra Turax mixer (13,000 RPM) and soaked overnight at room temperature. The extracts were then filtered through Whatman No.1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evator at 40°C to a constant

weight and then dissolved in respective solvents. The concentrated extracts were stored in an airtight container in the refrigerator below 10°C.

Phytochemical Screening of Leaf Extract of *Nothapodytes nimmoniana*

The phytochemical screening of leaf extracts of *N. nimmoniana* was assessed by standard method (Siddiqui and Ali 1997; Savithramma *et al.,* 2011; Sumathi and Vandana, 2018). Phytochemical screening was carried out on the leaf extract using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides**,** coumarins and steroids. General reactions in this analysis indicated the presence or absence of these compounds in the leaf extract tested.

Estimation of total phenol content in *N. nimmoniana*

Total phenolic content in the leaf extracts was determined by the Folin– Ciocalteau colorimetric method (Slinkard and Singleton, 1977). For the analysis, 0.5 ml aliquot of sample was added to 0.5 ml of Folin–Ciocalteau reagent (0.5 N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of sodium carbonate (2%) was added and the mixture was allowed to stand for 30 minutes after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolic contents were expressed as mg gallic acid equivalents (GAE)/g extract.

Estimation of Total Flavonoid Content in *N. nimmoniana*

The amount of total flavonoid content in the ethanolic leaf extract was determined by aluminium chloride colorimetric method (Mervat *et al*., 2009). 0.5 ml of leaf extracts of *N. nimmoniana* at a concentration of 1mg/ ml was taken and the volume was made up to 3ml. Then, 0.1ml of aluminium chloride (10%), 0.1ml of potassium acetate and 2.8 ml of distilled water were added sequentially. The test solution was shaken thoroughly and absorbance was measured at 415nm after 30 minutes of incubation. A standard calibration plot was generated at 415nm for known quercetin concentrations. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

Estimation of Total Alkaloid Content in *N. nimmoniana*

Quantitative estimation of alkaloid content in the ethanolic leaf extract of *N. nimmoniana* was carried out following the method of Edeoga *et al*., (2005) and Okwu and Josiah, (2006). 2.5 g of the leaf fine powder was extracted using 100 ml of 20% acetic acid in ethanol. The solution was allowed to stand at room temperature for almost 4 hours. Filtrate was concentrated to 25 ml, Concentrated ammonium hydroxide was added stepwise until precipitation appeared. The entire solution was kept at room temperature for the precipitate to settle completely. The collected precipitate was washed with dilute ammonium hydroxide and finally filtered. Filtrate was discarded, pellet obtained was dried and total alkaloid content recorded. Total alkaloid was calculated with the following formula: Alkaloid (%) = [final weight of the sample / initial weight of the extract] × 100

Quantitative Analysis of Free Radical Scavenging Activity of *N. nimmoniana*:

The antioxidant activities were quantitatively determined by using 2, 2 diphenyl-1-picryl hydrazyl (DPPH) as a free radical. 100µl of Leaf extracts of *N. nimmoniana* was mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al.,* 2003). Subsequently at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517 nm. The samples antioxidant ability were compared to the recognized synthetic standard, 0.16% Butylated Hydroxy Toluene (BHT) at 517nm. The experiment was carried out in triplicates. Free radical scavenging activity was calculated by the prescribed formula: % DPPH radical scavenging = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100.

HPLC analysis of Camptothecin compound

The fine powder of the fruit peel biomass was extracted with 75% of ethanol and then, the extract was evaporated. The residue of extract was mixed with n-butanol and water (2:1) and both the upper layer of n-butanol and lower layer of water were separated and evaporated under vacuum. The residues were washed with petroleum ether to remove fatty components and then extracted with methanol. The concentrated extract in methanol was separated and analysed using HPLC as per standard method (Janarthanam and Sumathi, 2015). The extracts were filtered through Sartorius regenerated cellulose membrane syringe filter (0.2 µ) and 20 µL of the filtrate was injected into the HPLC. Chromatography was performed using Shimadzu HPLC (Model SPD-10A UV-visible Detector) and Supelcosil LC-18 column (25 cm × 4.6 mm, 5 m) with mobile phase consisting of acetonitrile, water and acetic acid (50:50:0.1). Flow rate was maintained at 1.0 mL/min with a back pressure of 250 psi and the compounds were read at 270 nm using a UV detector. The total run time was 20 min, but preferably it was extended up to 40 min (Shimizu *et al.,* 1997). The results were compared with standard.

RESULTS AND DISCUSSION

The present study revealed that the polar (aqueous, ethanol, and acetone) and non-polar (petroleum ether and chloroform) solvent extracts were obtained from the leaf extracts of *N. nimmoniana* collected from different places namely Kolli Hills, Tirunelveli and Kodaikanal. The results of phytochemicals analysis are presented in Tables 1-3. The phytochemical screening was performed

with three wild accessions and five different solvent extracts recorded maximum presence of phytochemical detection of chemical constituents and showed that the ethanolic leaf extract of *N. nimmoniana* (Kolli Hill accession) was rich in secondary metabolites such as tannins, saponins, phenol, flavonoids, quinones, cardiac glycosides, terpenoids, steroid and alkaloids followed by leaf collected from Tirunelveli and Kodaikanal accession.

The results also recorded maximum phytochemical constituents present in ethanol extract of all three leaf extracts than other solvents such as aqueous, acetone, chloroform and petroleum ether extract. Thus, the preliminary screening tests would be useful in the detection of bioactive principles that would lead to the development and discovery of drugs. These secondary metabolites are reported to have many biological and therapeutic properties (Jamuna *et al.,* 2014). Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. The presence of alkaloids and saponins in the leaf extract, the biological function of alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and bactericidal activities (Stary, 1998). Saponins have properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties, formation of foams in aqueous solutions and hemolytic activity (Sodipo *et al.,* 2000) and traditionally saponins have been extensively used as detergents and molluscicides. In addition to their industrial applications as foaming and surface-active agents they also possess beneficial health effects (Shi *et al.,* 2004). Plant steroids are known important for their cardiotonic activities and also used in nutrition, herbal medicine and cosmetics.

Phenolics are among the most prevalent secondary metabolites in the plant kingdom. Plant phenolics are one of the most important classes of chemicals that can serve as natural antioxidants by scavenging free radicals. Phenolic compounds are a class of antioxidant agents which serves as a free radical terminator (Shahidi and Wanasundara, 1992). In our study, total phenol content (TPC) of *N. nimmoniana* leaf extract was estimated by using Folin- Ciocalteau colorimetric method and represented in terms of gallic acid equivalent (GAE). The result of the present study showed that the phenol contents of the ethanolic leaf extracts in terms of GAE were 39.13 ± 0.27 mg GAE/g (Table. 4). The higher content of total phenol was found in leaf extract of *N. nimmoniana* (Kolli Hill accession), followed by other accessions. Phenolic compounds are important plant antioxidants, which exhibited considerable scavenging activity against free radicals. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers (Rice-Evans *et al.,* 1997). Thus, antioxidant capacity of a sample can be attributed mainly to its phenolic compounds (Zheng and Wang, 2003; Chinnici *et al.,* 2004; Huang *et al.,* 2009).

Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. Flavonoids are a class of secondary plant metabolites with significant antioxidant and chelating properties (Rathanavel and Arasu, 2014). The results of the present study showed that the flavonoid contents of the ethanol leaf extract in terms of QE were found to be maximum 13.82 ± 0.19 mg QE/g (Table. 4). Alkaloids, widely existing in natural plants, are compounds containing basic nitrogen atoms. Most of alkaloids are pharmacologically active ingredients in many medicinal plants due to their significant physiological activity. Alkaloids have a wide range of pharmacological activities including anticancer and antibacterial activities, hence responsible for many healing properties in natural medicine (Xu *et al.,* 2009; Chandan and Krishna, 2024). Results in Table 4 recorded that the yield of alkaloids (12.71 ± 0.38 mg/g) is obtained from ethanol leaf extract of *N. nimmoniana* (Kolli Hill accession).

The percentage of DPPH radical scavenging activity of leaf extracts of *N. nimmoniana* from various accessions is shown in Fig. 2a, b, c. The results revealed that among three accessions and five different solvent extracts of *N. nimmoniana*, the ethanol leaf extract collected from Kolli Hill accession had maximum DPPH radical scavenging activity (90.18%), followed by Tirunelveli (83.29 %) and Kodaikanal accession (79.41 %), when compared with that of synthetic antioxidant BHT as a positive control (98.6%). In all accessions, ethanol leaf extracts recorded higher percentage of free radical inhibition followed by others. Scavenging activity for free radicals of DPPH (1,1-Diphenyl-2-picryl hydrazyl) has extensively used to assess antioxidant capacity of natural products from plant and natural sources. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants (Gálvez and Cordero, 2005; Tepe, *et al.,* 2005; Mammadov 2011).

The HPLC analysis of standard Camptothecin along with the camptothecin compound from *N. nimmoniana* leaf extract has been represented in Fig. 3a, b. Camptothecin compound eluted through HPLC analysis and based on standard retention time (Rt) 3.57 min. The *N. nimmoniana* leaf extract (Kolli Hill) used for HPLC analysis recorded a Rt of 3.52 minutes and standard camptothecin compound recorded a Rt of 3.57 minutes, thus confirming the presence of camptothecin compound in leaf extract of *N. nimmoniana*.

CONCLUSION

The present study revealed that ethanol leaf extract of *N. nimmoniana* was rich in phytochemical constituents and high levels of total phenolic, flavonoid and alkaloid

compounds. The leaf extract of *N. nimmoniana* also possessed strong antioxidant potential and was thus capable of inhibiting, quenching free radicals to terminate the radical chain reaction. The HPLC analysis revealed the identification of active compound, namely camptothecin, present in the leaf extract of *N. nimmoniana*. The results indicate that the plant material may become an important source of natural drug compounds with health protective potential and natural antioxidants of significant impact on the status of human health and disease prevention.

Conflicts of Interest

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENTS

The author Dr. Sumathi Ethiraj acknowledges the Tamil Nadu State Council for Higher Education (TANSCHE), Chennai-600 005, Tamil Nadu for the financial support provided under the TANSCHE sponsored project (File No. RGP/2019-20/MU/HECP-0041).

Table 1: Phytochemical screening from leaf extract of *Nothapodytes nimmoniana*. (Kolli Hill accession)

Phytochemicals	Leaf extract of *Nothapodytes nimmoniana*				
	Aqueous	Ethanol	Acetone	Chloroform	Petroleum ether
Tannins	++	++	++	+	-
Saponins	++	++	+	-	-
Quinones	+	++	+	-	-
Flavonoids	+	++	-	-	-
Phenols	++	++	+	+	+
Cardiac glycosides	-	++	+	+	+
Terpenoids	+	++	+	+	+
Coumarins	+	++	-	-	-
Steroids	++	++	+	+	+
Alkaloids	-	++	+	-	+
Anthocyanin	-	-	+	-	+
Betacyanin	+	+	-	-	-

Key: + Positive: ++ Strong positive: - negative

Table 2: Phytochemical screening from leaf extract of *Nothapodytes nimmoniana* (Tirunelveli accession)

Phytochemicals	Leaf extract of *Nothapodytes nimmoniana*				
	Aqueous	Ethanol	Acetone	Chloroform	Petroleum ether
Tannins	++	++	++	+	-
Saponins	++	+	+	-	-
Quinones	+	+	+	-	-
Flavonoids	+	++	-	-	-
Phenols	+	++	+	+	+
Cardiac glycosides	-	+	+	+	+
Terpenoids	+	++	+	+	+
Coumarins	+	+	-	-	-
Steroids	+	++	+	+	+
Alkaloids	-	+	+	-	+
Anthocyanin	-	-	+	-	+
Betacyanin	+	+	-	-	-

Key: + Positive: ++ Strong positive: - negative

Table 3: Phytochemical screening from leaf extract of *Nothapodytes nimmoniana.* (Kodaikanal accession)

Phytochemicals	Leaf extract of *Nothapodytes nimmoniana*				
	Aqueous	Ethanol	Acetone	Chloroform	Petroleum ether
Tannins	++	++	++	+	-
Saponins	++	+	+	-	-
Quinones	+	+	+	-	-
Flavonoids	+	++	-	-	-
Phenols	+	++	+	+	+
Cardiac glycosides	-	+	+	-	-

Terpenoids	+	++	+	+	+
Coumarins	+	++	-	-	-
Steroids	+	+	+	+	+
Alkaloids	-	+	+	-	+
Anthocyanin	-	-	+	-	+
Betacyanin	+	+	-	-	-

Key: + Positive: ++ Strong positive: - negative

Table 4: Quantification of Total Phenol, Flavonoid and Alkaloid Content in leaf extracts. of *Nothapodytes nimmoniana*

Samples	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE /g)	Total Alkaloid Content (mg/g)
Nothapodytes nimmoniana (Kolli Hill accession)	**39.13 ± 0.27**	**13.82 ± 0.19**	**12.71 ± 0.38**
Nothapodytes nimmoniana (Tirunelveli accession)	**27.91 ± 0.15**	**11.67 ± 0.31**	**10.43 ± 0.4**
Nothapodytes nimmoniana (Kodaikanal accession)	**26.36 ± 0.08**	**10.58 ± 0.25**	**9.67 ± 0.13**

Fig. 1a

Fig. 1b

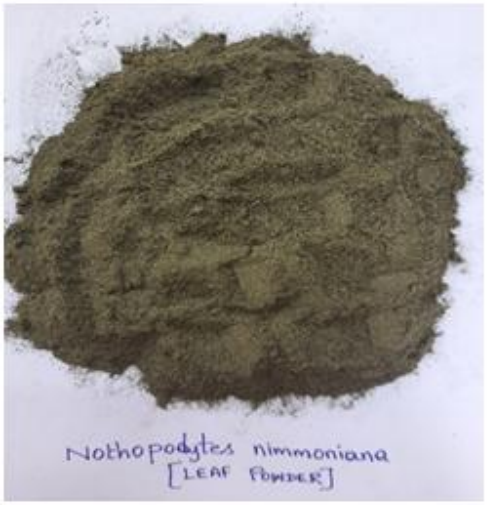

Fig. 1c

Figure 1a. Mother plant of *Nothapodytes nimmoniana* (Kolli Hills accession), 1b & 1c. *Nothapodytes nimmoniana.* dried leaf biomass and fine leaf powder.

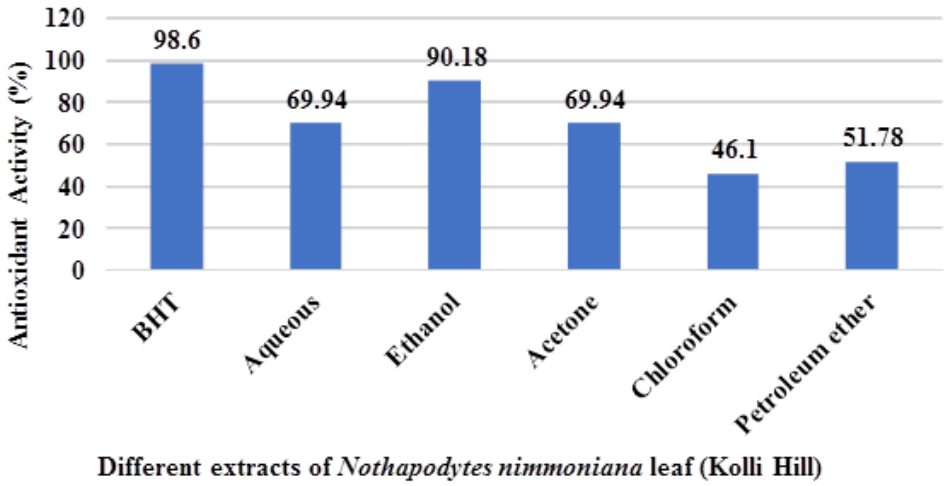

Figure 2a: Quantitative analysis of antioxidant activity from leaf extracts of *Nothapodytes nimmoniana* (Kolli Hill accession).

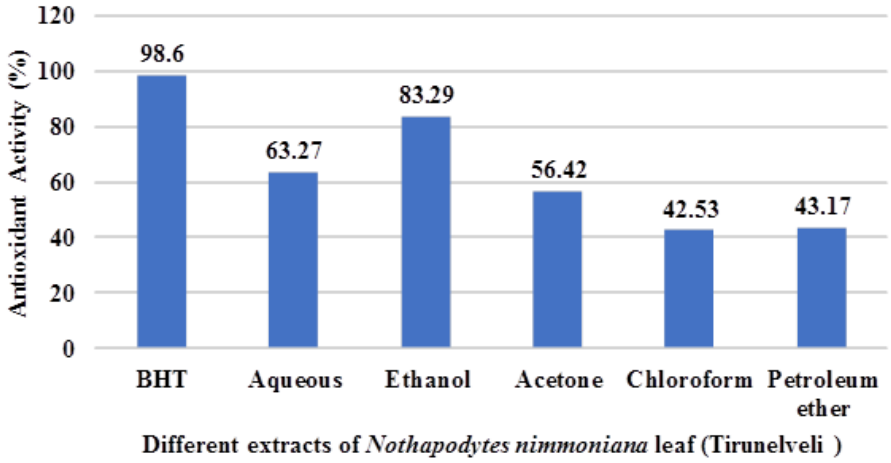

Figure 2b: Quantitative analysis of antioxidant activity from leaf extracts of *Nothapodytes nimmoniana* (Tirunelveli accession).

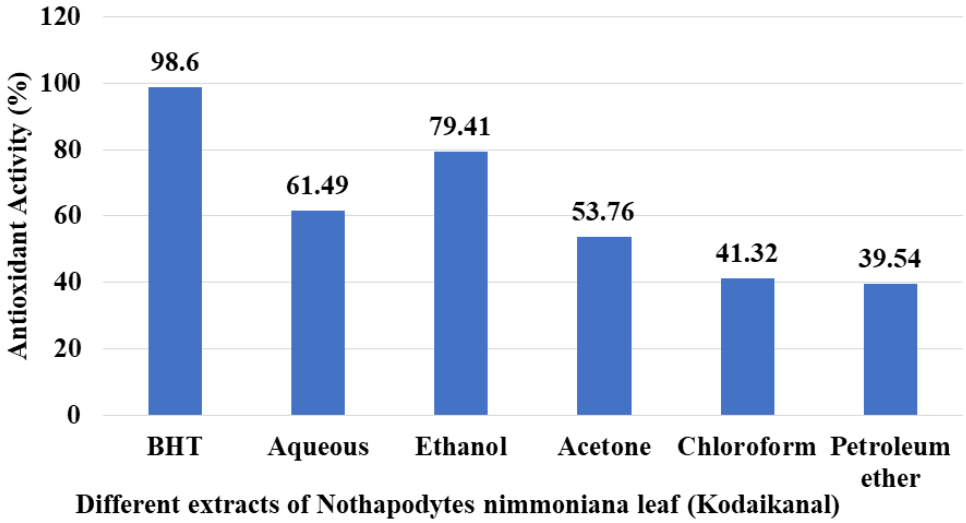

Figure 2c: Quantitative analysis of antioxidant activity from leaf extracts of *Nothapodytes nimmoniana* (Kodaikanal accession).

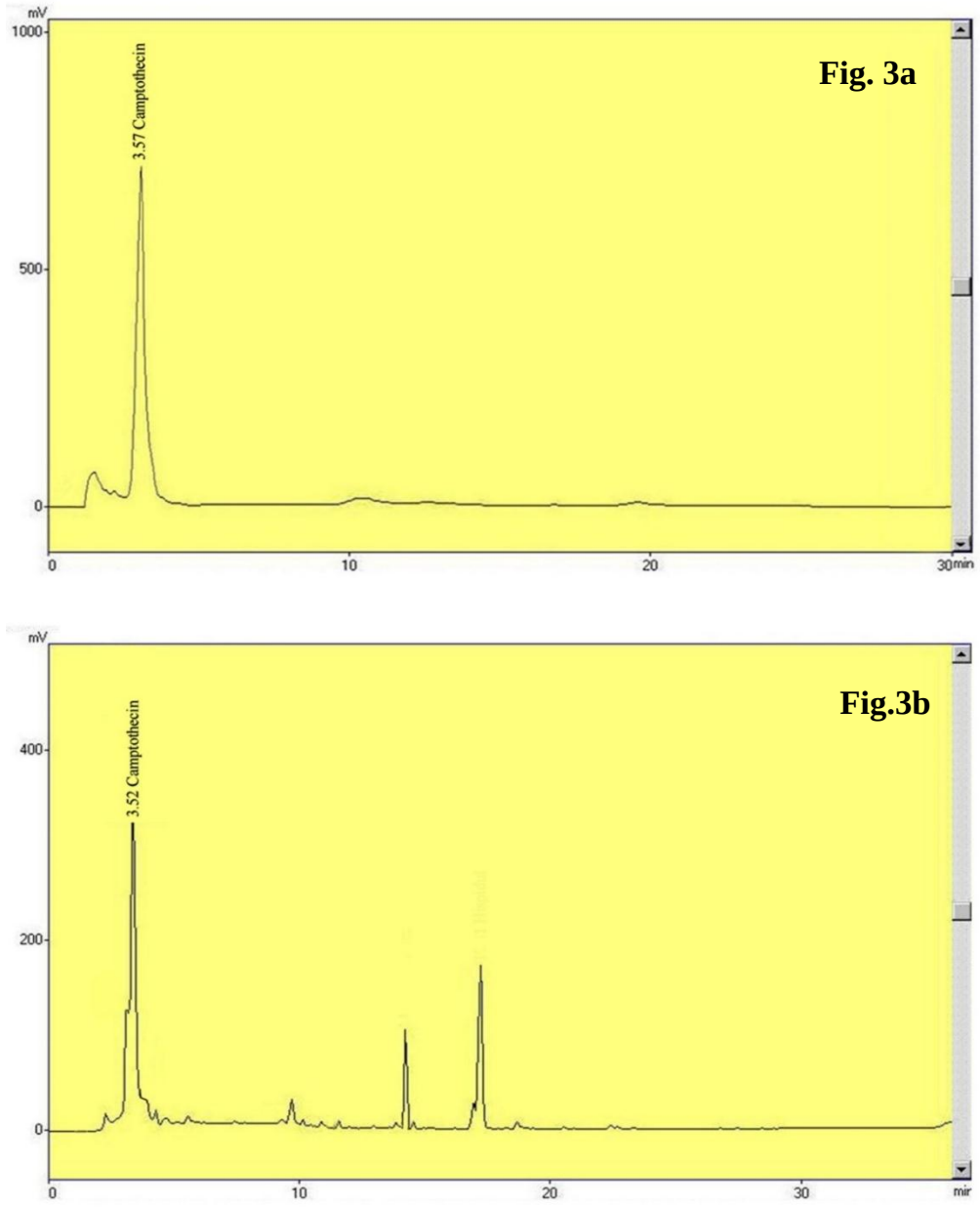

Figure. 3a, b. High-performance liquid chromatography (HPLC) analysis of Camptothecin. a) Camptothecin standard (1 mg / 1 mL), (b) HPLC of Camptothecin compound from the leaf extract of *Nothapodytes nimmoniana* (Kolli Hill accession).

REFERENCES

1. Momin RK, Kadam VB. Determination of ash values of some medicinal plants of genus *Sesbania* of Marathwada region in Maharashtra. J. Phytol, 2011; 3(2): 52-54.
2. Helen PA, Aswathy MR, Deepthi KG, Mol R, Joseph RJ, Jaya Sree, S. Phytochemical analysis and anticancer activity of leaf extract of *Mangifera indica* (*Kottukonam Varika*). IJPSR, 2013; 4(2): 819-824.
3. Jeeva S, Sheela JD, Shamila RMI, Lekshmi PJ, Brindha RJ. Antimicrobial activity and phytochemical analysis of *Sansevieria roxburghiana* leaf. Asian J. Plant Sci,, 2012; 2: 41-44.
4. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr. J. Biotechnol., 2008; 7(12): 1797-1806.
5. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine: a move towards nature. Biotechnol. Mol. Biol. Rev, 2007; 1(4): 97-104.
6. Akindele AJ, Adeyemi OO. Anti-inflammatory activity of the aqueous leaf extract of *Byrosocarpus coccineus*. Fitoterapia, 2007; 78(1): 25-28.
7. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-

activity relationships. J. Nutr. Biochem,, 2002; 13(10): 572-584.
8. Arika W, Kibiti CM, Njagi JM, Ngugi MP. *In vitro* antioxidant properties of dichloro methanolic leaf extract of *Gnidia glauca* (Fresen) as a promising anti-obesity drug. J. Evid. Based Integr. Med, 2019; 24: 1-17.
9. Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele, RD. Free radicals and antioxidants in human health: current status and future prospects. J. Assoc. Phys. India, 2004; 52: 794-804.
10. Iqbal S, Bhanger M. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. J. Food Comp. Anal, 2006; 19: 544-551.
11. Evans RC, Miller NJ, Bolwell GP, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Rad. Res, 1995; 22(4): 375-383.
12. Tiwari A. Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidant therapy. Curr. Sci., 2001; 81(9): 1179,
13. Miller AL. Antioxidant flavonoids: structure, function and clinical usage. Altern. Med. Rev, 1996; 1: 103-111.
14. Chu YH, Chang CL, Hsu HF. Flavonoid content of several vegetables and their antioxidant activity. J. Sci. Food Agric, 2000; 80(5): 561-566.
15. Sultana B, Anwar F. Flavonols (kaempferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. Food Chem, 2008; 108(3): 879-884.
16. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from *Licania licaniaeflora*. J. Ethnopharmacol, 2002; 79(3): 379-381.
17. Mythili K, Umamaheswara Reddy C, Chamundeeswari D, Manna PK. GC-MS analysis of phytocomponents and *in vitro* inhibitory effects of *Calanthe triplicata*. J. Nat. Prod., 2013; 6: 141-146.
18. Khan Nazeerullah KN, Kumar Sunil KS, Pal SR, Dhankhar Neelam DN. Preliminary phytochemical screening, fingerprinting and pharmacognostic evaluation of *Nothapodytes nimmoniana* leaves, stem and root collected from different geographical regions. J. Pharm., Biol. Chem. Sci., 2012; 3(1): 362-378.
19. Senthil RD, Rajkumar M, Srinivasan R, Venkata SM, Suresh S, Senthil Kumar K L. Anti-carcinogenic effects of *Nothapodytes nimmoniana* against Dalton's lymphoma ascites tumor model. Eur. J. Exp. Biol, 2014; 4(1): 527-533.
20. Sumitra P, Suman S, Arun DP, Shambhu C, Samiat O. Review on *Nothapodytes nimmoniana* and its nephroprotective, hepatoprotective and antioxidant activities. J. Emerg. Technol. Innov. Res, 2018; 5(11): 436-443.
21. Pizzale L, Bortolomeazzi R, Vichi S, Conte LS. Antioxidant activity of sage and oregano extracts related to their phenolic compound content. J. Sci. Food Agric, 2002; 82(14): 1645-1651.
22. Lu Y, Foo Y. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). Food Chem, 2001; 75(2): 197-202.
23. Siddiqui AA, Ali M. Practical pharmaceutical chemistry, 1st ed. New Delhi: CBS Publishers and Distributors., 1997; 126-131.
24. Savithramma N, Linga RM, Bhumi G. Phytochemical screening of *Thespesia populnea* L. Soland and *Tridax procumbens* L. J. Chem. Pharm. Res, 2011; 3(5): 2834.
25. Sumathi E, Vandana S. Phytochemical screening, antioxidant activity and extraction of active compound (Anonaine) from fruit peel extract of *Annona reticulata* L. Asian J. Pharm. Clin. Res, 2018; 11(11): 372-377.
26. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. Am. J. Enol. Vitic, 1977; 28(1): 49-55.
27. Mervat MM, Far El, Taie HAA. Antioxidant activities, total anthocyanins, phenolics and flavonoids contents of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol. Aust. J. Basic Appl. Sci, 2009; 3(4): 3609-3616.
28. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol, 2005; 4(7): 685-688.
29. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. Afr. J. Biotechnol, 2006; 5(4): 357-361.
30. Lee SE, Hwang HJ, Ha JS. Screening of medicinal plant extracts for antioxidant activity. Life Sci, 2003; 73(2): 167-179.
31. Janarthanam B, Sumathi E. Optimization of biomass culture yield and L-dopa compound in the callus culture from cotyledonary leaves of *Mucuna pruriens*. Asian J. Pharm. Clin. Res, 2015; 8(4): 282-286.
32. Shimizu T, Muroi T, Ichi T, Nakamura M, Yoshihira K. Analysis of red cabbage colors in commercial foods using high performance liquid chromatography with photodiode array detection–mass spectrophotometry. J. Food Hyg. Soc. Japan, 1997; 38(1):34-38.
33. Jamuna S, Subramaniam P, Krishnamoorthy K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb *Hypochaeris radicata* L. for *in vitro* antioxidant activities. Asian Pac. J. Trop. Biomed, 2014; 4(S1): 359-367.
34. Stary F. The natural guide to medicinal herbs and plants. London: Tiger Books International, 1998; 12-16.
35. Sodipo OA, Akiniyi JA, Ogunbamosu JU. Studies on certain characteristics of extracts of bark of *Pansinystalia macruceras* [K. Schemp] Pierre ex Beille. Glob. J. Pure Appl. Sci., 2000; 6: 83-87.

36. Shi J, Kakuda Y, Yeung D. Antioxidative properties of lycopene and other carotenoids from tomatoes: synergistic effects. Biofactors, 2004; 21(14): 203-210.
37. Shahidi F, Wanasundara PK. Phenolic antioxidants. Crit. Rev. Food Sci. Nutr, 1992; 32(1): 67-103.
38. Rice-Evans CA, Sampson J, Bramley PM, Holloway DE. Why do we expect carotenoids to be antioxidants *in vivo*? Free Radic. Res, 1997; 26(4): 381-398.
39. Zheng W, Wang SY. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. J. Agric. Food Chem, 2003; 51(2): 502-509.
40. Chinnici F, Bendini A, Gaiani A, Riponi C. Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition. J. Agric. Food Chem., 2004; 52(15): 4684-4689.
41. Huang Z, Wang B, Eaves DH, Shikany, JM, Pace RD. Total phenolics and antioxidant capacity of indigenous vegetables in the Southeast United States: Alabama collaboration for cardiovascular equality project. Int. J. Food Sci. Nutr., 2009; 60(2): 100-108.
42. Rathanavel C, Arasu PT. Antioxidant activity, phenol and flavonoid contents of some selected Indian medicinal plants. Int. J. Curr. Microbiol. Appl. Sci., 2014; 3: 830-838.
43. Xu XL, Zou J, Liu Y, Peng H, Wu Q. Medicinal function, separation and purification of alkaloids: a review. Chem. Eng. Sci., 2009; 48: 3835-3843.
44. Chandan GR, Krishna K. Qualitative and quantitative phytochemical analysis of leaf extracts of *Mirabilis viscosa*. Plant Arch., 2024; 24(SI): 65-72.
45. Gálvez M, Martín-Cordero C, Houghton PJ, Ayuso MJ. Antioxidant activity of methanol extracts obtained from *Plantago* species. J. Agric. Food Chem., 2005; 53(6): 1927-1933.
46. Tepe B, Sokmen M, Akpulat HA, Sokmen A. Screening of the antioxidant potentials of six *Salvia* species from Turkey. Food Chem, 2006; 95(2): 200-204.
47. Mammadov R, Ili P, Vaizogullar HE. Antioxidant activity and total phenolic content of *Gagea fibrosa* and *Romulea ramiflora*. Iran. J. Chem. Chem. Eng., 2011; 30(3): 57-62.