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Qualitative and quantitative evaluation of secondary metabolites of different plant extracts of *Nothapodytes foetida* (Wight) Sleumer an important endangered medicinal tree

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ABSTRACT

The present study of phytochemical screening has revealed valuable information about the chemical constituents of *Nothapodytes foetida.* Phytochemical analysis of leaves, stem, bark and root extracts of *Nothapodytes foetida* was performed using different solvent systems such as chloroform, butanol, hexane, methanol and acetonitrile for the presence of different phytochemicals with standard procedures. The phytochemical screening (both qualitative and quantitative) of variousplant extracts of *Nothapodytes foetida* revealed the presence of different phytoconstituents such as alkaloids, flavonoids, glycosides, tannins, phenols and triterpenoids. Among all the solvents tested, methanol, butanol and chloroform extract of leaf, stem, bark and root showed high concentration of all phytoconstituents compared to hexane and acetonitrile solvent extracts. The total quantity of secondary metabolites was evaluated by using the standard procedures and the line of regression and the regression coefficient estimated from the calibration curve of various standards. The highest quantity of phytoconstituent present in the plant extracts was identified to be alkaloids. The alkaloid content of leaf was evaluated to be 66.11±0.47, whereas stem possess 56.27±0.38, bark 59.02±0.17 and root 62.34±0.27 mg AE/gm of extracts and subsequent high amounts of phytoconstituents identified was flavonoids, tannins, phenols

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and saponins. Phytochemical screening of *Nothapodytes foetida*used in the medical field for the design of new drugs.

***Keywords*:** Camptothecin, phytochemicals, alkaloids, flavonoids, glycosides, tannins, phenols, triterpenoids, saponins, *Nothapodytes foetida*

1. INTRODUCTION

Nothapodytes foetida (Wight) Sleumer (synonym: *Mappia foetida*) commonly known as amruta, narkya, ghanera, durvasanemara, kalgur, kalagaura [1-2], belongs to the family Icacinaceae and one among the important medicinal plants, having enormous potential to cure cancer [3]. This plant species has gained considerable importance due to the presence of anticancer alkaloid (Camptothecin) and is considered as an endangered species because, its availability is restricted to Western Ghats and high pressure and demand for the potent drug exploitation of this plant is very fast [4]. In recent years, several independent groups have addressed the need to conserve the species and to explore the possibilities of identifying high-yielding species and development of *in vitro* production systems [5-8].

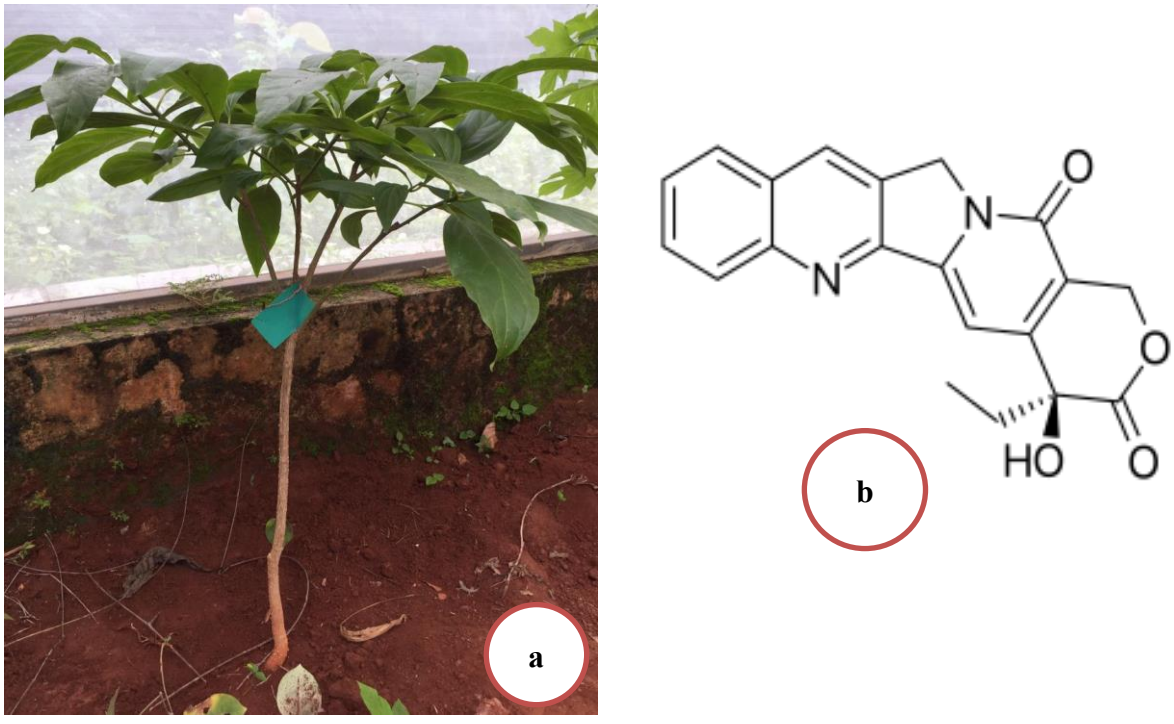

Figure 1(a,b). a. Young healthy plant of *Nothapodytes foetida*; **b.** Chemical Structure of Camptothecin

It is a small tree naturally distributed in many parts of the Western Ghats of India particularly found in Maharashtra, Goa, Karnataka, Kerala, Jammu and Kashmir and Tamil

Nadu areas. This plant is known for its rich source of potent cytotoxic quinoline alkaloids i.e., Camptothecin, 9-methoxy Camptothecin and its accumulation was reported in many parts of the plant [9]. The highest concentration of Camptothecin 0.3% (w/w) has been reported from *N. foetida* [10-11]. The patterns of accumulation of the alkaloids vary with age and seasonality as in *Nothapodytes nimmoniana* [12-13].

Camptothecin (CPT) possess a wide range of pharmacological activities like anti-cancer [14], anti-HIV, antibacterial,antimalarial [15], anti-inflammatory, anti-fungal, anti-oxidant [16] and also used to treat anaemia. CPT is a water insoluble pentacyclic monoterpene indole alkaloid, is regarded as one of the most promising antineoplastic agents of the twenty first century [14, 17]. Camptothecin (CPT) was isolated for the first time from the plant *Camptotheca acuminata* of Nyssaceae family [18] was further detected in *N. foetida.*

Camptothecin (CPT), 9-methoxy camptothecin and mappicine is a cytotoxic quinoline alkaloid used as an anticancer drug in the treatment of colon, bladder, lungs, breast, uterine and cervical cancers [19]. The cellular target of CPT is DNA topoisomerase-I. It also inhibits the replication of retroviruses such as human immunodeficiency virus (HIV) [16, 20-21].

Mechanism of action of CPT involves inhibition of Topoisomerase I through stabilization of physical barriers of DNA synthesis by Topoisomerase I- DNA cleavable complex stabilization, resulting in cell death due to collision of replication fork at the complex [22].

2. MATERIALS AND METHODS

Leaves, stem, bark and root parts were collected from *N. foetida* tree growing in its natural habitat of forest park of Amboli ghat, Sawantwadi taluka, Maharashtra for the experimental work. The plant materials i.e., leaves, stem, bark and roots were washed with tap water and shade dried for 15-30 days, afterwards ground to uniform powder and stored separately in plastic zip lock bags for further experimental work.

2. 1. Preparation of plant extracts

Preliminary Phytochemical screening was performed by taking each 5g powder of shade dried plant materials. These powders were extracted using a cold maceration technique with 50 ml of hexane, chloroform, acetonitrile, butanol and methanol separately. Later the extracts were filtered and concentrated using rotary evaporator and subsequently subjected to qualitative and quantitative phytochemical screening for the presence or absence of various secondary metabolites. The qualitative analysis of phytoconstituents was performed by following tests:

Alkaloids

Dragendorff's test: 2 ml of HCl was added to 0.5 ml of herbal extract followed by 1 ml of reagent. An orange red precipitate formation indicates the presence of alkaloids. Mayer's test: A few drops of the reagent were added to 1 ml of the herbal extract. The formation of a pale or cream color precipitate shows the presence of alkaloids. Wagner's test: 10 ml of herbal extract was acidified by adding 1.5% v/v HCl and a few drops of Wagner's reagent. The formation of a yellowish brown precipitate confirms the presence of alkaloids. Hager's test: Few drops of Hager's reagent were added to 0.5 ml of herbal extract, formation of yellow color precipitate indicated the presence of alkaloids. Tannic acid test: Few drops of 10% tannic acid

were added to 0.5 ml of herbal extract, formation of buff color precipitate indicated the presence of alkaloids.

Glycosides

Legal's test: Few drops of pyridine and alkaline sodium nitroprusside solution was added to 1 ml of herbal extract, the appearance of blood red color indicated the presence of glycosides. Bromine water test: Few drops of bromine water added to 1 ml of herbal extract. Formation of yellow color precipitation indicated the presence of glycosides. Kellar Kiliani test: 0.4 ml of glacial acetic acid containing trace amounts of ferric chloride was added to the extract followed by 0.5 ml of conc. H_2SO_4 along the walls of the tubes. Formation of blue color in the acetic acid layer indicated the presence of Dixie sugars. Molisch test: 1 ml of herbal extract was treated with a few drops of alcohol α- naphthol and 2 ml of Conc. H_2SO_4 along the walls of the tubes. Appearance of a brown ring at the junction of two liquids indicated the presence of carbohydrates. Conc. H_2SO_4 test: 1ml of conc. H_2SO_4 was added to 1ml of test solution and allowed to stand for 2 min. Red precipitate indicates the presence of glycosides.

Tannins

$FeCl_3$ test: Few drops of $FeCl_3$ solution was added to 1ml of herbal extract. Formation of blue or green color indicated the presence of tannins. Gelatin test: Few drops of 1% gelatin solution in 10% NaCl added to 1ml of herbal extract. Formation of white precipitation indicated the presence of tannins. Lead acetate test: Aqueous basic lead acetate was added separately to 1ml of test solution. Bulky red precipitate indicates the presence of tannins. Alkaline reagent test: The test solution was treated with sodium hydroxide solution. Yellow to red precipitate indicates the presence of tannins.

Flavonoids

Shinoda's test: 1 ml of herbal extract was treated with a few Mg turnings and few drops of conc. HCl. Formation of pink/ crimson red/ green color indicated the presence of flavonoids. Alkaline reagent test: 1 ml of herbal extract was treated with a few drops of NaOH solution. Formation of intense yellow color, which disappears on addition of dilute acid indicates the presence of flavonoids. Zn-HCl reduction test: 1 ml of herbal extract was treated with a mixture of zinc dust and Conc. HCl. Occurrence of red color indicated the presence of flavonoids. Lead acetate test: Aqueous basic lead acetate was added separately to 1ml of test solution. Bulky reddish brown precipitate indicates the presence of flavonoids. Ferric chloride test: Few drops of $FeCl_3$ solution was added to the test solution. Blackish precipitate indicates the presence of flavonoids.

Saponins

Foam test: The plant extract was diluted in 20 ml of distilled water and agitated for 10 - 15 minutes. Formation of foam indicates the presence of saponins.

Sterols/Triterpenoids

Libermann Burchard test: 1 ml of herbal extract was treated with a few drops of acetic anhydride, boiled and cooled at room temperature. Then conc. H_2SO_4 was added along the

walls. Occurrence of brown ring at the junction of two layers and conversion of upper layer into green color indicated the presence of steroids where as deep red color, triterpenoids. Salkowski test: 1 ml of herbal extract was treated with a few drops of conc. H_2SO_4 formation of lower red color indicated the presence of steroids whereas yellow color- triterpenoids.

Phenols

$FeCl_3$ test: 1 ml of herbal extract was treated with a few drops of $FeCl_3$ solution. Formation of blue color indicates the presence of phenols. Ellagic acid test: Few drops of 5% (w/v) glacial acetic acid and 5% (w/v) sodium nitrate solution added to 2 ml of test solution. Phenol is indicated by Niger brown precipitate.

Quantitative analysis of *N. foetida*

The present study was performed to determine the quantitative analysis of phytochemical constituents like alkaloids, phenols, flavonoids, tannins, saponins, and total antioxidants present in the different plant extracts (leaf, stem, bark and root) of *N. foetida*. The total quantification of secondary metabolites was done by using the standard procedures and the obtained line of regression and the regression coefficient estimated from the calibration curve of various standards. The absorbance of the standard and test samples was measured by using a spectrophotometer. The concentration of the test samples was expressed in terms of mg of the standard equivalent per gram of the plant extracts.

Analysis of total amount of Alkaloids

The total alkaloids content of *N. foetida* was estimated usingAjmalicine as standard and the standard curve is obtained Spectrophotometrically at 470 nm. For this, test samples and standard were prepared at a concentration of 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml in respective tubes. To this 5 ml of phosphate buffer (pH was set to 4.7) and 5 ml of BCG (Bromo Cresol Green) was added, mixed vigorously with 5 ml of chloroform and mixed again. Then chloroform fraction was transferred to 10ml measuring flask and the volume was made upto 10 ml by using chloroform. The quantification of plant extracts (leaf, stem, bark and root) was estimated by dissolving 1mg/ml of extract in 2N HCl (pH was adjusted to 4.7 by using 0.1N NaOH). The absorbance of the standard, blank and plant extracts were obtained by using spectrophotometer at 470 nm. Then the results were compared with the standard curve of ajmalicine equivalent per gram of extract (AE/gm of extract) [23].

Analysis of total amount of flavonoids

The estimation of total flavonoid content of *N. foetida* extracts was analyzed by using a spectrophotometer at 510 nm. For the quantification of flavonoids, quercetin is used as standard and it is determined by the aluminum trichloride method. The concentration of the different plant extracts (leaf, stem, bark and root) was 1 mg/ml and the concentration of standard was 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml. For each tube 75 µl of sodium nitrate (5% $NaNO_2$) was added and mixed thoroughly. For which, 150 µl of 10% aluminum chloride was added and stood for 5 minutes. To this reaction mixture 500 µl of NaOH (4%) was added and made the final volume up to 2.5 ml by using distilled water. The absorbance of plant extracts and standard was measured. The total flavonoid content of plant extracts is expressed in terms of quercetin equivalent per gram (QE/gm) [24].

Analysis of total amount of phenol

The estimation of total phenol content of the *N. foetida* plant extracts was determined by FC (Folin Ciocalteu) reagent method by using vanillic acid as standard. The concentration of different plant extracts (leaf, stem, bark and root) was prepared as 1mg/ml and the concentrations of standard were 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml. To which, 5 ml of FC reagent (1:10 diluted with distilled water) and 4ml of sodium carbonate (7.5 %) was added and incubated at 20 °C for 30minutes. The absorbance was measured by using spectrophotometer at 765 nm. The total phenol content of the *N. foetida* is expressed in terms of VAE/gm (vanillic acid equivalent per gram of plant extracts) [25].

Analysis of total amount of tannins

The estimation of the total tannin content in plant extracts of *N. foetida* was determined by using tannic acid as standard. For this, different concentrations of standard (50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml) and different plant extracts (1 mg/ml) were prepared accordingly. All the samples were diluted by addition of 7.5 ml of distilled water and followed by 0.5 ml of folin phenol reagent and 1ml of sodium carbonate (35%). The final volume of the reaction mixture was made to 10 ml by adding distilled water. All the samples are mixed vigorously and incubated at room temperature for 30 minutes. The absorbances of the samples are measured by using spectrophotometer at 725 nm. The total tannin content of the plant extracts was expressed in terms of TAE (tannic acid equivalent)/ gram of extract (as mg TAE/g of plant extracts) [26].

Analysis of total amount of saponins

The estimation of total saponins content of *N. foetida* plant extracts was determined with Oleanolic acid as standard. The concentration of standard is prepared as 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml accordingly. To this standard and test samples, 250 µl of vanillin reagent (8%), 2.5 ml of H_2SO_4 (72%) was added and mixed vigorously. The reaction mixture containing test tubes were incubated for 10 minutes in a water bath at a temperature of 60 °C, afterwards placed in ice cold water for 3-4 minutes. The absorbance of the samples was measured by using spectrophotometer at 544 nm. The total saponin content of the plant extracts is expressed in terms of OE (Oleanolic equivalent)/ gram of extract (as mg OE/g of extract [27].

Analysis of total amount of antioxidants

The estimation of total amount of antioxidants in different extracts of *N. foetida* was determined by ascorbic acid is used as standard. The concentration of the standard was prepared at 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml concentrations and the different plant extracts (leaf, stem, bark and root) at 1 mg/ml concentration. 300 µl of different concentrations of the plant extracts and standard were taken. To each test tube 3ml of reagent solution was added and incubated in a water bath at a temperature of 95 °C for one and half hour. The absorbance of the samples was measured by spectrophotometer at 695 nm [28].

3. RESULT AND DISCUSSION

Preliminary phytochemical screening was carried out by standard procedures [29-30] to screen and characterize the phytoconstituents available in *N. foetida* plant like alkaloids, flavonoids, glycosides, tannins, phenols and triterpenoids by cold maceration technique with acetonitrile, chloroform, butanol, hexane and methanol, in different plant parts like leaf, stem, bark and root.

3. 1. Qualitative analysis

Leaf extracts

Phytochemical analysis of leaf extracts of *N. foetida* were performed using various solvents like butanol, chloroform, acetonitrile, hexane and methanol. Analysis revealed, the presence of alkaloids, flavonoids, glycosides, tannins, phenols and triterpenoids in varying amounts in various solvents (Table 1). Similarresults of presence of tannins, flavonoids, lipids, glycosides, proteins, carbohydrates and phenolics were reported by Dixit et al. [31] in leaf extracts of *N. foetida*.

According to our analysis quinones, fat and oils were absent in leaf extract. The alkaloids more present in methanol, butanol, and chloroform solvent leaf extract followed by hexane and acetonitrile (Table 1). Very fewer positive results appeared in acetonitrile extract and our results are in accordance with the work was reported by Uma et al. [32] in leaf extract of *N. foetida*. Methanol extracts of leaves showed the presence of glycosides more effectively, followed by chloroform and butanol solvent extracts. Flavonoids were effectively observed in leaf methanolic extract which resembles the study carried out using methanol leaf extracts of *N. foetida* [15]. All the solvents showed the presence of tannins. Methanolextracts of leaves showed the presence of phenols more effectively, followed by other solvents. Only chloroform extracts showed the presence of triterpenoids. Chloroform and methanolic extracts showed the presence of saponins.

Stem extracts

Different stem solvent extracts of *N. foetida* showed presence of alkaloids, flavonoids, glycosides, tannins, phenols and steroids. The results showed that methanol, butanol, chloroform and hexane are more efficient solvent for alkaloids (Table 1). Our results coincided with results reported by Sharma et al. [33] in stem extracts of *N. foetida*, which showed the presence of alkaloids, carbohydrates, steroids, terpenoids and phenolics [47, 48].

The results revealed the presence of more amount glycosides in the butanol and methanol extract, followed by other solvent extracts. The same kind of results were reported by Sharma et al. [33] in the same plant. The flavonoid compounds were found excessive in all solvents except chloroform extract and followed by acetonitrile. Chloroform stem extract showed the presence of tannins more efficiently, followed by other solvents (Table 1) and similar results were reported by Uma et al. [32] in the stem extracts of the *Nothapodytes nimmoniana*.

The stem extracts also revealed to contain triterpenoids in less amounts in all the solvents, whereas saponins were identified only in methanolic extract. Butanol and hexane extracts showed the presence of phenols, similar results were reported by Das et al. [34] in stem extracts of *N. foetida.*

Table 1. Phytochemical constituents of *Nothapodytes foetida*.

Plant Parts	Component	Methanolic extract	Butanolic extract	Chloroform extract	Acetonitrile extract	Hexane extract
Leaf	Alkaloids	+++	+++	+++	+ +	++
	Glycosides	++	++	+	+	+
	Flavonoids	++	++	++	+	+
	Phenols	+++	++	+	+	+
	Tannins	+	+	+	+	+
	Triterpenoids	-	-	++	-	-
	Saponins	++	-	++	-	-
Stem	Alkaloids	+++	+++	+++	+	+++
	Glycosides	++	+++	+	-	+
	Flavonoids	++	++	+	++	++
	Phenols	-	++	+	-	++
	Tannins	+	+	+++	-	+
	Triterpenoids	+	+	+	+	+
	Saponins	++	-	-	-	-
Bark	Alkaloids	+++	+++	+++	+	+
	Flavonoids	++	++	+	+	-
	Glycosides	++	++	++	-	-
	Phenols	+	+	++	-	+
	Tannins	+	+	+++	-	-
	Triterpenoids	+	+	+	+	+
	Saponins	++	-	+	-	-
Root	Alkaloids	+++	++	+++	+	++
	Flavonoids	+	+	+	+	+
	Glycosides	++	+	-	-	-
	Phenols	++	++	++	-	++
	Tannins	++	+	+++	-	+
	Triterpenoids	++	++	++	-	-
	Saponins	+++	-	+	-	-

Bark extracts

Different bark extracts of *N. foetida* had shown the presence of alkaloids, flavonoids, glycosides, tannins, phenols and triterpenoids. The results of the extraction showed that methanol, butanol and chloroform are more efficient solvents for alkaloids extraction (Table 1) and similar results of occurrence of alkaloids, carbohydrates, phenols, tannins, glycosides, flavonoids, steroids, and triterpenoids were reported earlier in bark extracts of *N. foetida* [2].

According to our analysis quinones, fat and oils were absent in bark extracts of *N. foetida.* Similarly, the absence of fats and oils were reported in bark extracts of *N. foetida* [2]. Methanol, butanol and chloroform extracts of bark showed the presence of glycosides more efficiently and were not detected in hexane and acetonitrile fractions. The flavonoid compounds were found in methanol, butanol and chloroform bark extracts. Chloroform bark extract showed the presence

of tannins more effectively, followed by moderate presence in methanol and butanol. Phenols were shown to be in moderate amounts in chloroform bark extract, fewer amounts in hexane, butanolic and methanol bark extracts. The bark extracts also revealed to contain triterpenoids, which was more excessive in all the solvents.

Root extracts

The qualitative analysis of root extracts revealed the presence of different phytochemicals that considered as active medicinal chemical constituents. Essential medicinal phytochemicals such as alkaloids, flavonoids, glycosides, tannins, phenols and triterpenoids were detected in different solvent systems named butanol, chloroform, acetonitrile, hexane and methanol (Table 1). In *N. foetida* root extracts exhibited the presence of alkaloids, glycosides, flavonoids, tannins, phenols and triterpenoids, similar kind of work was done by Govindachari and Viswanathan[13]and reported that Camptothecin content was higher in the roots, followed by stem and leaves. Our current experiment results revealed that alkaloids are present in the root extract of *N. foetida*, in solvent systems like methanol and chloroform, followed by butanol, hexane and acetonitrile (Table 1). Glycosides were moderately present in all the solvents in the same plant, similar results were reported in root extracts of *N. foetida* [11]. All the solvents showed the moderate amounts of flavonoids. Chloroform showed the presence of copious amount of tannins followed by other solvents. Except for acetonitrile remaining, all the solvents showed the presence of phenols. Methanol, butanol and chloroform root extracts showed the presence of triterpenoids.

3. 2. Quantitative analysis

Table 2. Total quantity of phytochemical compounds in methanolic leaf, stem, bark and root extracts of *N. foetida*.

S.NO	Name of phytochemicals	Leaf	Stem	Bark	Root
1	Alkaloids	66.11±0.47	56.27±0.38	59.02±0.17	62.34±0.27
2	Flavonoids	48.04±0.58	35.13±0.24	38.63±0.29	43.21±0.19
3	Phenols	24.18±0.31	16.36±0.22	20.83±0.39	22.46±0.36
4	Tannins	32.13±0.44	18.13±0.36	26.27±0.12	28.44±0.54
5	Saponins	18.24±0.38	14.02±0.43	15.43±0.32	17.28±0.18
6	Total Antioxidants	40.21±0.23	33.08±0.18	36.24±0.16	38.09±0.26

Quantification of total alkaloid content

The total alkaloid content in methanolic extracts of *N. foetida* plant extracts was determinedspectrophotometrically by using calibration curve of Ajmalicine as standard. The

total alkaloid content is expressed as Ajmalicine equivalent (AE/gm). The regression line obtained was y = 0.0018x + 0.016 with a regression coefficient value of R^2 = 0.9925 (Figure 2). The quantitative analysis of total alkaloid content of leaf was found to be 66.11± 0.47, whereas stem possess 56.27±0.38, bark 59.02±0.17 and root 62.34±0.27 mg AE/gm of extracts (Table 2) [35]. Similar results ofquantification using ajmalicine was reported in *Rauvolfia serpentina* and *Rauvolfia tetraphylla* [36].

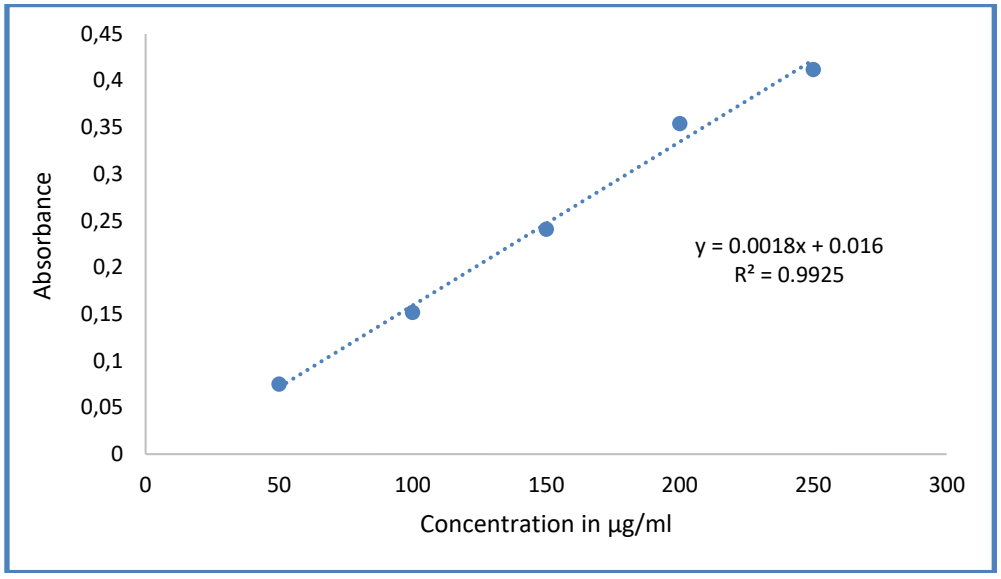

Figure 2. Calibration line for standard Ajmalicine.

Quantification of total flavonoid content

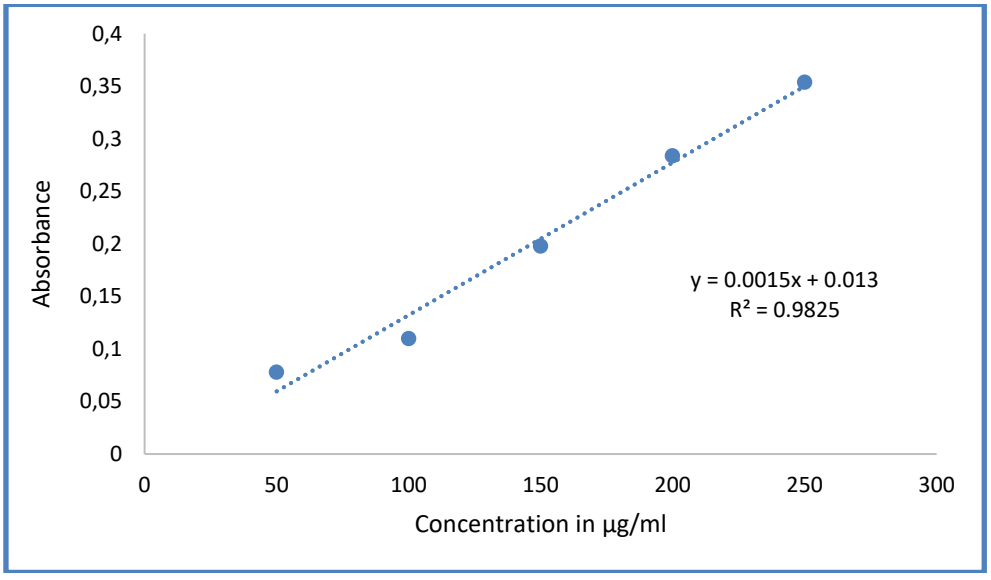

Figure 3. Calibration line for standard Quercetin.

The total flavonoid content of *N. foetida* plant extracts were determined spectrophotometrically by the calibration curve of Quercetin as standard and expressed as Quercetin equivalent per gram (QE/gm). The obtained regression line was y = 0.0015x + 0.013 and a regression coefficient value of R^2 = 0.9825 (Figure 3). The quantitative analysis of total flavonoid content of leaf, stem, bark and root was found to be 48.04 ±0.58, 35.13 ± 0.24, 38.63 ±0.29 and 43.21 ±0.19 mg QE/gm of extracts (Table 2) [37]. Comparative results were reported in quantitative analysis of quercetin indifferent parts of *Capparis spinosa* by HPLC [38].

Quantification of total phenol content

The total phenol content of *N. foetida* methanolic extracts was determined by spectrophotometer with the calibration curve of vanillic acid as standard. The total phenol content of the samples was expressed as VAE/gm. The obtained line of regression for total phenolic content was y = 0.0013x + 0.0298, whereas the regression coefficient is R^2 = 0.9971 (Figure 4). The obtained total phenol content of methanolic extracts of leaf, stem, bark and root was 24.18 ± 0.31, 16.36 ± 0.22, 20.83 ±0.39 and 22.46 ±0.36 mg VAE/gm of extracts (Table 2) [39]. Similar results were reported as determination of phenolic compounds in extracts of Amazonian medicinal plants [40].

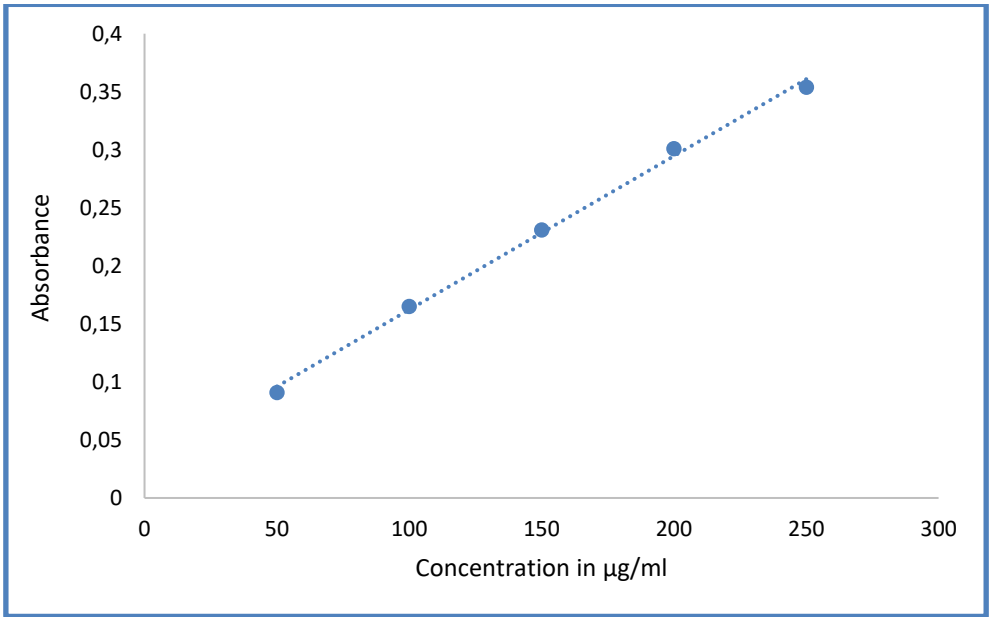

Figure 4. Calibration line for standard Vanillic acid.

Quantification of total tannins content

The total tannins content of *N. foetida* plant extracts was determined by spectrophotometer with the calibration line of tannic acid as reference standard. The total tannin content was expressed in terms of TAE/gm. The obtained line of regression for total tannins was y = 0.0016x + 0.0243 and the correlation coefficient value of R^2 = 0.9871 (Figure 5). The total tannin content of methanolic extracts of leaf, stem, bark and root was found to be 32.13

±0.44, 18.13 ±0.36, 26.27 ±0.12 and 28.44 ±0.54 mg TAE/gm (Table 2) [41]. Similar results of quantitative methods for the estimation of tannins were reported in plant tissues [42].

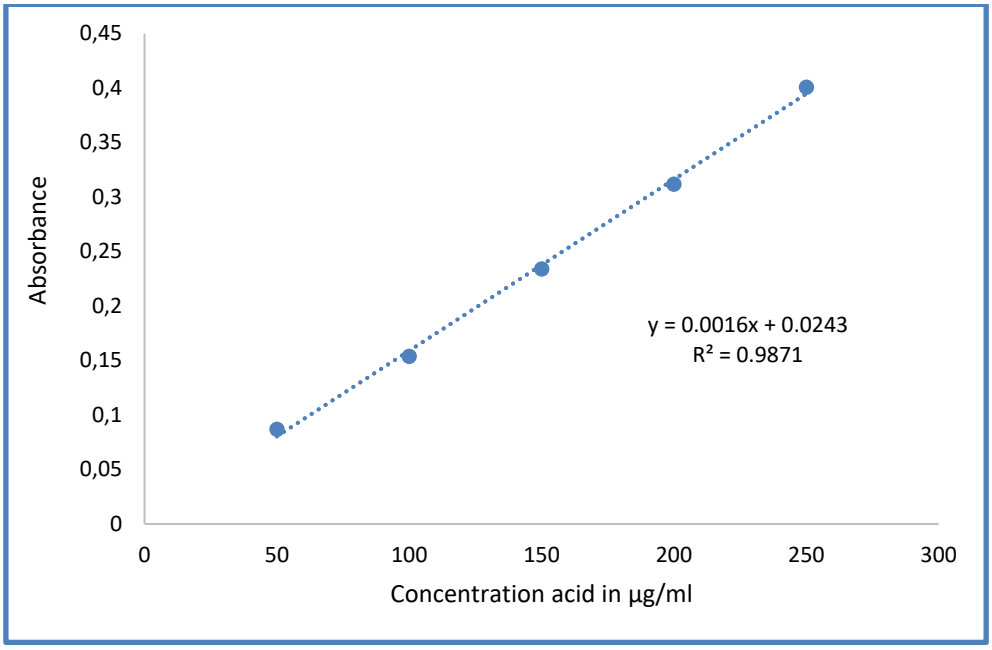

Figure 5. Calibration line for standard Tannic acid.

Quantification of total saponins content

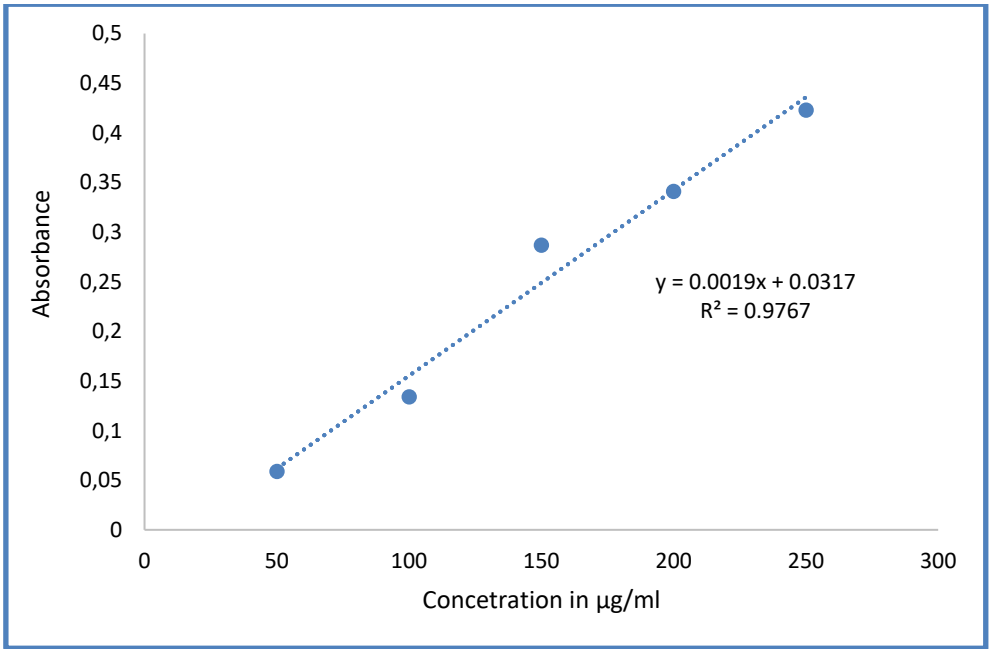

Figure 6. Calibration line for standard Oleanolic acid.

The total saponins content of methanolic extracts of *N. foetida* was determined by spectrophotometer using Oleanolic acid as reference standard. The total saponins content of samples was expressed as OEA/gm. The obtained regression line value was y = 0.0019x + 0.0317 and the correlation coefficient value was R^2 = 0.9767 (Figure 6). The total saponins contents of leaf, stem, bark and root was found to be 18.24 ± 0.38, 14.02 ± 0.43, 15.43 ± 0.32 and 17.28 ± 0.18 mg OAE/gm (Table 2) [43]. Comparative results were reportedby quantification of Oleanolic acid in the flower of *Gentiana olivier* Griseb by HPLC [44].

Quantification of total antioxidant activity

The total antioxidant activity of *N. foetida* plant extracts was determined by spectrophotometer by using ascorbic acid as standard. The antioxidant content was expressed as AAE. The obtained regression line value was y = 0.0021 + 0.0412 and the correlation coefficient value of R^2 = 0.9807 (Figure 7). The total antioxidant capacity of leaf, stem, bark and root was 40.21 ±0.23, 33.08 ±0.18, 36.24 ±0.16 and 38.09 ±0.26 mg AAE/gm (Table 2) [45]. Similar results of quantitative analysis of ascorbic acid in foods was assessed by HPLC [46].

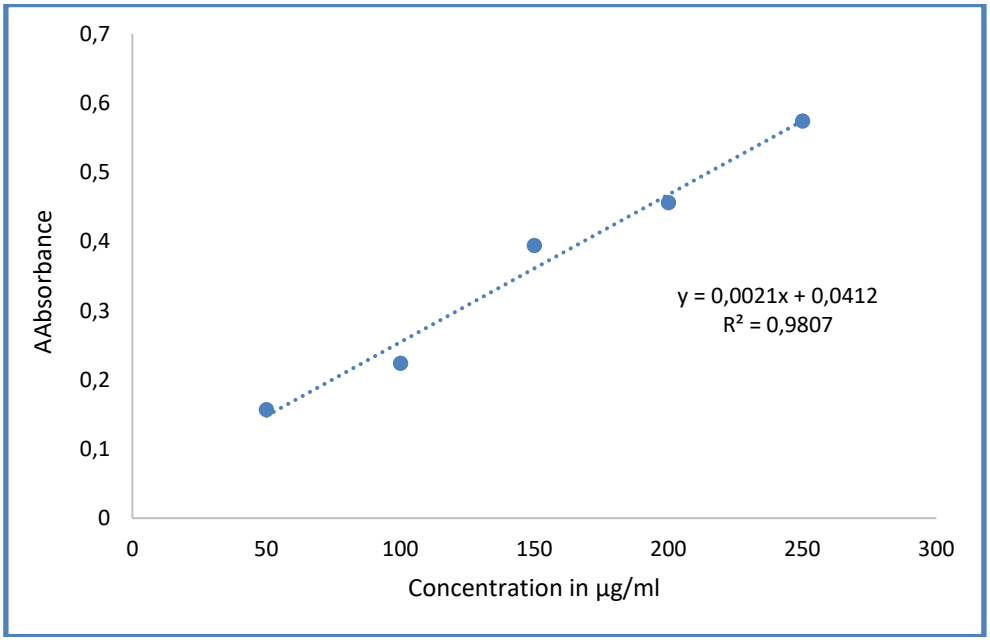

Figure 7. Calibration line for standard Ascorbic acid.

4. CONCLUSIONS

In the present study, preliminary phytochemical screening from different parts of *N. foetida* i.e., leaf, stem, bark and root extracts powder were conducted in different solvents systems like acetonitrile, butanol, chloroform, hexane and methanol. The phytochemical study of *N. foetida* has revealed valuable information about the chemical compounds present in various parts of the plant. The solvent system plays a key role in extracting bioactive compounds from different parts of the plant. Among all the solvents, methanolic and

chloroform extracts had shown best results. The qualitative analysis of leaf, stem, bark and root extracts of *N. foetida* showed the occurrence of alkaloids, glycosides, flavonoids, tannins, saponins, triterpenoids and phenols. Whereas the quantitative analysis of leaf, stem, bark and root showed highest concentration of alkaloids. Therefore, *N. foetida* has been used as an important medicinal plant due to the presence of these phytochemicals, and can be applied in medical fields for the designing of potential anti-cancer and anti-HIV new drugs.

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