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Phytochemical Screening and Metabolic Profiling of *Nardostachys jatamansi* and *Adhatoda vasica*

Rasha Irshad[1*], Rayees. A. Padder[1], Sayeed Ahmed[2], Mohammad Husain[1]

Department of Biotechnology[1], Molecular Virology Lab, Jamia Millia Islamia, New Delhi, India
Department of Pharmacognosy & Phytochemistry[2], Jamia Hamdard, New Delhi, India
*Corresponding Author

Abstract: **The traditional and alternative systems of medicine depend on large number of medicinal plants, which possess therapeutic properties. The medicinal plants, *Nardostachys jatamansi* and *Adhatoda vasica* are two such pharmacologically important plants widely grown in India, Tropical and Sub- Tropical countries. In view of its medicinal values, this study aimed to analyze the phytoconstituents of methanolic extracts of these plants through phytochemical screening and metabolic fingerprinting which was carried out by HPTLC and GC-MS analysis. Total phenol and flavonoid content was evaluated followed by measurement of antioxidant ability by radical scavenging assessment using 1,1,Diphenyl-2-picrylhydrazyl (DPPH) assay. The preliminary data showed the presence of significant amount of flavonoid, phenols and radical scavenging capability comparable to standard antioxidant Ascorbic Acid. The presence of diverse nature of secondary metabolites in methanolic extracts of these plants was confirmed by HPTLC analysis, whereas the GC-MS analysis revealed the presence of at least 23 and 24 metabolites in *N. jatamansi* and *A. vasica* respectively. These metabolites consisted of fatty acids, sesquiterpenes, alkane hydrocarbons and esters known to have potent pharmacological effects. Therefore, the present study demonstrates the pharmacological importance of these plants that can be exploited in conventional therapeutic use.**

***Keywords: Nardostachys jatamansi, Adhatoda vasica*, Phytochemical Analysis, HPTLC, GC-MS.**

I. INTRODUCTION

The *Nardostachys jatamansi* and *Adhatoda vasica* are two prominent medicinal plants with great usage in traditional and complimentary therapeutic regimens such as Unani, Ayurveda, Siddha and Homeopathy. The perennial herb, *Nardostachys jatamansi*, commonly known as Spikenard belongs to the Valerianaceae family and is widely present in Asian countries like India, Korea, and China[1]. In India, it is found in regions of Himalayas at an altitude of approximately 5000m and grows up to 1m in height. Accumulating data have validated that this herbal plant contains an exorbitant amount of phenolic content, lignans, sterols, alkaloids and flavonoids which accounts for its antioxidant, anti-spasmodic, anti-convulsive, anti-inflammatory, anti-Parkinson's, anti-depressant, anti- diabetic and antifungal properties. It has also shown protective effects against atopic dermatitis-like skin lesions and auto-immune diseases[2-5]. Similarly, *Adhatoda vasica* (vernacular name: Ardusi, Vasaka) is a therapeutically significant plant of the Acanthaceae family as it is reported to have numerous medicinal properties against diseases like asthma, tuberculosis, common flu, pulmonary discomfort, cardiac issues, dyspepsia, malaria, inflammation, piles, leporasy, jaundice and hypertension[6-9]. It is an evergreen, perennial shrub with a distinct smell widely distributed in Asia majorly in India, Pakistan and Afghanistan. It can also be found in regions of China, Taiwan, Hong Kong, Germany and Sweden[10]. It grows to a height of 1.5 – 2.5 m with leathery leaves present on ascending branches. Its leaves are mainly exploited for healing properties but other parts like stem, fruits and seeds are also being utilized for the same purpose[11]. The leaves of *A. vasica* have been described to possess quinazoline alkaloids such as vasicine and vasicinone, along with several steroids, sterols, essential oils, fatty acids and other glycosidic compounds[6].

The use of plants for medicinal purposes has existed since antiquity and still continues to be an exemplary source of drug development. Besides, the upsurge of drug resistance towards abundant chemically synthesized drugs and their potential side-effects attracts the attention of scientific community for utilization of medicinal plants for pharmacological intentions. The presence of various bioactive secondary metabolites is associated with the therapeutic potential of these plants[12]. The quality, variety and distribution of phytoconstituents like flavonoids, isoflavonoids, tannins, lignans, stilbenes, terpenes, phenols and alkaloids depends on factors like season, location and part of the plant[13]. Hence, identification and determination of constituents present in medicinal plants in different conditions will disclose the nature of bioactive compounds responsible for the healing nature of a particular plant. In this regard, this study aims to pin-point the major phytoconstituents present in *N. jatamansi* and *A. vasica* varieties found in Northern region of India. Also, metabolic profiling by HP-TLC and GC-MS analysis was done to expand and develop standard fingerprint of methanolic extracts of these two important medicinal plants.

II. MATERIAL AND METHODS

Collection and Identification of Plant Materials

The plants were collected from herbal drug market located in Old Delhi, India. The sample specimens of plants were deposited in National Institute of Science Communication And Information Resources (NISCAIR) under voucher herbarium specimen no. *Ref. NISCAR/RHMD/ Consult/- 2010-11/ 1671/ 269* for identification and authentication which was done by Dr. B. Singh, Raw Material Herbarium and Museum of NISCAIR.

Preparation of extract

Arial plant materials were accurately dried at 50°C in a hot air oven, powdered and passed through sieve no. 60 to acquire a uniform powder. Powdered plants were extracted in Soxhlet apparatus by methanol as an extraction solvent for 72 hours at 70-80°C. Further, the liquid extract was filtered through Whatman filter paper twice; concentrated in rotavapour; freeze-dried and stored at -80°C till further use. Later, respective stock solutions of each lyophilized powder were made in DMSO, the un-dissolved matter was removed by mild centrifugation at 2000 rpm/ 5min, followed by filtering the stock using 0.45 µm syringe filter.

Phytochemical Screening of the Plant Extract

1. Total phenolic content determination

Total phenol content was determined by Folin-Ciocalteu method with modifications. Briefly, 0.5 ml of each extract and phenolic standard was mixed with 5 ml FCR (diluted 1:10 v/v) and 4 ml of 1M aqueous Na_2CO_3. Solutions were incubated at 50 °C in a water bath for 15 minutes and total phenol content was determined spectrophotometrically at 765nm, by UV-Vis spectrophotometer (Shimadzu 1601). A calibration curve was constructed using Gallic Acid solutions as standard and total phenolic content of the extract was expressed in terms of mg of Gallic Acid equivalents per gram dry weight of extract.

2. Total Flavonoid determination

The Flavonoid compounds were determined by aluminum chloride calorimetric process. Briefly, 0.5 ml of extracts and flavonoid standard was added to 1.5 ml methanol, 0.1 ml each of 10% aluminum chloride and 1M potassium acetate and made up by 2.8 ml of double distilled water. The entire mixture was incubated at RT for 30 minutes and absorbance read at 415 nm, using a UV- Vis spectrophotometer (Shimadzu 1601). A calibration curve was plotted with quercetin solutions as standard and total flavonoid of the extract was expressed in terms of mg of quercetin equivalents per gram dry weight of extract.

3. Antioxidant activity determination

The antioxidant ability of the two plant extracts were evaluated by the (1,1-diphenyl-2-picryl-hydrazil DPPH) radical scavenging assay corresponding to Ismail *et al*[14]. The absorbance readings were measured at 515 nm and Ascorbic acid, a potential antioxidant was used as positive control. Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\% \text{ free radical scvanging activity} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Then, the percent inhibitions against concentrations were plotted and IC50 was calculated.

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Metabolite Profiling of Plant Extracts:

1. HPTLC fingerprinting

HPTLC studies were carried out following the method already described, with some modifications. After trying a number of TLC in different solvent systems by hit and trial method, the presence of spot/s was confirmed by TLC in a specific solvent system. Briefly, 10 mg of each extract was dissolved in 1 ml methanol and filtered via 0.45 µm membrane filter and then respective samples (8µl each) were spotted on pre-activated and a 0.2 mm thick pre-coated silica gel 60 F_{254} TLC plates (E. Merck, Germany). The Camag Linomat-V automated TLC applicator was used to apply the sample solution to 6 mm wide band having a delivery speed 150 nL/s from the syringe. A mobile phase of toluene: ethyl acetate: formic acid (9:4:1, v/v/v) was employed in a CAMAG glass twin trough chamber (20 × 10 cm), which was pre-saturated with the solvent before 15 minutes. The plates were acquired in Camag horizontal developing chamber (10 × 10 cm) at the room temperature. Plates were scanned at different wavelengths such as 250nm, 366nm. The plates were derivatized by 5% anisaldehyde sulphuric acid in methanol and scanned at 540 nm using a Camag TLC scanner III and the Wincats1.2.3 software.

2. Gas Chromatography and Mass Spectrometry (GC-MS) Analysis

The metabolomic fingerprinting of methanolic extracts were carried out to find out nature and content of polar metabolite/s to be used for the activity. The polar metabolomic fingerprinting of *Nardostachys jatamansi and Adhatoda vasica* was carried out as described. GC-MS was carried out using Gas Chromatograph system, equipped with dual split/ splitless inlet along with a recommended Mass spectrometer detector (Agilent technologies, 6890). A 30-m SPB-50 column having thickness of 0.25-mm film (Superlco, Bellfonte, CA) was utilized for performing GC. The ion source was maintained at 200 °C and the injection temperature was kept at 230 °C having the interface of 250°C. The temperature program given below was operated for the analysis: isothermal heating at 70°C for 5 minutes, then a 5°C/min oven temperature ramp to 310°C, finally heating at 310°C for 1 minute. This system was equilibrated for temperature at 70°C for 6 min before injecting the second sample. The mass spectra were then recorded at 2 scan/ sec with 50 to 600 m/z scanning range. Identification of individual components was achieved using the Wiley and National Institute of Standards and Technology (NIST) Library.

Statistical Analysis

All experiments were performed in triplicates at least three times independently and are presented as mean ± SD.

III. RESULTS AND DISCUSSION

Total Phenol and Flavonoid Content

Total Phenolic and flavonoid contents were measured for the estimation of total phyto constituents. The total phenol content was measured as gallic acid equivalent (GAE) and *N. jatamansi* showed slightly more phenol content than that of *A. vasica* whereas the flavonoid contents in *N. jatamansi* were significantly higher than *A. vasica* as shown in **Table 1**.

TABLE 1: THE TOTAL PHENOLIC AND FLAVONOID CONTENT OF METHANOLIC EXTRACTS OF *N. JATAMANSI AND A. VASICA*

Medicinal plants	Total Phenolic content (mg/g of GAE)	Total Flavonoids(mg/g of QE)
Nardostachys jatamansi	95.21±3.43	50.92±6.3
Adhatoda vasica	90.89±7.89	25.84±7

Phenol and flavonoids are vital phytochemicals that are present in fruits and vegetable and act as potent antioxidants possessing multiple positive effects on the biological machineries.

Evaluation of Antioxidant Activity

The antioxidant activities of methanolic fractions of both the plants were evaluated by the DPPH radical scavenging assay. The DPPH assay monitors the ability of extracts to scavenge or reduce DPPH by donating hydrogen ions to free radicals, thus reducing the sample absorbance. The concentration required to reduce 50% of DPPH known as IC_{50} value is presented in **Table 2**. The anti-oxidative ability of methanolic extract of *N. jatamansi* is significantly higher than *A. vasica*

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which might be correlated with greater phenolic and flavonoid contents in *N. jatamansi*. The effect of different concentration of the extracts on the percentage inhibition of DPPH in comparison to ascorbic acid is represented in the **Fig. 1**. The data shows a concentration dependent increase in the radical scavenging activity of both the methanolic extracts and the standard as well. Razack *et al.* have also reported IC_{50} values of ethanol and hexane extracts of *N. jatamansi* which is significantly higher than methanolic extract which we have found and it suggests that methanolic fraction of *N. jatamansi* is a more potent anti-oxidant than ethanol and hexane extracts[1]. Similarly, the methanolic extract of A. vasica displays considerable radical scavenging activity at concentrations above 25 µg/ml which was reported in other studies using different solvent system as well[15]. The presence of these phytochemicals was found to be positively concurrent with the antioxidative abilities of these medicinal plants showing anti- microbial, anti- inflammatory and cytoprotective properties which are reported earlier[16]. Indeed, *N. jatamansi* have been described to enhance memory, reduce stress and regulate hyperglycemia which can be associated with reduction of ROS[17-19]. In the same way, *A. vasica* has shown anti- diabetic potential by inhibiting α-glucosidase enzyme along with other pharmacological activites[20].

TABLE 2: *IN VITRO* ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *N. JATAMANSI* AND *A. VASICA IN TERMS OF RADICAL SCAVENGING EFFECT OF* DPPH FREE RADICAL ACTIVITY IN COMPARISON TO ASCORBIC ACID.

Medicinal plants	IC_{50} (µg/mL)	R^2
Ascorbic Acid	19.15	0.998
Nardostachys jatamansi	72.87	0.9621
Adhatoda vasica	270	0.9921

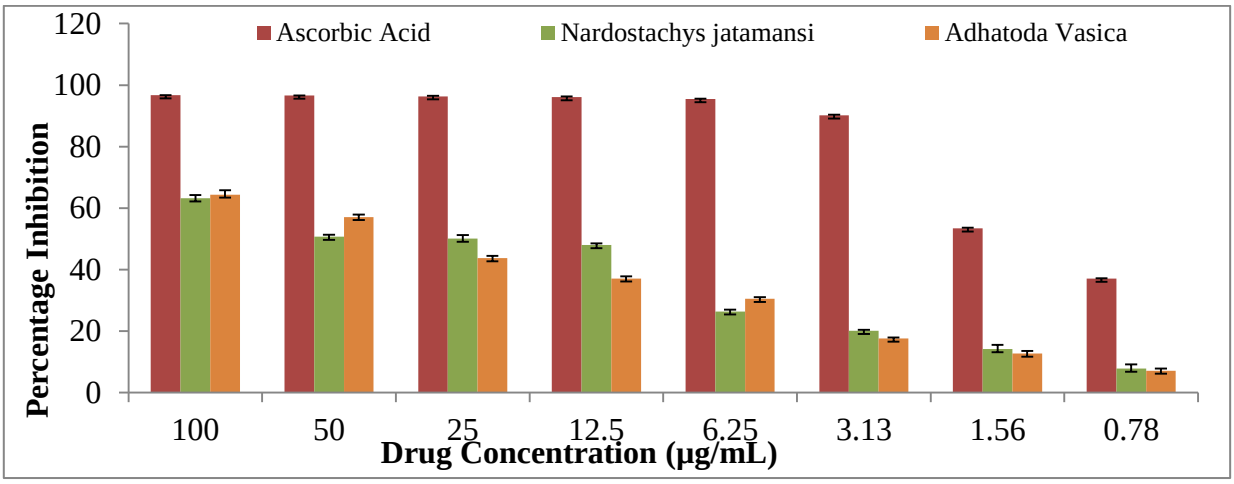

FIG. 1: DPPH FREE RADICAL INHIBITORY EFFECT OF *N. JATAMANSI* AND *A. VASICA* IN COMPARISON TO ASCORBIC ACID.

Metabolic Profiling of Methanolic Extracts of N. jatamansi and A. vasica

HPTLC Analysis

The HPTLC fingerprinting of methanolic extracts of *N. jatamansi and A. vasica* were optimized by utilizing diverse solvent systems and finally toluene: ethyl acetate: formic acid (9:4:1, v/v/v) was selected as the mobile phase due to proper resolution of peaks (**Figs. 2-3** and **Tables 3-4**). The prominent blue zones in each chromatogram were taken under UV C/A and visible light after derivatization with anisaldehyde sulfuric acid. The HPTLC chromatogram of *N. jatamansi* showed 11, 09, 10 numbers of metabolites at 250,366,540 nm respectively, out of which a total 3 metabolites were common (**Fig. 2** & **Table 3**) which showed strong peaks at R_f values of 0.01, 0.09, 0.39. This result is suggestive of the diversity of chemical compounds present in the methanolic fraction of this plant.

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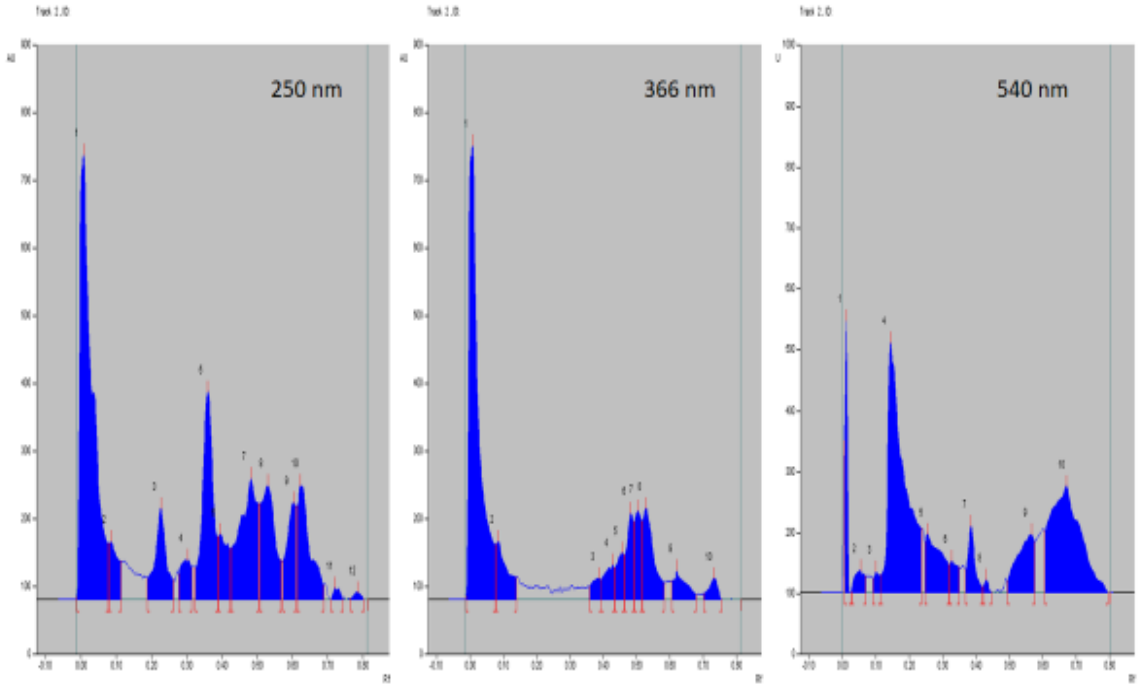

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FIG. 2: HPTLC CHROMATOGRAM OF *N. JATAMANSI* AS OBSERVED AT 250; 366 and 540 nm WAVELENGHTS, SHOWING 11, 09,10 METABOLITES OUT OF WHICH 3 METABOLITES WITH Rf VALUES OF 0.01, 0.09, 0.39 WERE COMMON.

TABLE 3: PEAK TABLE FROM CHROMATOGRAM OF *N. JATAMANSI ALONG* WITH AREA AND Rf OF THE CORRESPONDING PEAKS OBTAINED.

S.N0.	Rf	Area		
		250nm	366nm	540nm
1	0.01	21337.3	18312.4	2896
2	0.05			983.3
3	0.09	2036.9	2725	523.5
4	0.14			18457.9
5	0.24	3933.8		4199.7
6	0.30	1678.5		
7	0.33			1109.4
8	0.36	8540.9		
9	0.39	2479.5	797.2	2126.2
10	0.43			254.4
11	0.46		1385.7	
12	0.48	8064.2	2438.1	
13	0.50		2467.1	
14	0.53	6120.3	3959.3	
15	0.57			4457.6
16	0.61	3420.8	1419.4	
17	0.67			14414.5
18	0.72	288.7	780.8	
19	0.78	199.2		

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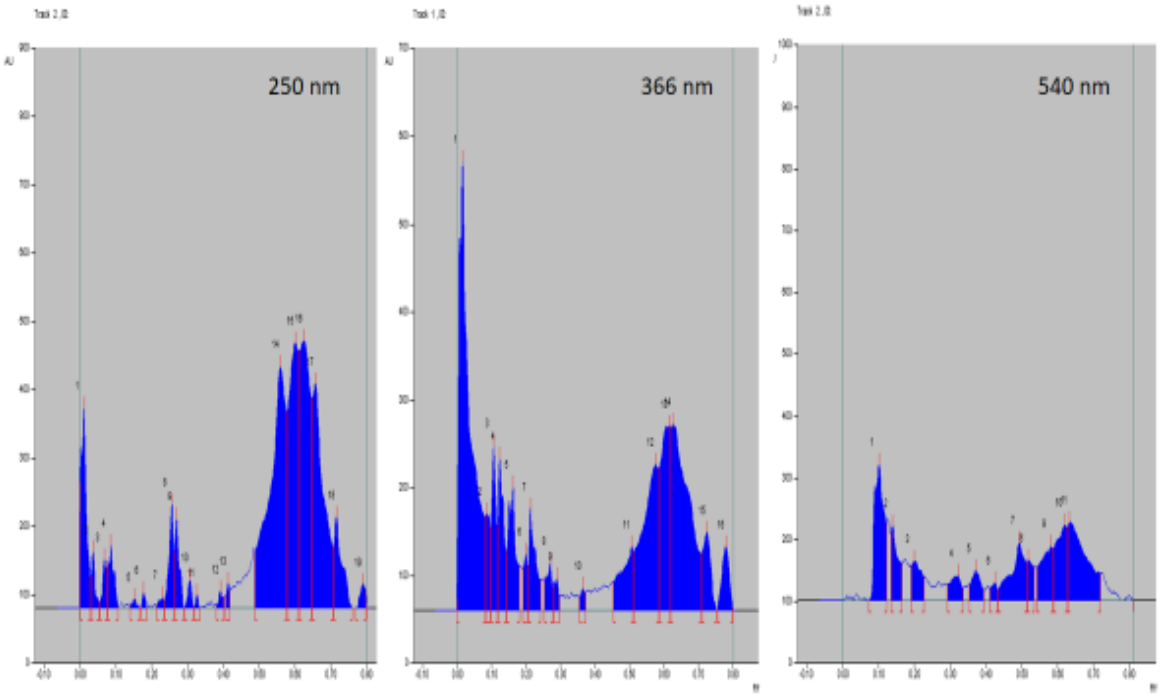

Fig 3: HPTLC CHROMATOGRAM OF A. VASICA AS OBSERVED AT 250; 366 and 540 nm SHOWING 18,17,11 METABOLITES OUT OF WHICH 4 METABOLITES WITH Rf VALUES OF 0.09, 0.37, 0.61 AND 0.63 WERE COMMON.

The HPTLC analysis of methanolic fraction of *A. vasica* revealed presence of compounds with varied nature having different R_f values when resolved using mobile phase of toluene: ethyl acetate: formic acid (9:4:1, v/v/v). The chromatogram showed 4 strong peaks having R_f values of 0.09, 0.37, 0.61 and 0.63 at different wavelengths along with other smaller peaks (**Fig. 3** & **Table 4**).

The HPTLC method for analysis utilized in this study is a quick and straightforward technique for qualitative evaluation of medicinal plants composition. The methanolic extracts of both the plants produced peaks having retention factors from 0.01 to 0.79 at an absorbance of 250, 366 and 540 nm. This confirms the presence of a variety of secondary metabolites in these plants which exhibits a range of potent biological activities.

TABLE 4: PEAK TABLE FROM CHROMATGRAM OF *A. VASICA* ALONG WITH AREA AND Rf OF THE CORRESPONDING PEAKS OBTAINED.

S.N0.	Rf	Area		
		250nm	366nm	540nm
1	0.02	3993.7	15151.8	
2	0.04	736.8		
3	0.07	736.7		
4	0.09	1250	1168.7	5375.6
5	0.11		2244.6	
6	0.12		2448.6	
7	0.14			2069.5
8	0.16	148.8	2689.9	
9	0.18	169.4		
10	0.20		712.8	1601.1
11	0.21		1916.1	
12	0.23		183.4	

13	0.26	1742.6		
14	0.27	1382.9	794.2	
15	0.29		452.9	
16	0.31	394.3		1147.9
17	0.33	120.8		
18	0.37	247.7	328.2	1151.5
19	0.41	283.6		
20	0.43			438.9
21	0.49			3298
22	0.51		2314	1012.6
23	0.56	14586.7		
24	0.58		6837.9	2661.9
25	0.61	9541.8	5025.4	3369.3
26	0.63	10465.2	10110.2	5920.9
27	0.66	9286.2		
28	0.72	2639.1	1858.1	
29	0.79	453.7	1418.7	

GC-MS Metabolomics

The GC-MS profiling was done for assessing the nature of polar compounds in methanolic extracts of *N. jatamansi* and *A. vasica* which aided in identification and characterization of an array of phytochemicals that contributes to the diverse medicinal properties of such plants. The total ion chromatogram (TIC) of the methanolic extract of *N. jatamansi* and *A. vasica* presented in **Fig. 4-5** repectively. The retention time, area and NIST library ID of the recognized compounds are listed in **Table 5-6**. The methanolic extract of *N. jatamansi* resulted in more than 20 different compounds in which the prominent ones were D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime;3,6,9-Trioxa-2,10-disilaundecane,2,2,10,10-tetramethyl;Benzo[c]thiophene-1-[(carbonyl)morpholide]; 3-(2-Thienyl)pyridine; Xylonic acid **(Fig. 4 & Table 5).** The compound, Benzo[b]thiophene-1 [(carbonyl) morpholide] was the most abundant and is a benzo[b]thiophene derivative and is known to have anti-microbial and anti-inflammatory properties as reported by Isloor *et a.l*[21]. Another compound, Hanegokedial present in *N. jatamansi* belongs to sesquiterpene group and is potent anti-oxidants with wide a range of pharmacological activities as reported in other studies[22]. The methanolic fraction of *N. jatamansi* displays a range of fatty acid compounds such as Octanoic acid, n-Pentadecanoic acid, n-Hexadecanoic acid, and Octadecanoic acid. Fatty acids are known to exhibit anti- inflammatory potential[23, 24].

TABLE 5: TOTAL POLAR METABOLITES IDENTIFIED FROM METHANOLIC EXTRACT OF *N. JATAMANSI* DETECTED BY GC-MS AFTER DERIVATISATION OF THE METHANOLIC EXTRACT

S.N0.	RT	Area %	Library ID
1	4.241	0.26	1-hydroxy-6-methyl-4-[(trimethylsilyl)methyl]-7-oxo-2,3-dioxabicyclo[4.3.0]n
2	4.416	1.6	d-Xylose, 2,3,4,5-tetrakis-O-(trimethylsilyl)-, o-methyloxime
3	4.537	1.1	Hanegokedial
4	4.972	0.75	2,3-Butanediol 2TMS PK A
5	5.075	2.45	2,3-Butanediol 2TMS PK A
6	5.486	0.26	Octanoic acid, trimethylsilyl ester
7	5.854	0.88	Hanegokedial
8	6.011	0.59	n-Pentadecanoic acid, trimethylsilyl ester
9	6.301	12.65	D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime
10	6.543	1.86	3,6,9-Trioxa-2,10-disilaundecane,2,2,10,10-tetramethyl
11	6.766	32.97	Benzo[c]thiophene-1-[(carbonyl)morpholide]
12	6.893	13.5	3-(2-Thienyl)pyridine
13	7.25	1.12	n-Hexadecanoic acid

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14	7.352	6.93	Xylonic acid
15	8.277	0.28	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-
16	8.96	9.2	Inositol,O,O,O,O,O,O-TMS
17	9.364	0.66	Octadecanoic acid
18	9.515	0.37	1-(1-Methyl-2,2-D2-2-Trimethylsilyloxyethyl)-4-(2-Methyl-2-Trimethylsilyloxy
19	10.174	0.38	Octadecanoic Acid
20	10.742	0.45	.beta.-Eudesmol, trimethylsilyl ether
21	16.107	1.46	Spiro[Cyclopropane-1,8'(1h')[3a.6]
22	19.128	0.45	Hexaethyldisloxane
23	20.01	1.52	Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene
24	22.276	8.31	Hexaethyldisloxane

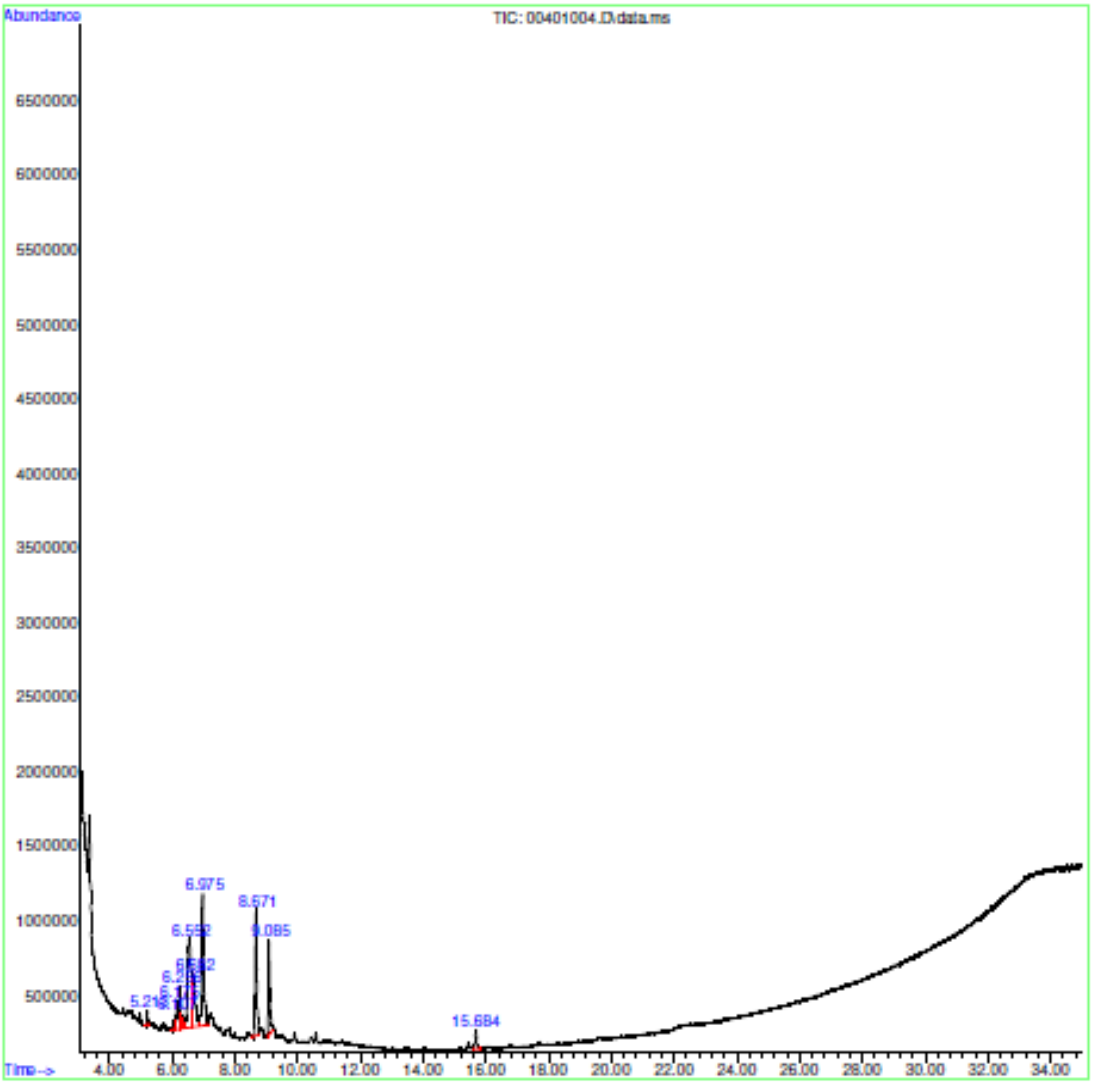

FIG 4: THE TOTAL ION CHROMATOGRAM (TIC) OBTAINED FROM THE METHANOLIC EXTRACT OF *N. JATAMANSI*

Similarly, The methanolic extract of *A. vasica* displays 29 peaks, out of which 3,7-Dioxa-2,8-disilanonan-5-one, 2,2,8,8-tetramethyl;Methyl Tetra-O-TMS-.Alpha.-D-Galacto-furanocide are the most prominent ones **(Fig. 5 & Table 6).** The Ionosolic compounds present in methanolic extract of *A. vasica* is known to possess anti- viral properties. Sadaka *et al* showed that the thiosemicarbazone metabolite is is linked with potent antimicrobial activities and this compound is present in significantly higher amount in the GC- MS chromatogram of *A. vasica*[25].

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TABLE 6: TOTAL POLAR METABOLITES IDENTIFIED FROM METHANOLIC EXTRACT OF A. VASICA DETECTED BY GC-MS AFTER DERIVITIZATION.

SN	RT	Area %	Library ID
1	5.757	0.54	Xylonic acid, 2,3,4-tris-O-(trimethylsilyl)-, .delta.-lactone, D-
2	5.842	1.77	1,2-O-Isopropylidene-3,5,6-tri-O-trimethylsilyl-D-glucofuranose
3	5.939	1.41	Sorbopyranose, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-, L Sorbose 5TMS
4	6.017	1.29	Sorbose 5TMS
5	6.162	2.37	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)
6	6.289	20.36	3,7-Dioxa-2,8-disilanonan-5-one, 2,2,8,8-tetramethyl
7	6.537	1.52	3-dibromomethylene-2,3-dihydrothiophen 1,1-dioxide
8	6.621	1.5	Farnesyl bromide
9	6.694	1.31	1-Ethyl-4-phosphorinanone thiosemicarbazone
10	6.748	15.63	1,2-O-Isopropylidene-3,5,6-tri-O-trimethylsilyl-D-glucofuranose
11	6.887	5.08	1,2-O-Isopropylidene-3,5,6-tri-O-trimethylsilyl-D-glucofuranose
12	7.099	1.71	D-Xylopyranose, 1,2,3,4-tetrakis-O-(trimethylsilyl)
13	7.244	0.77	Hexadecanoic acid
14	7.328	1.88	Ethylmalonate Mono Et Ester 1TMS
15	7.492	7.6	Isosteviol
16	7.969	1.03	Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, muco-
17	8.948	14.3	Inositol,O,O,O,O,O,O-TMS
18	17.745	4.1	3-(10-Methyl-10H-phenothizine)methylene propane-di-nitrile
19	17.95	0.87	Silane, (1,2,4,5-cyclohexanetetrayltetraoxy)tetrakis[trimethyl
20	19.183	0.97	d-(-)-Fructose, pentakis(trimethylsilyl) ether
21	19.878	12.37	Methyl Tetra-O-TMS-.Alpha.-D-Galactofuranocide
22	20.844	0.62	1-Ethyl-4-phosphorinanone thiosemicarbazone
23	22.228	0.99	Di-triethylsilyl ether

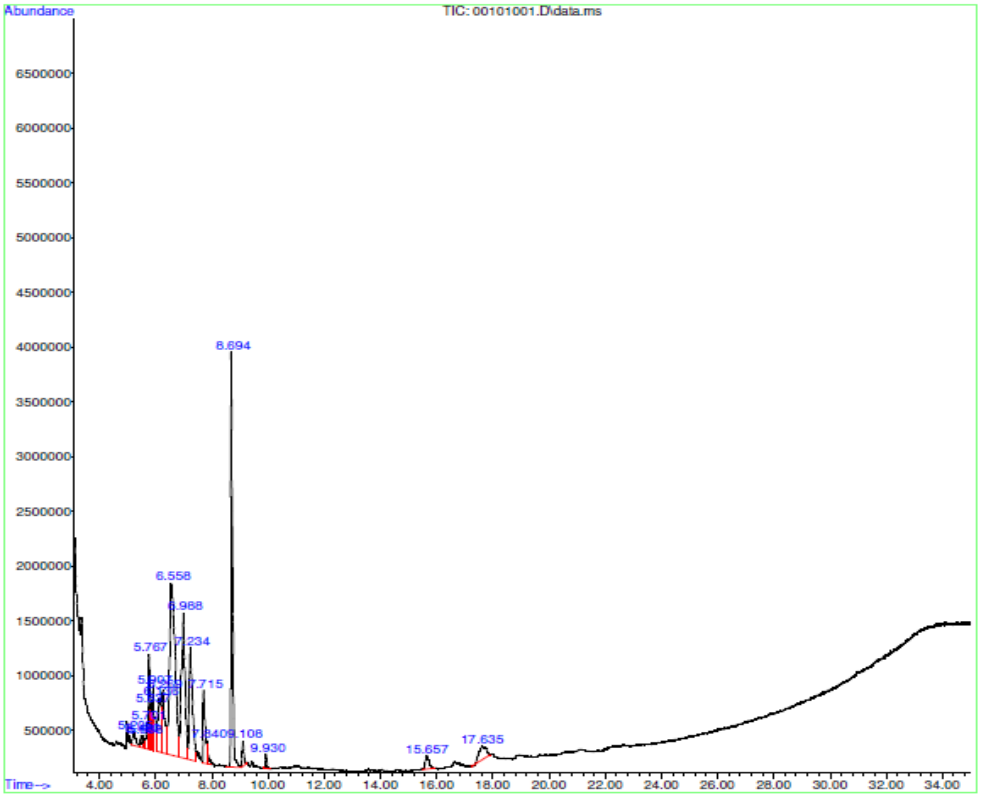

FIG. 5: THE TOTAL ION CHROMATOGRAM (TIC) OBTAINED FROM THE METHANOLIC EXTRACT OF A. VASICA.

In *A. vasica*, a compound known as Isosteviol also shows its presence which is a diterpenoid derivative having anti-inflammatory, antibacterial, and anti-hyperglycemic activities[26, 27]. Further, it also has fatty acids like Hexadecanoic acid which has multiple biological activities like cardioprotective and anti-inflammatory potential, in addition to its utilization in pharmaceutical and food industries[23, 24]. Therefore, it can be stated that the presence of this variety of bioactive compounds might be associated for the medicinal potential of *N. jatamansi* and *A. vasica*. Compounds derived from medicinal plants function as "biological response modifiers" and exhibits preventive activities against various ailments.

IV. CONCLUSION

Numerous medicinal plants are being used to develop novel drug candidates due to their cost- effectiveness, fewer side-effects and lesser toxicities. Hence, in this study, a comprehensive and in-depth investigation was carried out to identify and characterize the bioactive metabolites responsible for remedial properties of the two important plants from Northern India; *Nardostachys jatamansi* and *Adhatoda vasica*. On the basis of our study, it can be concluded that methanolic extracts of these two plants are rich in phenol and flavonoid contents, which furnishes higher free radical scavenging capabilities to these plants as well. The HPTLC and GC-MS analysis also confirmed the occurrence of bioactive compounds in appreciable amounts with large carbohydrates, sesquiterpenes and fatty acids being the most common and readily available. These findings substantially elucidate the therapeutic nature of these medicinal plants and open avenues for their utilization in the development of newer synthetic drugs and lead compounds.

V. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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