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Amrit Research Journal December 2023, Vol. 4, Issue 1, 30-39
ISSN: 2738-9553
RESEARCH ARTICLE

Evaluation of Chemical Constituents and Antioxidant Activity of Extracts of *Nardostachys jatamansi* (D. Don) DC.

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(Received: May 17, 2023, Received in revised form: November 3, 2023, Accepted: December 2, 2023, Available Online)

DOI: https://doi.org/10.3126/arj.v4i1.61185

Highlights:

- Roots of *Nardostachys jatamansi* were extracted in hexane, chloroform, ethyl acetate, and methanol
- GC-MS analysis of hexane, chloroform, and ethyl acetate extract confirmed the presence of 13, 10, and 6 different compounds, respectively
- IC_{50} values of chloroform and ethyl acetate extract were found as 824.13 µg/mL and 919.11 µg/mL, respectively on the DPPH scavenging assay
- TPC_s of chloroform extract (49.53 mg GAE/g), and ethyl acetate extract (44.41 mg GAE/g)
- TFC_s of chloroform extract (18.42 mg QE/g), and ethyl acetate extract (10.57 mg QE/g)

Abstract

Nardostachys jatamansi is an endangered medicinal plant of the Caprifoliaceae family. The main aim of this study was to evaluate the metabolites and their biological activities. The shade dried, powdered plant materials were subjected to successive ultrasonic extraction with solvents hexane, chloroform, ethyl acetate, and methanol in the increasing order of polarity. Flavonoids, terpenoids, saponins, phenolic volatile oils, and alkaloids were discovered in the phytochemical screening. Gas chromatography-mass spectrometry analysis identified 13, 10, and 6 chemical compounds in hexane, chloroform, and ethyl acetate extracts, respectively. Cyclic saturated hydrocarbons, organic acids, alcohol, and ester were the main constituents of extracts verified by GC-MS. Chloroform extracts had a higher phenolic content (49.53 mg GAE/g) than ethyl acetate extracts (44.41 mg GAE/g). Chloroform extracts had a higher total flavonoid concentration (18.42 mg QE/g) than ethyl acetate extracts (10.57 mg QE/g). The half-maximum inhibitory concentration (IC_{50}) of the chloroform and ethyl acetate extracts revealed moderate results to antioxidant activity.

Keywords: *Nardostachys jatamansi, phytochemical, GC-MS, TPC, TFC, antioxidant*

Introduction

Nepal is well-known for high-value Non-Wood Forest Products (NWFPs). Nepal is regarded as a land of immense diversity in terms of flora and wildlife due to its unique geographical location. Despite covering less than 0.1 % of the earth's land area,

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Nepal has a disproportionately large number of species. There are 26 species of gymnosperms, and 6,973 species of angiosperms [1]. Primary metabolites are necessary for plant growth and development, whereas secondary metabolites are essential for plant development [2,3]. They can function as protective substances against herbivores and pathogens, act as pigments in flowers to allure pollinators, or operate as hormones or signaling molecules [4] a critically endangered medicinal plant of alpine Himalayas. Illumina GAIIx sequencing of plants collected during end of vegetative growth (August). Besides their biological roles within plants, natural products have exerted a significant influence on human culture and have been employed throughout human history as flavor enhancers, colorants, and medicinal substances [5] providing protection against attack by herbivores and microbes and serving as attractants for pollinators and seed-dispersing agents. They may also contribute to competition and invasiveness by suppressing the growth of neighbouring plant species known as allelopathy.

Nardostachys jatamansi (D. Don) DC. *(N. jatamansi)* is a perennial rhizomatous plant in the Caprifoliaceae family. It is a hairy, herbaceous, endangered, and most primitive species within the family. It grows from 2300 m to 6000 m above sea level on steep, damp, rocky, undisturbed grassland slopes in Nepal, India, and China [6,7]. The roots of *N. jatamansi* are thick, short, and dark grey rhizomes topped with brown tufted fibrous remnants of the petioles of the radical leaves. The rhizomes has very important role in Ayurveda. It is effective as an antiepileptic, laxative, tonic, stimulant, and antispasmodic [8].

The plant consists of chemical compounds such as alkaloids, flavonoids, amino acids, protein, vitamin C, resins, and others. α-patchoulenese, angelicin, β-eudesemol, β-patchoulenese, β-sitosterol, calarenol, elemol, jatamansin, jatamansinol, jatamansone, and n-hexacosanyl isolverate are the chemicals found in *N. jatamansi* [9,10]. It has been used in medicine and perfumery for centuries [11].

Pharmacologically, *N. jatamansi* has demonstrated antihyperlipidemic, cardiotonic, antioxidant, hepatoprotective, and hair growth activities [2,12,13]. The plant has been reported to possess anti-anxiety, cardioprotective, anti-convulsant, antiparkinson, anti-depressant, and antimicrobial activities [14,15]. The aim of this research is to recognize the phytochemical and preliminary biological activities of the ultrasonic assisted extracts of *N. jatamansi* collected from Nepal.

Materials and Methods

Material collection

About 3 kg of fresh rhizome of the plant was collected in Rukum (East) district at altitudes ranging from 2300 to 6000 meters to represent the whole range of species for study. The Department of Botany, Amrit Campus, Lainchour, Kathmandu, identified the plant.

Extraction

The shade dried powdered plant materials were subjected extraction through ultrasonicator at room temperature for 1 hour with 1500 ml of hexane. The dissolved extracts were concentrated over the Rota Evaporator. For future usage, the concentrated extract was dried using a water bath, weighed, and stored in an airtight vial tube. Similarly, the filter residue was sonicated again with hexane. A similar technique was repeated three times to obtain the crude extract. Following the same process, the marc remained after each extraction was further subjected to successive extraction with 1500 mL each of chloroform, ethyl acetate, and methanol respectively, for three times in increasing order of the polarity of the solvents.

Phytochemical Screening

The phytochemical analysis of different extracts performed was based on standard protocol [16]. It helps to identify the bioactive compounds present in plants.

Gas Chromatography-Mass Spectrometry Method (GC-MS)

The following settings were utilized for GC-MS analysis on a Perkin Elmer GC Clarus 500 system, which includes an AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer apparatus: Column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1 Mdf, 100% Dimethylpolysiloxane), operated in electron impact mode at 70 eV; Helium gas (99.99%) was used as carrier gas at a steady flow of 1 mL/min and an injection volume of 0.5 mL (split ratio of 10:1). The injector temperature was 250°C, and the ion source temperature was 280°C. The oven temperature was programmed to rise from 110°C (isothermal

for 2 minutes) to 200 °C, then 5 °C/min to 280 °C, and finally 9 min isothermal at 280 °C. The mass spectra were collected at 70 eV with a 0.5 second scan interval with fragments ranging from 40 to 450 D. The total running time of the GC is 36 minutes. The relative percentage quantity of each component was calculated by comparing the average peak area of each component to the total areas. TurboMass Version 5.2.0 was used to handle mass spectra and chromatograms [17].

Antioxidant Activity

The investigation of antioxidant property was conducted using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging technique [18]. A stock solution of 1000 µg/mL concentration was prepared for chloroform and ethyl acetate extracts. By serially diluting this stock solution, a range of extract solutions ranging from 20 to 100 ppm were prepared. A 1000 µg/mL (ppm) ascorbic acid solution was made using ethanol. Through dilution, a series of ascorbic acid solutions of concentrations from 20 ppm to 100 ppm were also prepared. For each concentration of ascorbic acid solution, 2 mL was combined with 2 mL of the 0.2 mM DPPH solution in triplicate, and these mixtures were left for 30 minutes in the dark place. Subsequently, a spectrophotometer at 517 nm was used to measure the absorbance, with methanol and DPPH acting as the reference blank. The process was repeated for ascorbic acid. The curve of % scavenging vs concentration was created for both ascorbic acid and sample solution and half maximum inhibitory concentration (IC_{50}) values were computed using the equation in Excel.

The formula used to calculate the % DPPH scavenging is given below.

$$\text{DPPH scavenging (\%)} = \frac{A_o - A_S}{A_o} \times 100\ \%$$

Here,

A_0 = Absorbance of the control (DPPH solution + methanol)

A_S = Absorbance of the sample

Total Phenolic Content (TPC)

The TPC quantification of the extract was performed through the Folin-Ciocalteu colorimetric method, which relies on an oxidation-reduction reaction [19]. 1000 µg/mL stock solution of gallic acid and various concentrations of gallic acid such as concentrations ranging from 100 to 20 µg/mL were prepared through successive dilution of the stock solution. Each concentration's gallic acid solution (1 mL) was put into test tubes, 5 mL of 10% Folin-Ciocalteu reagent (FCR) and 4 mL of 7% sodium carbonate solution (Na_2CO_3) was added to these test tubes to make a total volume of 10 mL. The blue-colored liquid thus formed was vigorously shaken before being incubated in a 40°C oven for 30 minutes in a water bath. Finally, the solution's absorbance was measured at 760 nm. All the experiments were carried out in triplicate. A stock solution of 10,000 µg/mL of the extract was prepared. The triplicate concentrations of the 1000 µg/mL extract were made using serial dilution, and their absorbance values were measured using the same procedure employed for gallic acid. Gallic acid was used as a standard.

The TPC was calculated using the equation, $C = \frac{c \times V}{m}$

Here,

C = Total content of the phenolic compounds (mg/g) in gallic acid equivalent

c = Gallic acid concentration from the calibration curve (mg/mL)

V = Volume of extract (mL)

m = Dry weight of the plant extract (mg)

Total Flavonoid Content (TFC)

The TFC of the extracts were estimated by the aluminium chloride ($AlCl_3$) colorimetric assay method [19]. Quercetin stock solution with a concentration of 1000 µg/ml in methanol and various concentrations of quercetin were generated, including 100 to 20 µg/mL. A 1 mL aliquot of quercetin from each concentration was prepared and added to a 20 ml test tube containing 4 mL of distilled water. A test tube was filled with 0.3 mL of 5% $NaNO_2$, and following a 5 minute interval, an additional 0.3 mL of 10% $AlCl_3$ was introduced into the mixture. After 6 minutes, 1 M NaOH of volume 2 mL was added. To reach a total volume of 10 mL, distilled water of 2.4 mL was added. The pink-colored solution formed was then promptly subjected to absorbance measurement at 510 nm using a spectrophotometer. This measurement was taken in comparison to a blank solution that contained

all the reagents except for quercetin. Quercetin was used as a standard. The extract stock solution (10,000 µg/mL) was made, and using serial dilution, triplicates of extract concentrations of 1000 µg/mL were generated, and their absorbance values were evaluated as the technique used for quercetin.

The equation used to calculate the TFC is, $C = \frac{c \times V}{m}$

Here,

C = TFC (in mg/g) in Quercetin Equivalent (QE)

c = Concentration of quercetin established from calibration curve in mg/mL

V = Volume of the extract (mL)

m = Weight of the extract (mg)

Result and Discussion

Phytochemical screening

The presence of phytochemicals was established by the appearance of particular colours in the phytochemical screening [15]. The table below illustrates the phytochemicals present in the different extracts.

Table 1. Phytochemical analysis of extracts of *N. jatamansi*

S.N.	Class of phytochemicals	Hexane extract	Chloroform extract	Ethylacetate extract	Methanol extracts
1	Volatile oil	+	+	+	+
2	Alkaloids	+	+	+	+
3	Phenolic compounds	-	+	+	+
4	Flavonoids	-	+	+	+
5	Terpenoids	+	+	-	+
6	Saponin	-	+	-	-

Where (+) means presence (-) means an absence

GC-MS analysis

The spectrum of the compounds is shown in Figure 1, Figure 2, and Figure 3 of hexane, chloroform, and ethyl acetate extract, respectively. Structures of the bioactive compounds in *N. jatamansi* extract have been shown below.

GC-MS Analysis of Hexane Extract

The GC-MS chromatogram of hexane extract is given below

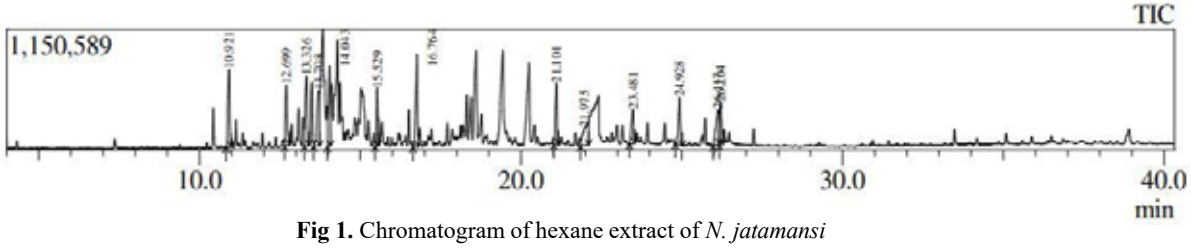
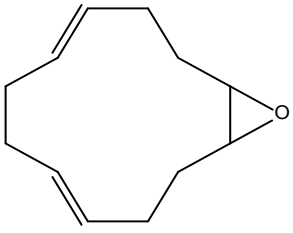


Fig 1. Chromatogram of hexane extract of *N. jatamansi*

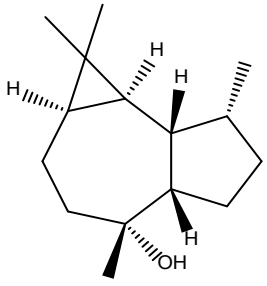
Table 2. The chemical compounds in hexane extract

S.N.	Chemical compounds	Molecular Formula	Area %	Mol. Wt. (g/mol)	Retention Time
1	1,2-Epoxy-5,9-cyclododecadiene	$C_{12}H_{18}O$	9.51	178	10.921
2	1H-Cyclopropa [a] naphthalene	$C_{15}H_{24}$	7.59	204	12.699
3	Spatulenol	$C_{15}H_{24}$	9.24	204	13.326
4	Naphthalene,1,2,3,4-tetrahydro-1,6-dimethyl-4- (1-methyl ethyl)	$C_{15}H_{22}$	9.32	202	13.708
5	1,5,9-Trimethyl-1,5,9-cyclododecatriene	$C_{15}H_{24}$	9.35	204	14.043
6	Bicyclo [2.2.1] heptane	$C_{15}H_{24}$	5.93	204	15.529
7	Ledol	$C_{15}H_{26}O$	11.09	222	16.764
8	Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7- (1-methylethenyl) pentanoic acid	$C_{15}H_{24}$	6.55	204	21.101
9	Oleyl Alcohol	$C_{18}H_{36}O$	7.25	268	21.975
10	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	5.04	256	23.481
11	Strophanthidin	$C_{23}H_{32}O_6$	5.92	404	24.928
12	Decahydronaphthalene	$C_{10}H_{18}$	7.57	138	26.117
13	9-Octadecenoic acid	$C_{18}H_{34}O_2$	5.63	282	26.204

Altogether thirteen compounds were found in the hexane extract of *N. jatamansi* rhizome. Unsaturated hydrocarbon, organic acids, aromatic compounds, alcohol, and ester were obtained. Among them, Ledol (11.09%) and 1,2-Epoxy-5,9-cyclododecadiene (9.51%) were the major compounds.



1,2-Epoxy-5,9-cyclododecadiene



Ledol

GC-MS Analysis of Chloroform Extract

10 major compounds in chloroform extract of *N. jatamansi* were analysed using GC-MS chromatogram from their different retention times.

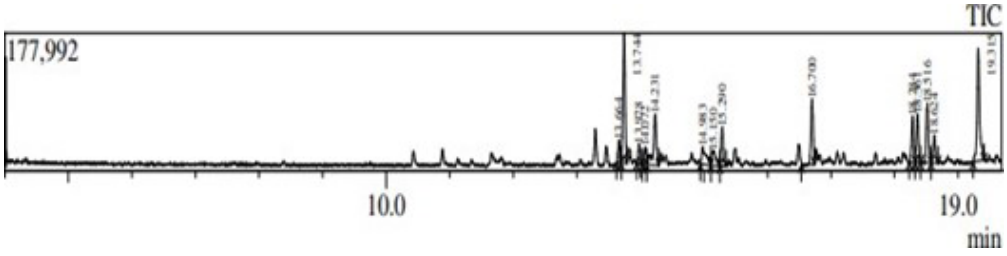
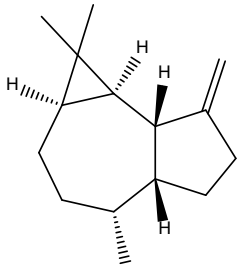


Fig 2. Chromatogram of Chloroform extract of *N.jatamansi*

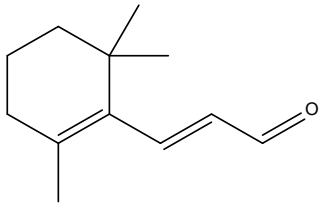
Table 3. The chemical compounds found in the chloroform extract with their retention time

S.N.	Chemical compounds	Molecular Formula	Area %	Mol. Wt. (g/mol)	Retention Time
1	Heptanal, 2-(phenyl methylene)	$C_{14}H_{18}O$	2.61	202	13.664
2	β-Gurjunene	$C_{15}H_{24}$	16.55	204	13.744
3	Methandrostenolone	$C_{20}H_{28}O_2$	2.36	300	13.978
4	Benzene,1-(1-methyl-2-propenyl)-4-(2-methylpropyl)	$C_{14}H_{20}$	2.36	188	14.072
5	Spathulenol	$C_{15}H_{24}$	10.01	204	14.231
6	Cyclopentene, 1-(3-methylbutyl)	$C_{10}H_{18}$	3.84	138	14.983
7	Phenol, 2,4-bis(1,1-dimethyl ethyl)	$C_{14}H_{22}O$	5.22	206	15.150
8	1H-Cycloprop[e]azulene	$C_{15}H_{24}$	9.16	204	15.290
9	Patchouli alcohol	$C_{15}H_{26}O$	5.97	222	16.700
10	2-Propenal,3-(2,6,6-trimethyl-1-cyclohexen-1-yl)	$C_{12}H_{18}O$	4.05	178	10

A total of ten significant chemicals were identified from their fragmentation pattern. 2-Propenal,3-(2,6,6-trimethyl-1-cyclohexen-1-yl) (18.14%) and β-Gurjunene (16.55%) were the main compounds found in chloroform extract.



ß-Gurjunene



2-Propenal,3-(2,6,6-trimethyl-1-cyclohexen-1-yl)

GC-MS Analysis of Ethyl Acetate Extracts

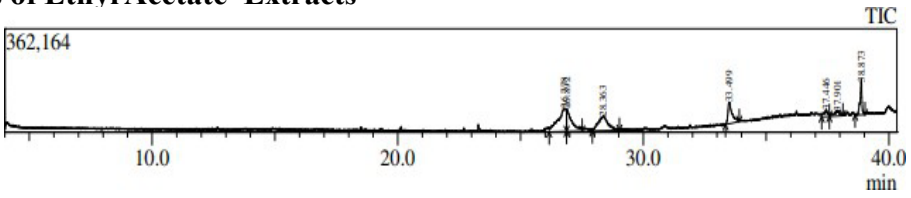


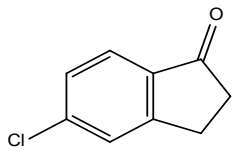
Fig 3. Chromatogram of EtOAc Extracts of *N. jatamansi*

The GC-MS chromatogram of ethyl acetate is given below:

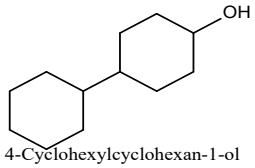
Table 4. The chemical compounds found in the ethyl acetate extract of *N. jatamansi*

S.N.	Chemical compounds	Molecular formula	Area%	Molecular weight	Retention Time
1	5-Chloro-1-indanone	C_9H_7ClO	17.11	166	26.778
2	Nonenylic acid	$C_9H_{16}O_2$	28.79	156	26.892
3	[1,1'-Bicyclohexyl]-4-ol	$C_{12}H_{22}O$	21.88	182	28.363
4	Cholesterol chloroformate	$C_{12}H_{45}ClO_2$	3.6	448	33.499
5	Stigmasta-5,22-dien-3-ol, acetate	$C_{31}H_{50}O_2$	4.76	545	37.446
6	β.-Sitosterol acetate	$C_{31}H_{52}O_2$	10.06	456	37.901

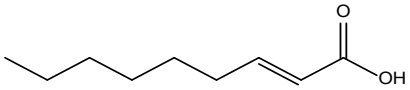
Table 4 illustrates the compounds present in ethyl acetate extract characterized by GC-MS analysis. A total of six compounds were found in ethyl acetate extract. The main compounds found in ethyl acetate extract were 2-Nonenoic acid (28.79%), [1,1'-Bicyclohexyl]-4-ol (21.88%), and 5-Chloro-1-indanone (17.11%) at different retention time.



5-Chloro-1-indanone



4-Cyclohexylcyclohexan-1-ol



2-Nonenoic acid

Quantification of Total Phenolic and Flavonoid Contents

The TPC of extracts was evaluated using the Folin Ciocalteu Calorimetric (FCR) method. The calibration was done using gallic acid as the standard. Among the extracts, TPC values were found comparable. The chloroform extract showed a TPC of 49.53 mg GAE/g while ethyl acetate displayed a TPC value, 44.41 mg GAE/g.

Using an aluminium chloride colorimetric assay and quercetin as a standard, the TFC of the extract was quantified. The TFC values were also found to be comparable among the extracts. The TFC value for chloroform extract was found 18.42 mg QE/g and 10.57 mg QE/g for ethyl acetate extract. The variation in data might be due to the extraction using the solid-liquid fractionation.

Table 5. The TPC and TFC in different extracts of *N. jatamansi*

Samples	TPC (mg GAE/g)	TFC (mg QE/g)
Chloroform extract	49.53	18.42
Ethyl acetate extract	44.41	10.57

Antibacterial Assay

Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia)* were used to test the antibacterial effects of the extracts. No zone of inhibition was observed in any extracts.

Antioxidant Activity Assay

The antioxidant property of the chloroform and ethyl acetate extract of the plant was tested using the DPPH scavenging method. The potential of the antioxidant extracts was calculated in terms of IC_{50} value. The antioxidant significance of the substance has an inverse relationship with the IC_{50} value, which can be calculated by using the logarithmic curve of % inhibition of DPPH vs concentrations [20]. The absorbance of the testing sample was measured with a spectrophotometer at room temperature at 517 nm. The comparative similarity of the antioxidant capacity was observed between the chloroform and ethyl acetate extracts. Ascorbic acid was taken as positive control ($IC_{50,}$ 22.4 µg/mL). The IC_{50} values for chloroform and ethyl acetate were 824.13 µg/mL and 919.11 µg/mL. Figure 4 and Figure 5 illustrate the DPPH scavenging assay of chloroform and ethyl acetate extract, respectively.

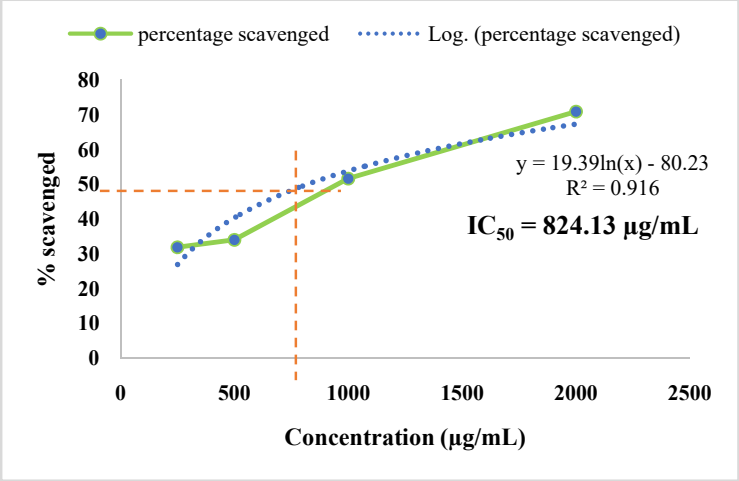


Fig 4. Graphic logarithm curve of the DPPH assay of the chloroform extract

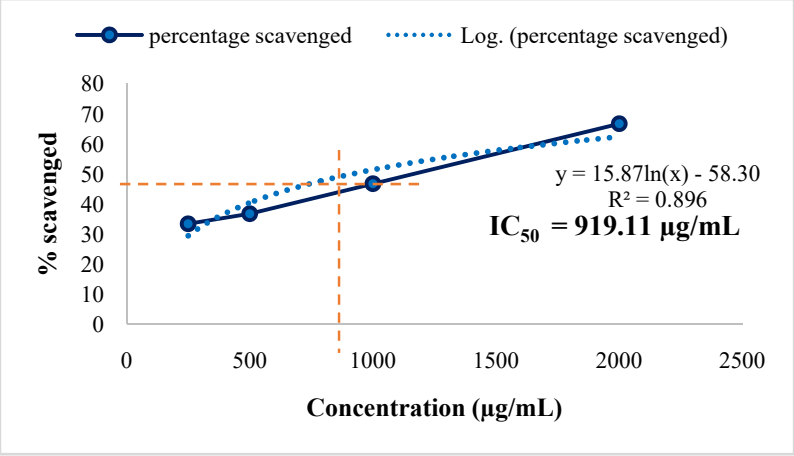


Fig 5. Graphical logarithm plot of the DPPH assay of the ethyl acetate extract

Conclusions

Phytochemical study of *N. jatamansi* rhizome extracts showed the presence of alkaloids, terpenoids, saponins, flavonoids, and volatile oils. The chloroform extract contained high phenolic content, compared to ethyl acetate extract. The total flavonoid content value was comparable in chloroform extract (18.42 mg QE/gm) with ethyl acetate extract. GC-MS analysis showed the presence of 13, 10, and 6 chemical compounds in hexane, chloroform, and ethyl acetate extract of *N. jatamansi* respectively. The determination of DPPH radical scavenging activities and the IC_{50} value of both chloroform and ethyl acetate extracts exhibited moderate result for natural antioxidants. The observed data variation might be due to the extraction using the solid-liquid fractionation method. The research findings further validate the scientific basis for the ethnomedicinal usage of *N. jatamansi*.

Acknowledgments

The authors express gratitude to the Department of Chemistry at Amrit Campus and Kathmandu Valley College for the laboratory resources. They also extend their appreciation for their assistance with GC-MS analysis to to the Department of Food and Technology. For their valuable contributions in conducting biological assays authors are thankful to the Department of Plant Resources and Research Institute of Bioscience and Biotechnology.

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