

## Research Article

Phytochemical and GC-MS Analysis of n-Hexane Extract of *Rauvolfia serpentina* L. Benth. ex Kurz.Anwar Hussain<sup>1,\*</sup>, Pradeep Bahadur Neupane<sup>1</sup>, Ram Narayan Jha<sup>1</sup><sup>1</sup>Department of Chemistry, Tri-Chandra Multiple Campus, Tribhuvan University, Ghantaghar, Kathmandu, Nepal**Abstract**

*Rauvolfia serpentina* which is also known as Indian snakeroot or sarpagandha is a member of the family Apocynaceae was popular from ancient times as an antidote to the stings of insects and bites of poisonous reptiles. Qualitative analysis of root extracts of *R. serpentina* indicates the presence of alkaloids, saponins, tannins, steroids, flavonoids, and phenols. The quantitative determination of phytochemical constituents of *Rauvolfia serpentina* indicates the presence of 2% fat, 12.4% alkaloid and 7.35% saponins in plant sample. n-Hexane extract of root of *Rauvolfia serpentina* was analyzed through GC-MS. The compounds were identified by comparison of GC-MS spectrum with library searches which led to identification of 18 compounds. The major constituents were found to be 2-methylheptane (3.48%), cis-1,3-dimethylcyclohexane (13.66%), 2-(propoxymethyl)oxirane (3.45%) Hexylene Glycol (14.28%), 3-methylheptan-2-one (15.56%), 2,5-dimethylhexan-3,4-diol (5.70%), 3,4-dimethylhexan-2-ol (7.47%), 1,1,2,3-tetramethylcyclopropane (24.33%), Diethyl Phthalate (2.11%) and other chemical constituents with less than 2% peak area.



**Keywords:** Apocynaceae, Phytochemicals, GC-MS analysis, n-Hexane extract, *Rauvolfia serpentina*

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**Introduction**

Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. The plant-based, traditional medicine systems continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care.[1] The medicine plant could be based on the antioxidant, antimicrobial, antipyretic effects of the phytochemicals in them.[2-3]

*R. serpentina* L. Benth. ex Kurz. is a species of flowering plant in the family Apocynaceae and commonly known as Sarpagandha, Chandrabagha, Snake root plant, Chotachand, Chandrika and Harkaya etc.[4] Member of this family are native to European, Asian, African and Australian with some temperate members.[5]

*Rauvolfia serpentina* is an evergreen, woody, glabrous and perennial shrub with maximum height up to 60-90 cm. The plant possess tuberous root with pale brown cork and elliptic to lanceolate or obovate leaves in whorls of three, 10 cm long and 5 cm broad.[6] Flowers pinkish white arranged in many flowered terminal cymes. Fruits drupe, 0.5 cm in diameter and shiny black when full ripen.

The roots, leaves and juice are of medicinal importance and have attracted the attention of practitioners of indigenous system of medicine, as it contain a large number of secondary metabolites (N- containing indole alkaloids)

localized mainly in the roots and rhizomes.[7-8] In Ayurvedic medicines, the roots of *R. serpentina* is used as a remedy for curing hypertension, insomnia, mental agitation, gastrointestinal disorders, excitement, epilepsy, traumas, anxiety, excitement, schizophrenia, sedative insomnia and insanity.[9]

## Materials and Methods

### Collection of plant materials

Roots of *R. serpentina* (**Figure1**) were purchased from ayurvedic shop in Bhedashing, Kathmandu and identified by Dr. Suman Bhattarai, Associate Professor, Department of Botany, Tri-Chandra Multiple Campus. Roots of *R. serpentina* were crushed into fine powder using mechanical grinder.



**Figure 1** Root of *Rouvolfia serpentina*

### Preparation of extract

Two extract of root of *R. serpentina* were prepared using soxhlet extraction technique. About 100 grams of fine powder of roots of *R. serpentina* was extracted with n-hexane (200 ml) for 24 hours in soxhlet followed by methanol (200 ml) for 23 hours. The collected methanol and n-hexane extract of root of *R. serpentina* was concentrated dried to solid on water bath.

### Phytochemical Analyses

#### Qualitative Phytochemical Analysis

The methanolic root extracts of *R. serpentina* was tested for the presence of different phytoconstituents like alkaloids, flavonoids, steroids, phenols and so on by method described by Tiwari P. et al.[10] and Rathore S. K. et al.[11] with slight modification.

#### Test for Alkaloids

Wagner's Test: About 1ml of plant extracts was acidified with hydrochloric acid in separate test tube, few drops of Wagner's reagent was added to each. A yellow brown precipitate indicated the presence of alkaloids.

#### Test for Saponins

Foam Test: About 0.5 gm of plant extracts was shaken with 2 ml of water. If foam produced persists for ten minutes then it indicates the presence of saponins.

**Test for Tannins**

Lead Acetate Test: To 5 ml of extract, few drop of 10% of lead acetate solution were added, formation of yellow or red precipitate indicated the presence of tannins.

**Test for Steroids**

Liebermann Burchard's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicates the presence of steroids.

**Test for Cardiac Glycosides**

Legal's Test: Plant extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

**Test for Carbohydrates**

Fehling's Test: About 2mg of plant extracts was shaken with 10 ml of distilled water, filtered and filtrate was concentrated in separate test tube. An equal part of Fehling's Solution A and B (1 ml) were added to each and allowed for boiling for few minutes. Formation of red or brick color precipitate indicated the presence of the reducing sugar.

**Test for Flavonoids**

Alkaline Reagent Test: Plant extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which became colorless on addition of dilute acid, indicated the presence of flavonoids.

**Test for phenols**

Phenolic test: About 2 ml of plant extracts was taken in test tube and few drops of  $\text{FeCl}_3$  was added to it. The formation of deep blue color indicated the presence of phenols.

**Quantitative Phytochemical Analysis****Determination of Fat Content**

About 4 gm of powder of root of *R. serpentina* were extracted with 100 ml of distilled water for 4-5 hours followed by 100 ml of anhydrous diethyl ether using soxhlet technique for 3-4 hours. The diethyl ether extract was dried in water bath and weight of dried sample was taken.[12]

**Determination of Alkaloid**

Ground root powder (5 gm) of *R. serpentina* was weighed and taken into a beaker (250 ml). To this 10% acetic acid in ethanol (200 ml) was added, covered, and allowed to stand for 4 hours. This was filtered and the filtrate extract was concentrated on a water bath to 1/4 of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.[13]

### Determination of Saponin

Ground root powder (20 gm) of *R. serpentina* was taken into a conical flask and 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of same 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the diethyl ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 5% aqueous sodium chloride (10 ml). The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight.[14]

### GC-MS Condition

Gas chromatography combined with mass spectroscopy is a preferable methodology for routine analysis of compounds. n-Hexane extract of root of *R. serpentina* was injected into the Gas chromatography unit Shimadzu GC-MS QP2010 was the instrument used for GC-MS analysis. It is separated into various constituents with different retention time which are detected by mass spectrophotometer. The chromatogram a plot of intensity against retention time was recorded by the software attached to it. From the graph the compounds are identified comparing the data with the existing software libraries like NIST, NIST08 and NIST08s. 1 µl of the n-hexane extract of root of *R. serpentina* was injected into GC. The injection mode was used split method with linear velocity of 28.3cm/sec for flow control. The injector temperature was maintained at 250°C. The pressure of the carrier gas was kept at 26.6 kPa. The oven temperature was set at 80°C to 230°C with a gradual increment of 8°C per min.

### Results and Discussions

#### Phytochemical Analyses

##### Qualitative analysis

The results of phytochemical screening of methanolic root extracts of *Rauvolfia serpentina* is presented in **Table 1**. Qualitative tests for alkaloids, carbohydrate, flavonoids, steroids, phenols and other phytochemicals were carried out in order to know the presence of primary and secondary metabolites in these crude extracts of the plant.

Qualitative analysis of methanolic root extracts of *R. serpentina* indicates the presence of alkaloids, saponins, tannins, steroids, flavonoids, and phenols. Deskmukh et. al. in 2012[6] reported the qualitative analysis of root and leaf extract of *R. serpentina* indicating the presence of tannins, saponins, flavonoids, alkaloids and starch in root extract whereas leaf extract gave positive test to reducing sugar, tannins, alkaloids, flavonoids and starch.

**Table 1** Phytochemical screening of methanolic root extract of *R. serpentina*

Phytochemical constituents	Qualitative analysis of <i>R. serpentina</i>
Alkaloids	+
Saponins	+
Tannins	+
Steroids	+
Cardiac Glycosides	-
Flavonoids	+
Phenols	+

## Quantitative Analysis

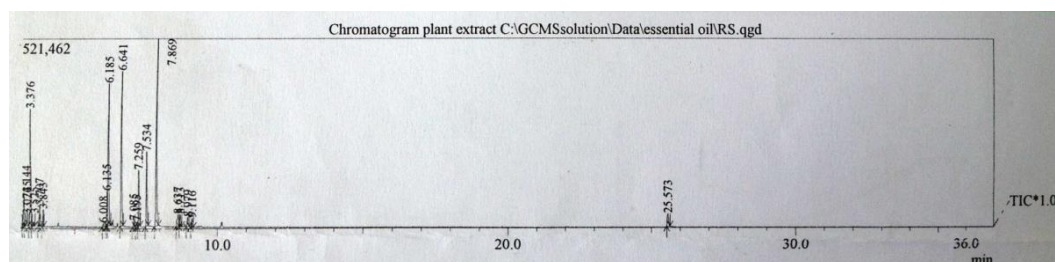
The quantitative determination of phytochemical constituents of *Rauvolfia serpentina* was summarized in **Table 2**. High quantity of alkaloids and saponins were found on root of *Rauvolfia serpentina* whereas as the fat content is very trace in root of *Rauvolfia serpentina*. The percentage of fat content in 4 gm of plant sample was found 2 %. The percentage of Alkaloid content in plant sample (5 gm) was 12.4 % and the percentage of saponin content in plant sample (20 gm) was 7.35 %.

**Table 2** Percentage of phytochemicals constituents in plant sample

Phytochemicals	Plant Sample Taken	Wt of extracted phytochemicals	Percentage content
Fat	4 gm	0.08 gm	2
Alkaloids	5 gm	0.62 gm	12.4
Saponins	20 gm	1.47 gm	7.35

## GC-MS Analysis

GC-MS chromatogram of the n-hexane extract of root of *R. serpentina* (**Figure 2**) showed twenty one peaks indicating the presence of 18 different compounds, besides a number of peaks with very narrow retention time. The chemical compounds identified in the n-hexane extract of root of the *R. serpentina* are presented in **Table 3**.



**Figure 2** Chromatogram of n-Hexane extract of root of *R. serpentina*

**Table 3** GC-MS Data of n-Hexane extract of root of *R. serpentina*

SN	Compound name	Mol. wt.	Mol. formula	Area %	Ret. time (min)	S. I.
1	2,3,4-trimethylpentane	114	C <sub>8</sub> H <sub>18</sub>	1.32	3.074	91
2	2-methylheptane	114	C <sub>8</sub> H <sub>18</sub>	3.48	3.144	95
3	3-methylheptane	114	C <sub>8</sub> H <sub>18</sub>	1.68	3.265	94
4	cis-1,3-dimethylcyclohexane	112	C <sub>8</sub> H <sub>16</sub>	13.66	3.376	94
5	1-Decanol	158	C <sub>10</sub> H <sub>22</sub> O	1.61	3.707	87
6	trans-1,3-dimethylcyclohexane	112	C <sub>8</sub> H <sub>16</sub>	1.39	3.843	91

7	Isobutane	58	C <sub>4</sub> H <sub>10</sub>	0.27	6.008	93
8	2-(propoxymethyl)oxirane	116	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	3.45	6.135	83
9	2-methylpentane-2,4-diol	118	C <sub>6</sub> H <sub>14</sub> O <sub>2</sub>	14.28	6.185	84
10	3-methylheptan-2-one	128	C <sub>8</sub> H <sub>16</sub> O	15.56	6.641	86
11	1-nitropropane	89	C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub>	0.54	7.085	82
12	3,3-dimethylbutan-2-ol	102	C <sub>6</sub> H <sub>14</sub> O	0.58	7.195	81
13	2,5-dimethylhexan-3,4-diol	146	C <sub>8</sub> H <sub>18</sub> O <sub>2</sub>	5.70	7.259	84
14	3,4-dimethylhexan-2-ol	130	C <sub>8</sub> H <sub>18</sub> O	7.47	7.534	87
15	1,1,2,3-tetramethylcyclopropane	98	C <sub>7</sub> H <sub>14</sub>	21.19	7.869	82
16	2-hydroperoxy-2-methylpropane	90	C <sub>4</sub> H <sub>10</sub> O <sub>2</sub>	1.34	8.979	87
17	3-methylpentan-2-one	100	C <sub>6</sub> H <sub>12</sub> O	0.81	9.116	84
18	Diethyl Phthalate	222	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	2.11	25.573	90

The major constituents were found to be 2-methylheptane (3.48%), cis-1,3-dimethylcyclohexane (13.66%), 2-(propoxymethyl)oxirane (3.45%) Hexylene Glycol (14.28%), 3-methylheptan-2-one (15.56%), 2,5-dimethylhexan-3,4-diol (5.70%), 3,4-dimethylhexan-2-ol (7.47%), 1,1,2,3-tetramethylcyclopropane (24.33%), Diethyl Phthalate (2.11%) and other chemical constituents with less than 2% peak area.

## Conclusions

Phytochemical investigation of root extracts of *R. serpentina* indicates the presence of alkaloids, saponins, tannins, steroids, flavonoids, and phenols. The quantitative determination of phytochemical constituents of *Rauwolfia serpentina* indicates the presence of 2% fat, 12.4% alkaloid and 7.35% saponin in plant sample. GC-MS chromatogram of the n-hexane extract of root of *R. serpentina* showed different peaks indicating the presence of different compounds. The compounds were identified by comparison of GC-MS spectrum with library searches which led to identification of 18 compounds. The presence of various phytochemicals contributes many medicinal values of the plant. This is first time reporting of GC-MS analysis of n-hexane extract of *R. serpentina* and indicated the presence of important phytochemicals. Further studies are required to confirm the compound detected by GC-MS analysis.

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