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Extraction of alkaloids from *Rauwolfia serpentina* medicinal plant

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Abstract
Rauwolfia serpentina L. Benth Kurz, known as Sarpagandha, is mainly known for its chemical constituent's reserpine, which was widely used as an antihypertensive drug and a powerful sedative. I has important medicinal values. During present study phytochemical analysis of root and leaves of plant. The evaluation of presence and absence of indole alkaloids were carried out by TLC and HPLC methods. The methods also focused on the quantitative and qualitative determination of indole alkaloids. This work represents a first report of antiproliferative activity of *Rauwolfia serpentina*.
Plant leaves and roots were extracted by using solvent like ethanol and the quantity of crude extracts obtained was 12.05%. The evaluation of indole alkaloids were done by using the methods like TLC and HPLC that indicated the presence of four different indole alkaloid derivatives like ajmalicine, ajmaline, yohimbine and reserpine in root extract of *Rauwolfia serpentina.* Further quantitative determination of *Rauwolfia* alkaloids was carried out by spectrophotometric analysis which resulted that Ajmalicine content was greater in leaf extract whereas reserpine, ajmaline and yohimbine were greater in root extract of plant.

Keywords: extraction, alkaloids, *Rauwolfia serpentine*, medicinal plant

1. Introduction
India has a rich heritage of traditional Ayurvedic medicine, and a recent surge in the demand for plant-derived drugs has gained momentum. Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years (Pandey *et al.* 2013) [1]. The World Health Organization estimated that <80% of the world's population relies on traditional medicine for their primary health-care requirement most of them are derived from plant extracts or their active components.
Reserpine, the Indol alkaloids is the most active compound isolated from root and leaves of the plant. It is an indole alkaloid, and chemically it is 11, 17 α-Dimethoxy-18 β-[(3, 4, 5-Trimethoxybenzoyl) Oxy]-3 β, 20 α-yohimban-16 β-carboxylic acid methyl ester (Glynn, 1955) [2]. The concentration of reserpine varies from 1.8% to 3.0% depending on geographical location and the season of plant collection with December being the favorite month for maximum alkaloid yield (Gawade and Fegade, 2012) [3].
Thus, the present study was deal with phytochemical analysis of *Rauwolfia serpentina* which were carried out by using methods like extraction, TLC and HPLC. These methods focused on the presence and absence of indole alkaloids and their quantitative and qualitative determination.

2. Material and Methods
Plant Material
The plant of *Rauwolfia serpentina* was collected from Amarkantak district Anuppur (M.P.), State of India. The roots of *Rauwolfia serpentina* were also collected from a local herbal drug store. All the botanical aspects of the whole plant were studied in detail. After the samples were identified and authenticated by the scientists of SFRI, Polypather Jabalpur (M.P.) and the voucher specimens were deposited in chemistry department.

Chemicals
3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Acetonitrile (HPLC grade), Phosphate buffer, Basal Salt Solution medium (BSS), Dimethyl Sulfoxide (DMSO), Phosphate Buffer Saline (PBS), potassium dihydrogen phosphate, Orthophosphoric acid, ethanol, 1,10- phenanthroline, ferrous chloride, ferric chloride and antibiotics were purchased from Sigma, Merck and Qualigen. All chemicals and solvents of analytical grade were used.

Preparation of Extract
Ten gram powder of leaf and root were mixed in 40 ml of ethanol in 250 ml of conical flask and was kept at 25ºC for 12hrs. After 12 hours, the suspension was filtered through Whatman's filter paper and collected in large Petri plates. These were allowed to dry completely in water bath set at 40 ± 0.2ºC for 30 min. Dried extracts were scraped out by using scalpels and was collected in pre weighed vials separately. Extracted powders were made available to use as per requirements by resuspended in ethanol every time. The quantity of crude extracts obtained by this method was 12.05%.

Phytochemical Screening
The powdered samples of leaf and root cultures of *Rauwolfia serpentina* were screened for phytochemical constituents using standard procedures of analysis (Preiyasamy *et al.* 2010, Koche *et al.* 2010, Ajayi *et al.* 2011 and Igbinosa *et al.* 2009) [4-7].

Qualitative Analysis
Thin Layer Chromatography
The qualitative analysis of major groups of indole alkaloid derivatives of *Rauwolfia serpentina* was initially done by thin layer chromatography (TLC) technique on preparative

silica gel (silica gel-60). Mobile phase or solvent system used for alkaloid estimation was chloroform: methanol (97:3). Bands were visualized by spraying Dragendorff's reagent uniformly over the plates or also observing the plates under UV- transilluminator. Identification was done on the basis of color of bands and their Rf values under UV light (Kumar *et al.* 2009 and Panwar and Guru, 2011) [8-9].

High Performance Liquid Chromatography

HPLC of crude extract of *Rauwolfia serpentina* plant was carried out by Lichrosorb C–18 (25 X 0.5cm 10A) column. Mobile phase used was Acetonitrile: Phosphate Buffer (35:65). 20 µl of the volume was injected with the flow rate of 1ml/min. Detection wavelength was 268 nm and the method was carried out at ambient temperature. Isocratic method was used for obtaining chromatogram of metabolites of *Rauwolfia serpentine* (Kumar *et al.* 2010 and Goel *et al.* 2009) [10-11].

Analysis of alkaloids extracted from *Rauwolfia serpentina*

Aliquots of each alkaloid sample (root and leaf) were transferred into the test tubes in duplicates. To each of these test tubes, 1ml of $FeCl_3$ solution and 1ml of 1, 10 - phenanthroline solutions was added and final volume was marked up to 8 ml. Out of 5 test tubes, 2 tubes were containing root extract and other 2 were with leaves extract. 1 test tube was kept as a blank without sample extract. These tubes were then placed in water bath maintained at 70 ± 2ºC for 30 min. The absorbance of orange- red colored product was measured at 510 nm against the blank solution by UV-Visible spectrophotometer (Systronic Visiscan 167) Singh *et al.* (2004) [12].

Statistical analysis

All experiments were conducted in triplicate and statistical analysis was done by using the MS Excel (CORREL Statistical function) and Graphpad Prism 4 softwares. The data were presented as mean ± SD.

3. Results

Phytochemical Screening

The results of phytochemical screening of leaf and root extracts of *Rauwolfia serpentina* is presented in table 1. Qualitative tests for carbohydrates, free reducing sugars, alkaloids, saponnins, tannins, flavonoids and starch soluble compounds were carried out in order to know the presence of primary and secondary metabolites in these crude extracts of the plant.

Table 1: Phytochemical analysis of crude leaf and root extracts of *Rauwolfia serpentina*

S.No.	Secondary metabolites	*Rauwolfia serpentina* Root extract	*Rauwolfia serpentina* Leaf extract
1.	Molisch's test	-	+
2.	Barfoerd's test	-	-
3.	Fehling's test (free reducing sugar)	-	+
4.	Fehling's test (combined reducing sugar)	-	-
5.	Tannin's test	+	+
6.	Liebermann- burchard test	-	-
7.	Saponin's test	+	+
8.	$FeCl_3$test for flavonoids	-	-
9.	NaOH test for flavonoids	+	+
10.	Mayer's test for alkaloids	+	+
11.	Starch soluble test	+	+
12.	Phlobatannins test	-	-

Qualitative analysis

Thin Layer Chromatography

The qualitative analysis of alkaloid was done by Thin Layer Chromatography (TLC). In TLC, the qualitative analysis of alkaloids was done on preparative silica gel plates using specific solvent systems for secondary metabolite's group. When the alcoholic extracts of leaf and root of *Rauwolfia serpentina* were subjected to the solvent system chloroform: methanol (97:3) both the samples showed fluorescent green and blue bands on preparative silica gel plates under ultraviolet light indicating the presence of various alkaloid derivatives. Calculated Rf values were found to be very close with standard Rf values which indicated the presence of different indole alkaloid derivatives which might indicate the presence of ajmaline, ajmalicine, yohimbine and reserpine.

High Performance Liquid Chromatography

HPLC method was carried out by providing all the suitable experimental conditions and the peaks from root and leaf crude extract were obtained. HPLC of root and leaf extract was performed by Isocratic method. Acetonitrile: Phosphate Buffer (35:65) was the mobile phase used for detection of indole alkaloids from root and leaf sample of *R. serpentina* and the analysis was done at 268 nm. Various numbers of peaks were observed in the HPLC chromatogram that revealed the presence of different indole alkaloids. Some of the peaks were identified on the basis of retention time (Rt). In crude leaf extract, 11 peaks were observed at different retention time. The crude leaf extract had shown a peak at 7.01 min that indicated the presence of ajmaline at Rt 7.0 minutes.

In crude root extract, 12 peaks were observed at different retention time at same conditions. Out of these 12 peaks, the two peaks which were obtained at Rt 8.18 min and 13.26 min indicated the presence of reserpine and ajmalicine respectively at exactly same conditions.

Quantitative Analysis of *Rauwolfia* alkaloids

Alkaloid content of *Rauwolfia serpentina* was measured by using spectrophotometric analysis. The absorbance was measured at 510 nm on visible spectrophotometer (Systronics Visiscan 167). In this assay, four indole alkaloids were measured viz. reserpine, ajmaline, ajmalicine

and yohimbine. The root as well as leaf extract showed the presence of all four indole alkaloids. The leaf extract showed the presence of reserpine, ajmalicine, ajmaline and yohimbine as 0.880, 0.753, 0.485 and 0.537 respectively. However, the root extract showed the presence of these all four indole alkaloids as 0.955, 0.440, 0.817 and 0.584 respectively. Ajmalicine content was found to be 0.753 mg/g in leaf extract which was more than the root extract of *R. serpentina* whereas the quantity of reserpine, ajmaline and yohimbine was more in root extract as compared to leaf extract**.**

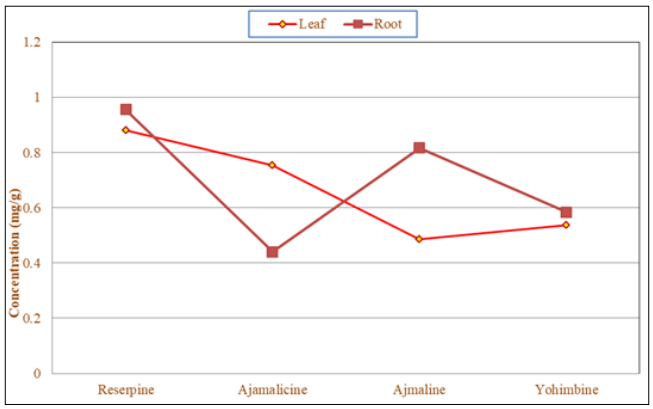

Fig 1: Graphics Analysis or Indole Alkaloids in Leaf and Root Extracts of *Rauwolfia serpentina*

4. Discussion

The preliminary phytochemical screening was done by some standard chemical tests. These classes of compounds such as carbohydrates, tannins, saponins, flavonoids, alkaloids and starch soluble compounds are present in the plant extracts used. Presence of saponins and flavonoids like compounds showed the justified use of extracts from *R. serpentina* plant extract. The study suggested that pure isolated alkaloids and their synthetic derivatives can be used as basic medicinal agents for their analgesic, antispasmodic effects (Harisaranraj *et al*. 2009) [13].

Alkaloid analysis by TLC showed the presence of four different indole alkaloid derivatives in root extract of *R. serpentina.* Leaves of plant were found to contain very low amount of these indole alkaloid derivatives after the detection by TLC. The report of HPLC analysis of *R. serpentina* plant samples showed the higher presence of indole alkaloid contents in the root extract. Leaf extract also detected the presence of those alkaloids which were present in very low amount.

From the spectrophotometric analysis alkaloid content of *Rauwolfia serpentina* was measured which reported that Ajmalicine content was more in leaf extract as compared to root extract of *Rauwolfia serpentina* whereas ajmaline, reserpine and yohimbine were found to be more in root extract as compared to leaf extract.

5. Conclusion

Thus, the presence of high level of indole alkaloids in the leaf and root extracts of *Rauwolfia serpentina* may be responsible for the biological activity of the samples. Therefore, this study reveals that the *R. serpentina* plant can be used as a natural therapy.

6. References

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