ABSTRACT

Rauvolfia serpentina (Apocynaceae) is used among rural Indian communities to treat arthritis, skin cancer, burns, eczema, psoriasis, digestive problems, high blood pressure, sedative and diabetes, despite very little supporting scientific evidence. Due to increased interest by both the scientific community and industry regarding the medicinal uses of this plant species, we identified, quantified and compared the phytochemical contents and antioxidant capacities of extracts of Rauwolfia serpentina. Each medicinal plant and the specific plant part used as crude drug material contain active or major chemical constituents with a characteristic profile that can be used for chemical quality control and quality assurance. So the increasing demand for herbal medicines has inevitably led to maintaining the quality and purity of herbal raw materials and finished products. A sensitive and reproducible reversed-phase high-performance liquid chromatography (HPLC) method using photodiode array detection is established for the simultaneous quantitation of important root alkaloids of Rauvolfia serpentina, namely, reserpine, ajmaline, and ajmalicine. Rauvolfia serpentina plant species is used since ancient time. Now days it has been became endangered plant species in India due to climacteric changes and increased demand in drug industry. Hence comparative physiochemical analysis of wild and cultivated species is necessary. The TLC analysis of all the samples, including roots, leaves and callus, showed that roots are rich in reserpine and they also contain other alkaloids besides reserpine.

KEYWORDS: Rauvolfia serpentina, Medicinal herbs, Phytochemicals, Pharmacognosy, Pharmacological activities.
INTRODUCTION
Rauvolfia serpentine is also known as 'Sarpagandha’, ‘Indian snakero’t, ‘Chandrabhaga’ or ‘Chhota Chandis’. It is an important medicinal plant distributed in the foothills of the Himalayan range. It has long tapering snake-like roots which are a rich source of reserpine alkaloids. This alkaloid is used in the manufacture of anti-hypertensive and sedative medicines. The roots are also used in Ayurvedic and other systems of medicines for curing a wide range of ailments. The plant is used in the treatment of hypertension, anxiety, and insomnia. In the traditional medicinal system, it is also used orally for snake bites, insect bites, fever, constipation, and malaria. It is also considered useful in treating liver disease, rheumatoid arthritis, edema, and epilepsy. Along with that, it also acts as a uterine stimulant which helps the uterus to contract. In some cases, oral intake of Indian snakeroot may cause some side effects due to the presence of small amounts of harmful elements (like yohimbine). Most adverse effects with the use of Sarpagandha appear to be mild. Higher doses can cause cardiovascular side effects including bradycardia (slower than normal heart rate) and hypotension (low blood pressure). Also, its long-term use may cause depression in some people. Rauvolfia serpentina, the Indian snakero, devil pepper, or serpentine wood, is a species of flower in the milkweed family Apocynaceae. It is native to the Indian subcontinent and East Asia (from India to Indonesia). Rauvolfia is a perennial undershrub widely distributed in India in the sub-Himalayan regions up to 1,000 metres (3,300 ft).

A. Plant description
Rauvolfia serpentina is identified as a critically endangered species Due to high demand and unavailability, the roots of Rauvolfia serpentina are usually adulterated with other species like Ophiorrhiza mungo’s, white-flowered and red-flowered Clerodendrum species and
Taberna Montana divaricata. Substitutes were used and recommended in the ancient texts for plants that were scarce to obtain. In this way, different regions in the country have come to use different botanical sources for some of the classical plants. In industry, Sarpagandha is commonly substituted with the root of other Rauvolfia species. Identification of a scientifically validated substitute from allied/related species for the endangered medicinal plant has a great importance in the herbal drug industry as it exterminates the unauthorized substitution and adulteration. The unscientific substitution may affect the quality of herbal preparations adversely. No previous scientific studies are available regarding the identification of substitute for this selected species. The objective of the present study is to find out an appropriate substitute for the root of R. serpentina by evaluating the phytochemical and pharmacological properties of its allied species

**Synonym:** *Ophioxylon album* Gaertn, *Ophioxylon obversum* Miq, *Ophioxylon salutiferum* Salisb.

**Common name:** *Sarpagandha, Snake root plant, Chotachand, Chandrika.*

### Table 1: Taxonomic classification of *rauvolfia serpentine.*

<table>
<thead>
<tr>
<th><strong>Taxonomy</strong></th>
<th><strong>Rauvolfia Serpentina</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Domain</td>
<td>Eukaryote</td>
</tr>
<tr>
<td>Kingdom</td>
<td>Plantae</td>
</tr>
<tr>
<td>Sub kingdom</td>
<td>Viridiplantae</td>
</tr>
<tr>
<td>Infra Kingdom</td>
<td>Streptophyta (Land Plants)</td>
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<tr>
<td>Class</td>
<td>Magnoliopsida</td>
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<tr>
<td>Super order</td>
<td>Asteranae</td>
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<tr>
<td>Order</td>
<td>Gentianales</td>
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<tr>
<td>Family</td>
<td>Apocynaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Rauvolfia L. (Devil’s Pepper)</td>
</tr>
<tr>
<td>Species</td>
<td>Rauvolfia Serpentina (Indian Snake Wood or Serpentine Wood)</td>
</tr>
<tr>
<td>Botanical name</td>
<td>Rauvolfia serpentina</td>
</tr>
</tbody>
</table>

### B. Phytochemical composition

The phyto constituents present in the organic extracts were determined qualitatively according to Sofowora (1993), Trease and Evans (1989) and Harbone (1973) as well as by thin layer chromatography (TLC). In TLC, the extracts spotted on silica coated plates, were developed using butanol-glacial acetic- water (100: 10 :10) as the solvent system. The developed plates were then sprayed with with vanillin solution (1% (w/v) in 50% phosphoric acid) for steroid detection, Dragendorff’s reagent for alkaloid detection and sodium metaperiodate (0.1%) followed by ethanolic benzidine for glucose detection. Nicotinic acid,
cholesterol D-glucose and tannic acid at 1% solution were prepared accordingly and used as standards in the TLC assay. The TLC results were further used to validate the presence of tannins based on positive reaction (brownish green – blue black coloration) with 0.1% FeCl3, alkaloids based on positive reaction (brown coloration) with Dra-gendorff’s reagent (Trease and Evans, 1989; Sofowora, 1993), steroids based on positive reactions (violet to blue or green) with acetic anhydride and H2SO4, steroidal glycosides by Keller-Killani test and cynogenic glycoside based red coloration of picrate paper (Harbone, 1973; Trease and Evans, 1993). The observation of persistent frothing in distilled water (2 ml) by 1% standard saponin solution (3 ml) followed by formation of emulsion with olive oil (0.5 ml) was used to indicate the presence of saponin in the extract (Trease and Evans, 1989).

Chemical structures of some alkaloids present in Rauvolfia serpentina

**MATERIALS AND METHODS**

**Preparation of plant extract of rauvolafia serpentina**

**Extraction method**

500 gms of dry powdered bark of Rauvolfia serpentina was extracted using soxhlet apparatus with chloroform for 5 hours per day and continued for 3 days. The extracted chloroform part was separated, and evaporated to dryness.
Chromatography technique

Phytochemical analysis of the extracts

A. Detection by TLC

Total alkaloid contents were estimated from different plant parts of R. serpentina, (e.g., callus, leaves and roots) collected from different habitats as well as in vitro regenerated plants. Hundred grams of powdered dry samples of R. serpentina were soaked in 10.0 mL methanol and left for 30 min. After 30 min, the soaked plant material was filtered. The residue obtained after filtration is further dissolved in 5.0 mL methanol and filtered after 10 min, the same step is repeated once again, and the final filtrate is collected in 50 mL conical flask. The extract was evaporated to dryness in the soxhlet evaporator. The crude extract was dissolved in 100 mL of 0.01 M HCl. The pH of filtered solutions were adjusted to 6.0 with 0.01 M NaOH. The crude extracts obtained were used for TLC, HP-TLC and HPLC analysis (Klyushnichenko et al., 1995). The crude extract obtained, was concentrated to dryness to yield Crude Alkaloid Fraction (CAF). The purified samples were spotted on TLC-pre-coated silica gel plate. Each plate contains five samples and two standards of reserpine and recinnamine (purchased from Hi-media Laboratories) dissolved in methanol (1.0 g mL⁻¹). The mobile phase was chloroform (CHCl₃) and methanol (CH₃OH) in 97:3 ratio (v/v) or chloroform, methanol and aqueous ammonia (NH₃) in 95:4.5:0.5 (v/v/v) ratio. Spots were visualized by the spray of Dragendorff’s reagent. The sprayed plates develop orange spots. Spots intensify if the plates further sprayed with HCl, or 50% water-phosphoric acid and finally the Rf value was calculated.

B. Detection by HPLC

A sensitive and reproducible reversed-phase high-performance liquid chromatography (HPLC) method using photodiode array detection is established for the simultaneous quantitation of important root alkaloids of Rauvolfia serpentina, namely, reserpine, ajmaline, and ajmalicine. A Chromolith Performance RP-18e column (100 x 4.6-mm i.d.) and a binary gradient mobile phase composed of 0.01 M (pH 3.5) phosphate buffer (NaH₂PO₄) containing 0.5% glacial acetic acid and acetonitrile are used. Analysis is run at a flow rate of 1.0 mL/min with the detector operated at a wavelength of 254 nm. The calibration curves are linear over a concentration range of 1-20 microg/mL (r = 1.000) for all the alkaloids. The various other aspects of analysis (i.e., peak purity, similarity, recovery, and repeatability) are also validated. For the three components, the recoveries are found to be 98.27%, 97.03%, and 98.38%, respectively. The limits of detection are 6, 4, and 8 microg/mL for ajmaline,
ajmalicine, and reserpine, respectively, and the limits of quantitation are 19, 12, and 23 microg/mL for ajmaline, ajmalicine, and reserpine, respectively. The developed method is simple, reproducible, and easy to operate. It is useful for the evaluation of R. serpentina. This technique is used for separation, quantification, and identification of inorganic and organic solutes in industrial, environmental, biological, or pharmaceutical samples. It is based on solute interactions in the mobile phase (usually polar combinations of water and other solvents) with tightly packed solid particles of the stationary phase (usually non-polar particles like C18). High pressure of between 250 to 400 bars is required for analyte elution through the column to the detector.

Standardization of rauvolfia serpentina

a. **Determination of total ash content**

1g of each test drug was incinerated in a crucible at 4500 C in a muffle furnace and cooled, weighed, and % of total ash was calculated.

b. **Determination of acid insoluble ash**

Ash was further boiled for 5 min with 25 ml of 0.1 N HCl and filtered using ashless filter paper. Insoluble matter retained on filter paper was washed with hot water and dried to a constant weight and finally, the percentage of acid insoluble ash was calculated.

c. **Determination of extractive value**

Extractive values of each test drug were determined using following methods

d. **Determination of alcohol soluble extractives**

The powdered test drugs were macerated with 100 ml of alcohol in a closed flask for 24 h. It was then allowed to stand for 18 h and filtered. 25 ml of each filtrate was evaporated to dryness in a porcelain dish at 1050 C to constant weight. The percentage of alcohol soluble extractive was calculated.

e. **Determination of water soluble extractives**

The powder test drugs were macerated with 100 ml of water in a close flask for 1 h. Then, it was boiled gently for another hour on water bath, cooled and weighed and the weight was re-adjusted. 25 ml of each filtrate was evaporated to dryness in a porcelain dish at 1050 C to constant weight. The percentage of water-soluble extractive was calculated.

f. **Loss on drying**

1g of each drug was taken in a tarred glass bottle and heated at 1050 C in an oven till a constant weight. The percentage of moisture content was calculate.
CONCLUSION

Rauwolfia Serpentina root powder is useful for lowering and managing the blood pressure. Reserpine presents in the roots binds the vesicular monoamine transporters and inhibits the uptake of norepinephrine into secretory vesicles and depletes serotonin and catecholamines from the central and peripheral axon terminals. It results in depletion of neurotransmitters and reduces promulgation of the nerve impulses occurring in the postsynaptic nerve cells. This depletion results in suppression of sympathetic nerve function, which decreases arterial blood pressure and heart rate. This action reduces the blood pressure. This action may also cause many side effects for which rauvolfia is used in modern medicine.

REFERENCES

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