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SUBSTITUTION OF ROOTS WITH SMALL BRANCHES OF *RAUWOLFIA SERPENTINA* FOR THERAPEUTIC USES - A PHYTOCHEMICAL APPROACH

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KEYWORDS: *Rauwolfia serpentina*, HPTLC profile, physicochemical analysis, phytochemical analysis.

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ABSTRACT

Rauwolfia serpentina commonly called *Sarpagandha* is a medicinal plant widely used in Ayurveda. As per the Ayurvedic literature, roots of this plant are used in cardiac disorder, cancer, mental illness and psychiatric disorder. To collect roots for medicinal purpose whole plant is uprooted on a mass scale from their natural habitat which is leading to depletion of resources, due to which plant may be difficult in near future for use in traditional systems of medicine. Present study was carried out to assess possibilities of using small branches of *R. serpentina* in place of its roots which will help in conservation of this plant and availability of raw material for therapeutic purposes. Roots and small branches of *R. serpentina* are compared on the basis of physicochemical analysis, phytochemical analysis, total phenolic contents, total flavonoid contents and high performance thin layer chromatography (HPTLC) to evaluate the possibilities of using small branches in place of its roots. Results of phytochemical analysis and HPTLC of *n*-hexane, ethyl acetate and ethanol extracts showed many similarities which suggest that small branches may have nearly similar active constituents like roots and may be used as a substitute of roots after comparison and confirmation of same for pharmacological activities.

INTRODUCTION

Medicinal plants are playing an important role in meeting the global health care needs and hence the numbers of plant species are individually under attack to accomplish the global demand. In Indian system of medicine the most commonly used medicinal plants are slow-growing trees, with bark and underground parts being the parts mainly utilized. To collect the underground parts for medicinal purpose the whole plant is uprooted on a mass scale from their natural habitat which is leading to depletion of resources, due to which plant may be difficult in near future for use in traditional system of medicine. Substitution of underground parts with suitable alternate is therefore the need of the hour to safeguard the survival of such plants and to ensure the availability of raw material for the use in traditional systems of medicine. It will provide greater scope for the physician to utilize raw materials that are easily available, cost effective and most appropriate for the therapeutic uses.

Rauwolfia serpentina (Family: Apocynaceae) commonly called *Sarpagandha* is a medicinal plant widely used in Ayurveda. As per the Ayurvedic literature, roots of this plant are used in *Sula, Anidra, Apasmara, Bhutabadha, Bhrama, Jvara, Krmiroga, Madaroga, Unmada, Yonisula, Raktavita, Manasaroga, Visucika, Vrana*.[1] Roots are also reported for various pharmacological activities likes antimicrobial[2, 9], genotoxic, mutagenic, recombinogenic[3], hyperglycemic, haematinic, antioxidant[4], hypolipidemic[5, 7], glucose tolerance[6], hypoglycaemic[7], antifungal potential[8], antiproliferative[9], breast cancer[10], high blood pressure[11], various psychiatric diseases[12], mental disorders.[13] Roots mainly contain reserpine[14], recinnamine[15], serpentine, ajmaline, ajmalcine, serpentinine, ajmalinine[16], ajmacilidine[17], ajmalimine.[18]

Present study is carried out in *R. serpentina* to assess the possibilities of using small branches in place of roots. Standard physicochemical parameters of small branches of *R. serpentina* have not been prepared yet. So work is also carried out to establish preliminary physicochemical standards of small branches.

MATERIAL AND METHODS

Plant material

Roots and small branches of *R. Serpentina* were collected from National Research Institute for Ayurveda Siddha Human Resource Development, Aamkho, Gwalior (M.P) and India. Identified and authenticated by the botanist of NRIASHRD, Gwalior.

Instrumentation

A CAMAG HPTLC system (Muttenz, Switzerland) equipped with a semi automatic TLC applicator Linomat IV, twin trough plate development chamber, Win CATS software version 1.4.2. and Hamilton (Reno, Nevada, USA) Syringe (100 µl).

Material and reagents

All chemicals, reagents and solvents used during the experiments were of analytical grade and HPTLC plates were purchased from E. Merck Pvt. Ltd. (Mumbai, India).

Physicochemical parameters

Roots and small branches were studied for various physicochemical standards like foreign matter, loss on drying at 105°C, total ash, acid-insoluble ash, alcohol soluble extractive, water-soluble extractive and pH of 10% aqueous solution using standard methods.[19, 20]

Preliminary phytochemical screening

n-Hexane, ethyl acetate and ethanol extract of both roots and small branches were screened for the presence of phenols, tannins, alkaloids, carbohydrates, saponins, amino acids, steroids, flavonoids, coumarins, quinone, furanoids and triterpenoids by the standards methods of Harborne[21] and Kokate et al.[22]

Estimation of total phenolic and flavonoid content

Five grams of each of shade-dried plant material was pulverized into coarse powder and subjected to ethanolic extraction using soxhlet apparatus. Extracts were concentrated to dryness. Dried residues were then dissolved in 100 ml of 95% ethanol. Extracts were used for total phenolic and flavonoid assay.

Total phenolics content was determined by using Folin-ciocalteu assay.[23] An aliquot (1 ml) of extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 µg/ml) was added to a 25 ml volumetric flask, containing 9 ml of distilled water. A reagent blank was prepared using distilled water. One millilitre of Folin-ciocalteu phenol reagent was added to mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. Volume was then made up to the mark. After incubation for 90 min at room temperature, absorbance against reagent blank was determined at 550 nm with an UV/Vis spectrophotometer. Total phenolics content was expressed as mg gallic acid equivalents (GAE).

Total flavonoid content was measured by aluminum chloride colorimetric assay.[24] An aliquot (1 ml) of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) was added to a 10 ml volumetric flask containing 4 ml of distilled water. To the flask, 0.3 ml of 5% $NaNO_2$ was added and after 5 min, 0.3 ml of 10% $AlCl_3$ was added. After 5 min, 2 ml of 1M NaOH was added and volume was made up to 10 ml with distilled water. Solution was mixed and absorbance was measured against blank at 510 nm. Total flavonoid content was expressed as mg quercetin equivalents (QUE).

HPTLC profiles

HPTLC studies were carried out by following methods of Sethi [25], Stahl[26] and Wagner et al.[27] Roots and small branches were powdered coarsely. Ten gram powdered samples of each of roots and small branches were accurately weighed and exhaustively extracted by *n*-hexane, ethyl acetate and ethanol (each 100 ml) separately using soxhlet apparatus. Extracts were filtered and concentrated under reduced pressure and made up to10 ml in standard flasks separately. Mobile phases used for developing the *n*-hexane, ethyl acetate and ethanol extract were toluene: ethyl acetate (7:3 v/v), respectively.

Samples were spotted in form of bands of width 10 mm with a 100 µl Hamilton syringe on aluminum TLC plates pre-coated with Silica gel 60 F_{254} of 0.2 mm thickness with the help of TLC semi-automatic applicator Linomat IV attached to CAMAG HPTLC system, which was programmed through Win CATS software version 1.4.2. 10 µl of each extracts of roots and small branches were applied in two tracks as 10 mm bands at a spraying rate of 10 seconds/µl. Track 1 was roots and track 2 was small branches for each of extracts applied.

Development of plate up to a migration distance of 80 mm was performed at 27 ± 2°C with mobile phase for each extracts in a CAMAG HPTLC chamber previously saturated for 30 min. After development the plate was dried at 60°C in an oven for 5 min and visualized under wavelength 254 nm and 366 nm for ultra violet detection. Developed plate was then dipped in anisaldehyde sulphuric acid reagent for derivatization and dried at 105°C in hot air oven till colour of band appears and visualized under white light. Images were captured by keeping plates in photo documentation chamber and R_f values were recorded by Win CATS software.

RESULTS AND DISCUSSION

Physicochemical parameters like foreign matter, loss on drying at 105º C, ash values, acid insoluble ash, extractive values and pH are given in Table 1. These data may be useful to pharmaceutical industries for the authentication and batch to batch consistency of the commercial samples. Both the parts of *R. serpentina* were found to possess little moisture and hence can be stored at room temperature without fear of spoilage.

Table 1: Physicochemical parameters of roots and small branches of *R. serpentina*.

S. No.	Parameters	Results	
		Roots	Small branches
1.	Foreign matter (% w/w)	Nil	Nil
2.	Loss on drying (% w/w)	6.31	6.87
3.	Total ash (% w/w)	3.49	3.14

4.	Acid insoluble ash (% w/w)	0.17	0.34
5.	Alcohol soluble extractive value (% w/w)	1.62	2.12
6.	Water soluble extractive value (% w/w)	5.52	9.45
7.	pH of 10 % aqueous solution	5.55	6.01

Phytochemical analysis of different extracts of roots and small branches are shown in Table 2. Results revealed the presence of similar phytochemicals in roots and small branches of *R. serpentina* in various extracts tested.

Table 2: Phytochemical analysis of extracts of roots and small branches of *R. serpentina*.

Phytochemicals	Roots extracts			Small branches extracts		
	***n*-Hexane**	**Ethyl acetate**	**Ethanol**	***n*-Hexane**	**Ethyl acetate**	**Ethanol**
Phenols	-ve	+ve	+ve	-ve	+ve	+ve
Tannins	-ve	+ve	+ve	-ve	+ve	+ve
Alkaloids	+ve	+ve	+ve	+ve	+ve	+ve
Carbohydrates	-ve	-ve	+ve	-ve	-ve	+ve
Saponins	-ve	-ve	-ve	-ve	-ve	-ve
Amino acid	-ve	-ve	+ve	-ve	-ve	+ve
Steroids	-ve	+ve	+ve	-ve	+ve	+ve
Flavonoids	-ve	-ve	-ve	-ve	-ve	-ve
Coumarins	-ve	-ve	+ve	-ve	-ve	+ve
Quinone	-ve	-ve	-ve	-ve	-ve	-ve
Furanoids	-ve	-ve	-ve	-ve	-ve	-ve
Triterpenoids	-ve	-ve	-ve	-ve	-ve	-ve

Total amount of phenolic and flavonoid content of ethanolic extract of roots and small branches of *R. serpentina* are summarized in Table 3. Results indicate that in comparison to roots, small branches had the high total phenolic and flavonoid content.

Table 3: Total phenolic and total flavonoid content of ethanol extracts of roots and small branches of *R. serpentina*.

S. No.	Plant parts	Total phenolics mg of GAE/10g dry weight*	Total flavonoids mg of QUE/10g dry weight*
1.	Roots	17.26±0.09	12.11±0.01
2.	Small branches	54.36±0.19	18.12±0.22

*Values are expressed as Mean ± SD

Comparative HPTLC profile of *n*-hexane, ethyl acetate and ethanol extracts of roots and small branches of *R. serpentina* were recorded to reveal the chemical pattern of each extract. HPTLC profile of *n*-hexane extract of roots and small branches (Table 4 and Figure 1) showed no band when visualized under UV at 254 nm. At UV 366 nm roots and small branches showed five and six bands, respectively out of which four bands at R_f 0.38 (florescent green), 0.52 (blue), 0.63 (blue), 0.78 (red) were found similar. Visualization under white light after derivatization with anisaldehyde sulphuric acid reagent, roots and small branches showed two and four bands, respectively out of which two bands at R_f 0.50 (blue), 0.83 (blue) were found similar. Similarities in HPTLC profile indicate the presence of many similar compounds in *n*-hexane extract of roots and small branches.

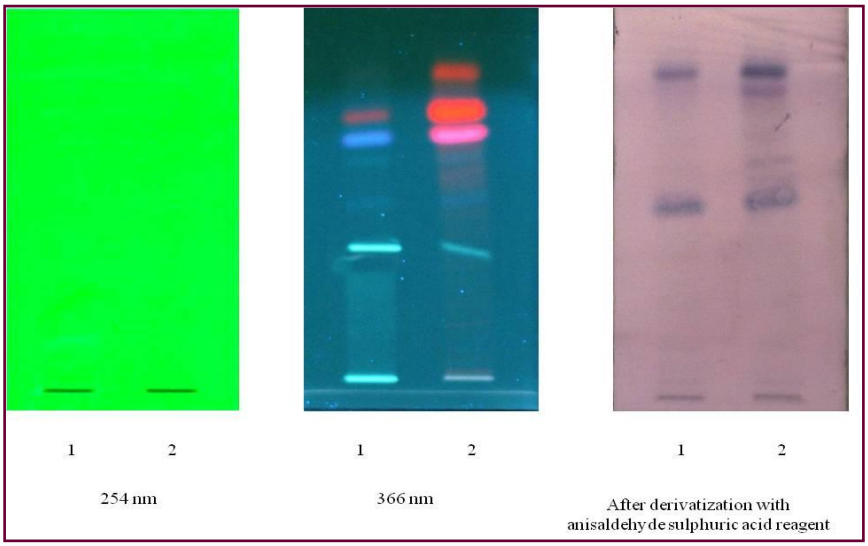

Figure 1: HPTLC profile of *n*-hexane extracts of roots and small branches of *R. serpentina.* (Track 1: roots, track 2: small branches)

Table 4: R_f values of *n*-hexane extract of *R. serpentina*.

S. No.	Wavelength	R_f values	
		Roots	Small branches
1.	254 nm	No band	No band
2.	366 nm	0.38, 0.52, 0.63, 0.70, 0.78	0.38, 0.52, 0.63, 0.72, 0.78, 0.89
3.	Visible light after derivatization	0.50, 0.83	0.50, 0.61, 0.79, 0.83

The HPTLC profile of ethyl acetate extract of roots and small branches (Table 5 and Figure 2) showed no band when visualized under UV at 254 nm. At UV 366 nm roots and small branches showed eight and ten bands, respectively out of which five bands at R_f 0.14 (florescent white), 0.26 (red), 0.37 (florescent white), 0.74 (red), 0.82 (red) were found similar. Band at R_f 0.60 (red) was found common to both parts but with different color. Visualization under white light after derivatization with anisaldehyde sulphuric acid reagent both roots and small branches showed two bands and all were found similar. Similar HPTLC profile of ethyl acetate extract under UV 254 nm, 366 nm and after derivatization with anisaldehyde sulphuric acid reagent indicates the presence of many similar compounds in ethyl acetate extract of roots and small branches.

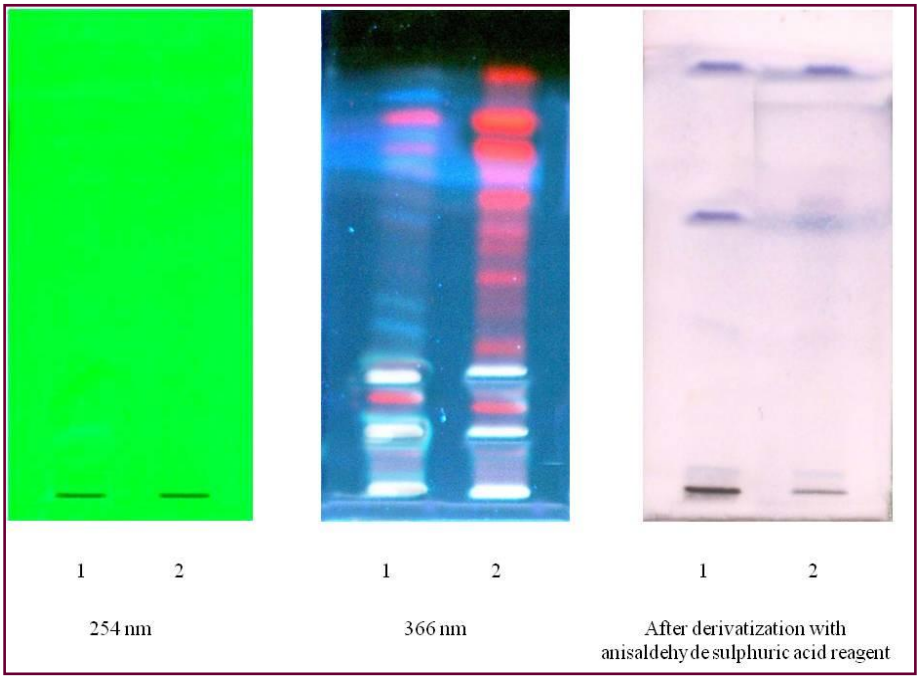

Figure 2: HPTLC profile of ethyl acetate extracts of roots and small branches of *R. serpentina.* (Track 1: roots, track 2: small branches)

Table 5: R_f values of ethyl acetate extract of *R. serpentina*.

S.No.	Wavelength	R_f values	
		Roots	Small branches
1.	254 nm	No band	No band
2.	366 nm	0.14, 0.26, 0.37, 0.43, 0.60, 0.74, 0.82, 0.87	0.14, 0.26, 0.37, 0.48, 0.56, 0.60, 0.65, 0.74, 0.82, 0.91
3.	Visible light after derivatization	0.62, 0.89	0.62, 0.89

HPTLC profile of ethanol extract of roots and small branches (Table 6 and Figure 3) showed no band when visualized under UV at 254 nm. At UV 366 nm roots and small branches showed six and ten bands, respectively out of which one bands at R_f 0.76 (red) was found similar. Band at R_f 0.18 (red), 0.37 (red), 0.68 (red) were also found common to both parts but with different color. Visualization under white light after derivatization with anisaldehyde sulphuric acid reagent, roots and small branches showed four and six bands respectively, out of which four bands at R_f 0.06 (blue), 0.26 (blue), 0.50 (blue), 0.85 (blue) were found similar. Many similarities in HPTLC profile of ethanol extracts of roots and small branches again indicates the presence of many similar compounds in roots and small branches.

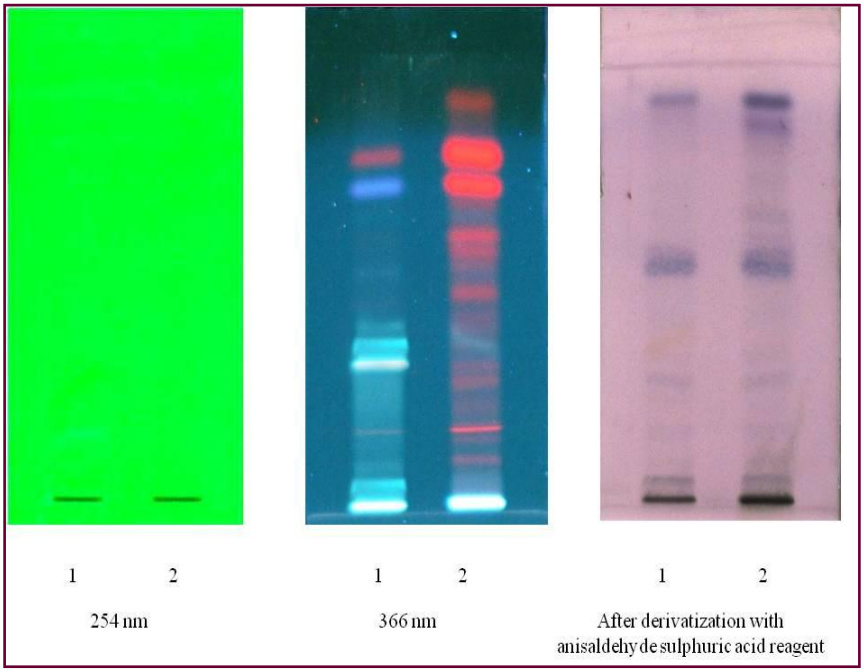

Figure 3: HPTLC profile of ethanol extracts of roots and small branches of *R. serpentina.* (Track 1: roots, track 2: small branches)

Table 6: R_f values of ethanol extract of *R. serpentina*

S.No.	Wavelength	R_f values	
		Roots	Small branches
1.	254 nm	No band	No band
2.	366 nm	0.05, 0.18, 0.35, 0.37, 0.68, 0.76.	0.12, 0.18, 0.28, 0.37, 0.47, 0.53, 0.58, 0.68, 0.76, 0.88
3.	Visible light after derivatization	0.06, 0.26, 0.50, 0.85	0.06, 0.26, 0.50, 0.60, 0.79, 0.85

CONCLUSION

Evaluation of the results of the present investigation indicates that small branches of *R. serpentina* may have same active potency like its roots and therefore may be used in place of its roots and vice-versa after comparison and confirmation of same for pharmacological activities. The results of qualitative evaluation of HPTLC profile will also be helpful in the identification and quality control of the drug and can provide standard HPTLC profiles of roots and small branches of this plant with selected solvent system. Investigations like this may protect many plant species from extinction, and may allow the recovery of rare and threatened medicinal plants.

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