Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 2442-2445
Received: 13-07-2020
Accepted: 15-08-2020

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Quality evaluation of *Rauvolfia serpentina* by physicochemical analysis

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DOI: https://doi.org/10.22271/phyto.2020.v9.i5ah.12710

Abstract
Objective: *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. *Rauvolfia serpentina* mother tincture is having specific target action on hypertension. Thus before initiating phytochemical standardization and *in vivo* study, validation of manufacturing process and physicochemical analysis is essential.
Methods: According to HPI guidelines, two samples of *Rauvolfia serpentina* mother tincture were prepared from wild and cultivated species in GMP certified homoeopathic manufacturing unit. Further validation and comparative physicochemical analysis were done for both samples.
Results: The physicochemical parameters of *Rauvolfia serpentina* mother tincture prepared from wild and cultivated species were almost alike.
Conclusion: This analytical data will be possibly considered as quality standard for *Rauvolfia serpentina* mother tincture prepared from wild and cultivated species in further research. The process of manufacturing and physicochemical values of wild and cultivated samples are validated and established in the study.

Keywords: *Rauvolfia serpentina,* physicochemical analysis, organoleptic, microscopic, standardization, quality evaluation

1. Introduction
Herbal medicines are at great demand globally for primary healthcare due to their higher safety margins and cost effectiveness. Herbal medicines are being manufactured on large scale where manufacturers face many problems such as low-quality raw material, lack of authentication of raw material, non-availability of standards, lack of proper standardization methodologies, and lack of standardization parameters. At the same time consumers prefer to choose products with established standards. [1, 2]. *Rauvolfia serpentina* (L.) Benth. ex Kurz is well-known plant species since ancient time for therapeutic efficacy. *Rauvolfia serpentina* is used in Homeopathy, Ayurveda, Unani and siddha systems of medicine. It is officially covered by both Homoeopathic Pharmacopoeia of India and German Homoeopathic Pharmacopoeia [3, 4]. *Rauvolfia serpentina* has a potent anti-arrhythmic effect, control nerve impulses, reduce the heart rate, and dilates blood vessels thus lower blood pressure [5-8]. Now days it has been became endangered plant species in India due to climacteric changes and increased demand in drug industry. Government of India has prohibited the collection of plants growing as wild in forests and exported since 1969 [9, 10]. That's why need to search another source that is cultivated species. But it is depicted in homoeopathic literatures we have to trust on wild species for therapeutic efficacy as it contains more amount of phytoconstituents [11, 12]. So comparative study of wild and cultivated species is needed in today's era.
Characterization of physicochemical properties attained strong interest in the pharmaceutical research area and is now a standard method.Thus before initiating phytochemical standardization and *in vivo* study validation of manufacture process and physicochemical analysis of *Rauvolfia serpentina* mother tincture prepared from wild and cultivated species is essential. Hence the present study was planned to explore the changes in physicochemical parameters of prepared *Rauvolfia serpentina* mother tinctures from wild and cultivated species with respect to HPI.

2. Material and Methods
2.1. Raw drug collection: Dried roots of *Rauvolfia serpentina* from wild and cultivated were collected from Agasti Agroved Farms, A/P- Sarola Kasar, Tal/Dist - Ahmednagar. This farm is following Good Agricultural Practices [13].

2.2. Raw drug authentication: The raw drug is analyzed, identified and authenticated as '*Rauvolfia serpentina* (L.) Benth. ex Kurz.' Family – Apocynaceae by the Taxonomist in Late Prin. B. V. Bhide Foundation Pune.

2.3. Manufacture: Drug was prepared at renowned 'Medisynth Ch. Pvt. Ltd., Homoeopathic Medicines' at Turbhe, Navi- Mumbai. (GMP certified homoeopathic manufacturing unit) [15-17]. Two samples of *Rauvolfia serpentina* mother tinctures from wild and cultivated species were prepared as per standard guidelines stated in HPI. Standard operating procedures were followed throughout the preparation of both mother tincture. Preparation of *Rauvolfia serpentina* mother tincture was done by the new method of preparation of mother tincture that is percolation. To prepare one liter of mother tincture *Rauvolfia serpentina* coarse powdered roots - 100 gm., purified water – 200 ml and strong alcohol – 824 ml were used.

2.4 Organoleptic, Macroscopic and Microscopic Studies: Wild and cultivated roots as well as mother tinctures prepared from it were evaluated for organoleptic characters like color, odor, taste and texture. Macroscopic and microscopic characterization of roots and root powder were carried out according to standard methods [18].

2.5 Physicochemical Analysis: The determination of physicochemical parameters for a crude drug is helpful in setting standard data for quality control of a crude herbal drug, as these parameters are mostly constant for a medicinal plant. Therefore, these are important for the detection of drug adulteration or improper handling of raw materials [19]. The different physicochemical parameters such as mother tincture appearance, alcohol percentage, pH, and weight per ml and total solids were determined by standard procedures [20].

3. Results

Organoleptic, macroscopic, microscopic as well physicochemical parameters of the present study are compared with each other and thus interpreted as below (Table 1, Table 2, Table 3 and Table 4).

3.1 Organoleptic evaluation of roots: Comparative organoleptic evaluation of wild and cultivated roots were done by visual inspection. This evaluation provides the simplest and quickest means to establish the identity and purity and thereby ensure quality of a particular drug.

Table 1: Comparative organoleptic and macroscopic evaluation of *Rauvolfia serpentina* wild and cultivated roots

Wild roots	Cultivated roots
	
Color - yellow to brown, Odor– Slight, Taste – bitter Size - 8 to 15 cm long and 0.5 to 2 cm in diameter Shape – sub cylindrical, curved, stout, thick and rarely branched , irregular longitudinal surface Fracture - Short	Color - greyish yellow to light brown, Odor– Slight, Taste – bitter Size - 6 to 10 cm long and 1 to 2.5 cm in diameter Shape – sub cylindrical, slightly curved, stout, thick and rarely branched , irregular longitudinal surface Fracture - Short

3.2 Microscopic evaluation of roots: Comparative microscopic evaluation of *Rauvolfia serpentina* wild and cultivated roots were done. Free hand sections were taken and then stained. Sections and root powder were examined under compound microscope. They were compared and evaluated.

Table 2: Comparative microscopic evaluation of *Rauvolfia serpentina* wild and cultivated roots

Wild roots	Cultivated roots
Roots comprises of stratified cork; phelloderm perenchyamatus, some cells backed with starch grains and prismatic and cluster crystals of calcium oxalate; Secondary phloem tissue consist of sieve cells, companion cells and parenchymatous cells contains starch grains and crystals of calcium oxalate; phloem fibers absent; phloem parenchyma occasionally filled with granular substances; starch grains mostly simple but compound granules secondary xylem is traversed by well-developed lignified medullary rays.	Roots comprises of stratified cork; phelloderm perenchyamatus, some cells backed with starch grains and prismatic and cluster crystals of calcium oxalate; Secondary phloem tissue consist of sieve cells, companion cells and parenchymatous cells contains starch grains and crystals of calcium oxalate; xylem consist of vessels, wood fibers and lignified parenchyma. The vessels appear rounded, polygonal or at times radially elongated and occurs inner single or in pairs.

3.3 Organoleptic evaluation of mother tincture: Comparative organoleptic evaluation of mother tincture prepared from wild and cultivated root were done by visual inspection. This evaluation provides the simplest and quickest means to establish the identity and purity and there by ensure quality of a particular mother tincture.

Table 3: Comparative organoleptic evaluation of *Rauvolfia serpentina* mother tincture from wild and cultivated species

Test Parameter	Wild	Cultivated
Appearance	Clear liquid	Clear liquid
Color	Dark brown	Dark brown
Odor	Characteristic	Characteristic
Taste	Bitter	Bitter

3.4 Physicochemical analysis: Physicochemical parameters of mother tincture from wild and cultivated species such as mother tincture appearance, alcohol percentage, pH, and weight per ml and total solids were determined by standard procedures and compared with each other.

Table 4: Comparative physicochemical analysis of *Rauvolfia serpentina* mother tincture from wild and cultivated species

Test Parameter	Wild	Cultivated
Mother Tincture appearance	Clear Dark Brown Liquid	Clear Dark Brown Liquid
Alcohol percentage	75 % v/v	73 % v/v
pH	6.15	6.10
Weight per ml	0.876 gm. per ml	0.880 gm. per ml
Total solids	1.714	1.354

3.4.1 Alcohol percentage: It was observed that wild sample having alcohol percentage 75 % v/v and cultivated sample having 73 % v/v.

3.4.2 pH: It is the measure of hydrogen ion concentration; a measure of the acidity or alkalinity of a solution. The pH scale ranges from 0 to 14. pH less than seven are acidic, while those with pH greater than seven are basic or alkaline. It was observed that pH of wild sample 6.15 and cultivated sample 6.10. So both samples were acidic in nature.

3.4.3 Weight per milliliter: The weight per ml of a liquid is the weight expressed in gm. of 1 ml of a liquid, when weighed in air at the specified temperature. The weight per ml of mother tincture was determined by dividing weight in air expressed in grams, of the quantity of liquid in specific gravity bottle at 25 ^{O} by the capacity of specific gravity bottle at 25 ^{O} expressed in milliliter. It was observed that wild sample having 0.876 gm. per ml and cultivated sample having 0.880 gm. per ml.

3.4.4 Total solids: The term total solids applied to the residue obtained when the prescribed amount of mother tincture is dried to constant weight. It was observed that total solids in wild sample 1.714 and in cultivated sample 1.354.
It was observed that obtained values for the parameters such as mother tincture appearance, alcohol percentage, and pH, weight per ml and total solids are alike of wild and cultivated sample.

4. Discussion
Standardization of herbal medicines is greatest area of challenge. WHO involves in standardization and quality control of herbal crude drugs to monitor the physicochemical evaluation of drugs [21, 22]. Correct identification and authentication of starting raw material of herbal drugs is necessary before its use as a drug because the therapeutic efficacy of herbal drugs depends significantly on the use of unadulterated and authenticated raw materials. Herbal drugs standardization in volve entire field of study starting from cultivation of medicinal plant to its clinical application [23- 25]. The macroscopic and microscopic studies of a medicinal plant is the preliminary step toward establishing its identity and purity [26]. Physicochemical parameters serve as standard data for the quality control of the herbal preparation. By using these standards, the plant can be differentiated from other related species [27, 28].
In accordance to classical standard *Rauvolfia serpentina* mother tincture should be prepared from wild roots. However, wild roots are not available in ample quantity and in existing pharmaceutical practice *Rauvolfia serpentina* mother tinctures are prepared by cultivated source. Thus there is a need to develop and generate specific quality standard values for *Rauvolfia serpentina* mother tincture prepared from cultivated source and its comparison with parameters given in monograph of HPI.
In present study, two samples of *Rauvolfia serpentina* mother tinctures were prepared (one from wild and one from cultivated source). They were compared with organoleptic, macroscopic, microscopic and physicochemical analysis to provide standard values for individual mother tincture. Analytical values of wild samples against cultivated samples were compared with HPI parameters and discussed.

5. Conclusion
In present study the process of manufacture with analytical values of *Rauvolfia serpentina* mother tincture prepared from wild and cultivated species are validated and established in the study. To conclude, there is high possibility that difference among *Rauvolfia serpentina* mother tinctures prepared from wild and cultivated species not significantly identified on the basis of physicochemical analysis. This Analytical data will be possibly considered as quality standard for wild and cultivated sample. Further researchers can use these analytical values for wild and cultivated sample as standard values.

5.1 Scope for further study
A detail further research is needed for scientific validation and further establishment of data by using higher instrumental techniques of qualitative and quantitative standardization.

6. Acknowledgments
The authors wishes to thank Bharati Vidyapeeth (Deemed To Be University) Homoeopathic Medical College and Hospital for providing necessary infrastructure and research conveniences. The authors also express their gratitude to Prof. Dr. (Mrs.) Anita S. Patil, Post Graduate Co-ordinator Bharati Vidyapeeth (Deemed To Be University) - Homoeopathic Medical College, Pune- 411043, for their flawless support in present study. Authors are thank full to renowned 'Medisynth Ch. Pvt. Ltd., Homoeopathic Medicines'at Turbhe, Navi-Mumbai. (GMP certified homoeopathic manufacturing unit) for providing required infrastructure and facilities for manufacturing and quality evaluation of the drug.

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