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Identification of suitable substitute for *Sarpagandha* (*Rauvolfia serpentina* (L.) Benth. ex Kurz) by phytochemical and pharmacological evaluation

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Abstract

Background: *Sarpagandha* is an important Ayurveda drug used for treating many diseases including high blood pressure. *Rauvolfia serpentina* is the genuine source plant for *Sarpagandha* and it is a critically endangered species belonging to the family Apocynaceae. The present study is aimed at finding out an appropriate substitute for the endangered species *R. serpentina* by evaluating the phytochemistry and biological activities of allied species such as *Rauvolfia tetraphylla* L, *Rauvolfia hookeri* S.R.Sriniv. & Chithra, *Rauvolfia micrantha* Hook.f., and *Rauvolfia verticillata* (Lour.) Baill.

Results: The result indicated that the root of *R. serpentina* is phytochemically similar with that of *R. tetraphylla*. Chemical profiling using HPTLC showed similar chemical profiles for *R. serpentina* and *R. tetraphylla*. LC/MS characterization of various species showed that most of the active alkaloids are common for both *R. serpentina* and *R. tetraphylla*. Anti-hypertensive activity and analgesic activity were evaluated in experimental animal model. *Rauvolfia serpentina* and *R. tetraphylla* showed comparatively significant reduction in systolic and diastolic pressure. Comparable analgesic activity was also shown by *R. serpentina* and *R. tetraphylla*.

Conclusion: On the basis of phytochemical and pharmacological evaluation, it was concluded that the root of *R. tetraphylla* can be used as a validated substitute for *Sarpagandha*.

Keywords: *Rauvolfia*, HPTLC, LC/MS, Anti-hypertensive activity, Analgesic activity

1 Background

In *Ayurveda*, *Sarpagandha* is used for the treatment of high blood pressure, insomnia, asthma, acute stomach ache and painful delivery and for mental illness such as neuropsychiatric disorders, psychosis, and schizophrenia. The root of *Rauvolfia serpentina* is the genuine source drug of *Sarpagandha*. It is also used in the treatment of snake-bite, insect stings, mental disorders, gastric tumor, general weakness, goiter, hysteria, insomnia, insanity, lipoma, paraplegia, paratyphoid, piles, pneumonia,

splenomegaly, stomach disorder, tonsillitis, traumatic wound, tuberculosis, and vertigo [1].

Genus *Rauvolfia*, belonging to the family Apocynaceae, comprises more than 80 species, which are distributed in tropical climatic conditions. *Rauvolfia serpentina* is well known for the presence of indole alkaloids. Reserpine is the most prominent of these alkaloids and is useful in the treatment of hypertension, cardiovascular diseases, nervous disorders, and as a tranquilizing agent that is in great demand by modern pharmaceutical industries [2].

Rauvolfia tetraphylla popularly known as “devil pepper” is a woody shrub commonly available in India. It is native to West Indies but naturalized in South India [3].

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It is an ethnomedicinally important plant and is used against snakebite, to stimulate uterine contraction and to facilitate difficult childbirth cases. *Rauvolfia hookeri* is a large dichotomously branched shrub belonging to the family Apocynaceae. It is an endemic south Indian species containing many indole alkaloids [2]. *Rauvolfia micrantha* is an annual shrub, reaching up to 1.5 to 2 m high. Stems are sparingly branched. Leaves are simple, alternate, elliptic lanceolate, 5-10 cm × 3-4 cm, acuminate, peduncle terminal, erect, 4-6 flowered. *Rauvolfia verticillata* is an erect shrub growing up to 3 m tall and also a prolific source of heterocyclic indole alkaloids [4].

Nowadays, the herbal drug industry is getting often disrupted due to non-availability of good quality raw materials for manufacturing drugs. The acute shortage of plant raw materials is adversely affecting the quality of finished herbal products, as manufacturers are forced to buy sub standard raw material at double the prices pushing many manufacturers at the brink of shutting down of their industry. The habitat loss by export of medicinal plants collected from wild sources finally leads to severe and irreplaceable loss of genetic stock of many of these species. In India, the Ministry of Environment and Forests has banned export of 29 species including some popularly used drugs in Ayurvedic formulations like *Sarpagandha* [5].

Rauvolfia serpentina is identified as a critically endangered species [6]. Due to high demand and unavailability, the roots of *Rauvolfia serpentina* are usually adulterated with other species like *Ophiorrhiza mungos*, white-flowered and red-flowered *Clerodendrum* species and *Tabernaemontana 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 [7]. 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.

2 Methods

2.1 Chemicals and reagents

Folin-Ciocalteu reagent was procured from Sisco Research Laboratory (SRL), Mumbai, India. LC/MS grade

solvents and analytical standards such as DOCA, enalapril maleate (powder), gallic acid, quercetin, reserpine, ajmalicine, and atropine were procured from Sigma Chemicals Co. (Bangalore, India). Aspirin tablets (Cipla, India) were procured from the local pharmacy. All other chemicals employed were of standard analytical grade from Merck India.

2.2 Collection of plant materials

Various species such as *Rauvolfia serpentina*, *R. hookeri*, *R. micrantha*, *R. tetraphylla*, and *R. verticillata* were collected from different zones of south India (Table 1). The materials were authenticated and voucher specimens were added to the herbarium.

2.3 Extraction of materials

The plant materials were shade dried and pulverized. Five grams each of the sample was successively extracted with various solvents like n-hexane, chloroform, and methanol by refluxing for 6 h. Crude extracts were prepared separately with methanol, water, and hydro alcohol (ethanol: water 50:50) using the reflux extraction method. The process was repeated in triplicates. The final extracts were pooled and concentrated at 40 °C using a rotary evaporator (Heidolph, Germany) and it was made up to 100 ml with respective solvents in standard flasks. The extracts were kept under refrigerator until the various phytochemical analyses were over.

Two hundred fifty grams of various samples were separately extracted for pharmacological studies with water using the soxhlet extraction method for 72 h. The final extracts were concentrated to dryness under reduced pressure using a rotary evaporator (Heidolph, Germany). The sample details are presented in Table 2.

2.4 Estimation of total alkaloids

Total alkaloid was estimated spectrophotometrically based on the reaction of alkaloids with bromocresol green [8]. The method consisted of extraction of alkaloid by acid treatment of the extracts followed by basification and extraction with chloroform. Different concentrations of atropine were used to plot the calibration curve. The absorbance of the complex in chloroform was measured at 470 nm against blank using a spectrophotometer.

2.5 Estimation of total poly phenolics

Polyphenols such as phenolics and flavonoids were estimated spectrophotometrically. The total phenolic content (TPC) was determined using Folin-Ciocalteu reagent [9, 10]. TPC was expressed as gallic acid equivalents (GAE) in mg/g of sample. Total flavonoid content (TFC) was measured by aluminum chloride colorimetric assay [11] and expressed as milligram quercetin equivalents (mg EQ).

Table 1 Details of plant collections

Sl. no.	Species name	Locality	Voucher specimen no.
1	<i>Rauvolfia serpentina</i> (L.) Benth. ex Kurz	Kasargode, Kerala Kozhikode, Kerala Thrissur, Kerala	CMPR 9997 CMPR 8922 CMPR 8909
2	<i>Rauvolfia hookeri</i> Sriniv. & Chithra	Breymore, Agasthyamalai Biosphere Reserve, Trivandrum, Kerala	CMPR 9671
3	<i>Rauvolfia micrantha</i> Hook. f.	Wagamon, Idukki, Kerala	CMPR 9548
4	<i>Rauvolfia tetraphylla</i> L.	Thenhipalam, Malappuram, Kerala	CMPR 9395
5	<i>Rauvolfia verticillata</i> (Lour.) Baill.	Wagamon, Idukki, Kerala	CMPR 9549 CMPR 9593

2.6 High-performance thin layer chromatographic (HPTLC) analysis

HPTLC analysis was performed using CAMAG HPTLC system (Switzerland). Samples were applied using CAMAG ATS 4 on aluminum-backed pre-coated silica gel 60F₂₅₄ HPTLC plate (Merck India). Mobile phase was standardized as toluene, ethyl acetate, and methanol in the ratio of 7:3:1. The chromatogram was developed in a saturated Twin Trough chromatographic chamber (Camag, Switzerland). The developed plate was visualized under UV at 254 nm and 366 nm and in visible light after derivetizing with anisaldehyde-sulfuric acid reagent followed by heating at 105 °C for 5 min.

2.7 Characterization of alkaloids

Five grams each of dried root of five species was extracted with ethyl alcohol (100 ml) by cold percolation up to 72 h with frequent stirring. It was then concentrated to dryness under reduced pressure in a rotary evaporator (Heidolph, Germany) and treated with dilute HCl. The acid layer was washed with CHCl₃ three times (10 × 3). Basified the extract with ammonia and extracted with CHCl₃ (10 ml). The final alkaloid fraction was re-dissolved in HPLC grade chloroform. The preliminary analysis was carried out by HPTLC profiling. Characterization was done by LC/MS-MS analysis.

2.8 LC/MS characterization of alkaloid fractions

LC-ESI/MS analysis was conducted on Agilent 6520 accurate mass Q-TOF-LC/MS coupled with Agilent LC 1200 equipped with Extend-C18 column of 1.8 μm, 2.1 × 50 mm. Isocratic elution was performed with ACN with 0.1% ammonium formate at a constant flow rate of 0.8

ml/ min. The MS analysis was performed using ESI in the positive mode. The conditions for mass spectrometry were drying gas (nitrogen) flow 5 L/min; nebulizer pressure 40 psig; drying gas temperature 325 °C; capillary voltage +3000 V; fragmentor volt 125 V; Oct RF Vpp 750 V. The mass fragmentation was performed with varying collision energy 4 V/100 DA with an offset of 8 V.

2.9 Evaluation of anti-hypertensive activity

DOCA-salt-induced hypertension model was used for the evaluation of anti-hypertensive activity [12]. Wistar albino rats ($n = 6$) of weighing 220-250 g were obtained from animal house of Karpagam University, Tamil Nadu, India, and used for the study. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 20 °C and relative humidity of 30-70%. A light and dark cycle was followed. All animals were fed on a standard balance diet and provided with water ad libitum. The study protocol was approved by the Institutional Animal Ethics Committee (Approval No: KAHE/IAEC/2018/21-04/002). All institutional and national guidelines for the care and use of laboratory animals were followed.

All animals were maintained for 2 weeks and BP was measured. The systolic and diastolic blood pressure was determined by the tail-cuff method (BP-2000-MR-2, blood pressure analysis system for mice and rats). The animals were placed in the chamber at an ambient temperature of 30–34 °C for 15 min, and from each animal, blood pressure values were recorded. At the end of the experiment, animals were not sacrificed and were shifted to separate cages.

Animals were given, weekly twice, subcutaneous injections of DOCA (20 mg/kg BW sc) in olive oil and salt were administered by substitution of 1% NaCl solution for drinking water ad libitum throughout the experimental period for 4 weeks then BP was measured. Deoxycorticosterone acetate (DOCA) is one of the most common high-salt diet used for anti-hypertensive experimental model.

Table 2 Sample design

Sl. no	Sample code	Sample details
1	RSR	<i>R. serpentina</i> root
2	RHR	<i>R. hookeri</i> root
3	RMR	<i>R. micrantha</i> root
4	RTR	<i>R. tetraphylla</i> root
5	RVR	<i>R. verticillata</i> root

The rats were randomly divided into nine groups of six rats each. Group one served as normal control and group II treated with DOCA-salt and served as hypertension control. Group III treated with enalapril (48 mg/kg, oral), group IV to group IX were hypertensive rats which received different extracts at a dose of 150 mg/kg BW for 5 weeks (Table 3). The extracts were prepared from a plant material having a high safety margin and the dose was fixed based on the previous methods [13].

2.10 Evaluation of analgesic activity

2.10.1 Formalin test

The Wistar albino rats weighing between 150 to 180 g were obtained from animal house of Karpagam University, Tamil Nadu, India, and were used for the study. The study protocol was approved by the Institutional Animal Ethics Committee (Approval No: KAHE/IAEC/2018/21-04/001). All institutional and national guidelines for the care and use of laboratory animals were followed. Fifty microliter of a 5% formalin solution was injected subcutaneously into the dorsal surface of the right or left hind paw of the rat using a 29-gage needle (U-100 Insulin Syringe, Beckton and Dickinson, Dublin, Ireland). The 5% formalin solution was prepared by dilution with normal saline of the commercial 10% formalin solution. The total number of flinches of the hind paw and/or the hind quarters was recorded by visual observation for 10 min periods for total observation duration of 2 h following injection of formalin. In addition, licking/biting of the injected paw was recorded using a digital time-out stopwatch as total licking time (s) per 10 min observation period for a total duration of 2 h following injection of formalin. Control rats, injected subcutaneously in the dorsal surface of the right hind paw with 50 μ l normal saline, did not show these typical nociceptive behaviors [14].

2.10.2 Hot plate method

The Wistar albino rats weighing between 150 to 180 g were used for the study. The analgesic activity was examined using the hot plate method. Animals were

divided into VIII groups, each group containing six animals each. Group I served as the positive control with no protection. Group II animals received the standard drug of aspirin 150 mg/kg body weight, whereas group III to VIII animals were orally administered the various plant extracts at the dose of 150 mg/kg body weight respectively. The temperature of the hot plate was maintained $55 \pm 1^\circ\text{C}$, rats were placed on the hot plate, and time in seconds for paw licking or jumping was recorded as basal reaction time. Cutoff time in the absence of a response was 15 s to prevent the animals from being burnt. The reaction time in seconds (latency period) was observed on a hot plate, the time taken for the rat to react to the thermal pain by licking its paw or attempting to jump out. Observations were made after 60 min, the animals are placed on the hot plate and the observations were recorded, and at the time interval of 90 and 120 min. After the experiment, animals were not sacrificed and were shifted to separate cages.

2.11 Data analysis

For determination of significant intergroup differences of each parameter one-way analysis of variance (ANOVA) was carried out. Dunnet's test was used for individual comparisons after significant ANOVA results. The differences with $p < 0.05$ was considered statistically significant. The Graphpad Prism-6 software (Graphpad software, Inc., USA) was used for the statistical analysis.

3 Results

3.1 Phytochemical characterization

Various extracts of selected species were subjected to different phytochemical analyses including estimation of important class of compounds like alkaloids, phenolics, and flavonoids. Chemical profiling of different extracts such as n-hexane, chloroform, methanol, hydro alcohol, and water were done using HPTLC analysis. Metabolite profiling of selected species was carried out by LC-MS/MS analysis.

Rauvolfia is well known for its alkaloid content [4] and the same was estimated based on the reaction with bromocresol green (BCG). The highest alkaloid content was showed by *R. tetraphylla* (0.90%), followed by *R. serpentina* (0.48 %). *Rauvolfia verticillata* showed the least alkaloid content (0.22%). The alkaloid content of *R. serpentina* is in agreement with the previous report [15]. When compared to allied species, the quantity of alkaloids in *R. tetraphylla* is much higher than that of the genuine drug (Table 4).

Total polyphenolic content of various *Rauvolfia* species was estimated spectrophotometrically. Total phenolic content (TPC) and total flavonoid content (TFC) were calculated from the calibration curve of gallic acid ($R^2 = 0.994$) and quercetin ($R^2 = 0.995$) respectively (Table 4).

Table 3 Experimental design

Group	Treatment
Group I control	Normal
Group II BP group	DOCA treated (20 mg/kg BW)
Group III (standard)	DOCA treated +Enalapril inj. (48 mg/kg, oral)
Group IV (RSR)	DOCA treated +150 mg/kg of RSR
Group V (RHR)	DOCA treated +150 mg/kg of RHR
Group VI (RMR)	DOCA treated +150 mg/kg of RMR
Group VII (RTR)	DOCA treated +150 mg/kg of RTR
Group VIII (RVR)	DOCA treated +150 mg/kg of RVR

Table 4 Estimation of major phytochemicals in various *Rauvolfia* species

Sl. no	Sample	Total alkaloids	Total phenolics	Total flavonoids
1	<i>R. serpentina</i>	0.48	5.20	1.80
2	<i>R. hookeri</i>	0.33	5.67	5.50
3	<i>R. micrantha</i>	0.36	7.57	6.70
4	<i>R. tetraphylla</i>	0.90	8.10	6.10
5	<i>R. verticillata</i>	0.22	12.33	10.30

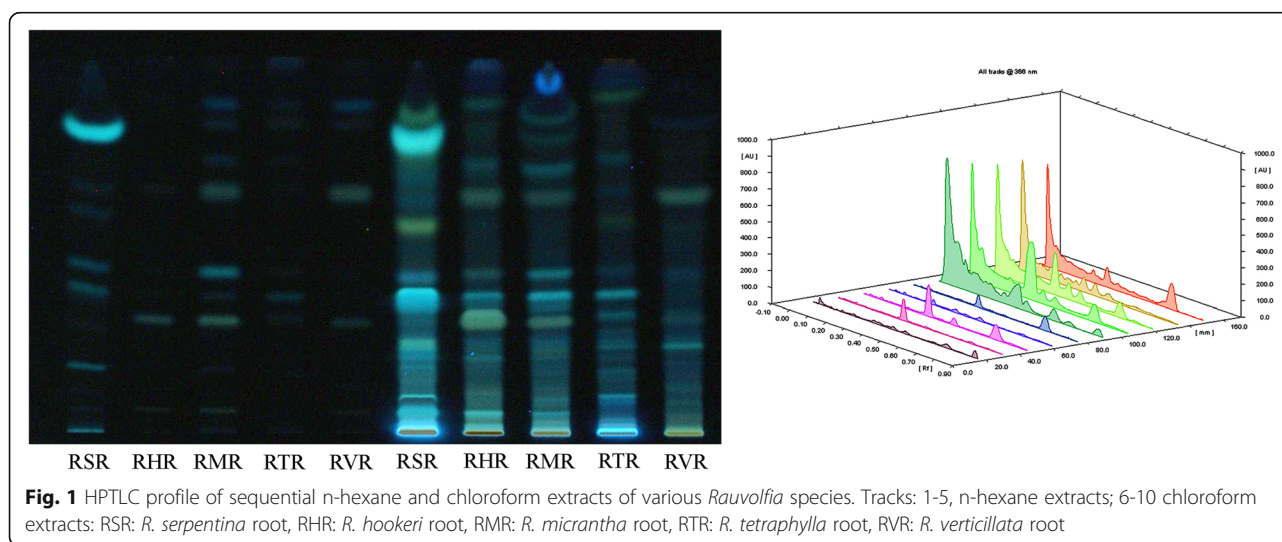
The highest phenolic content was observed for *R. verticillata* (12.33 mg Eq Ga) followed by *R. tetraphylla* (8.10 mg Eq Ga). The phenolic content of *R. serpentina* is found to be 5.20 mg equivalence of gallic acid. In the case of total flavonoids, *R. verticillata* showed the highest value (10.30 mg Eq QE) followed by *R. tetraphylla*. The chemical contents of *R. tetraphylla* are much higher than that of *R. serpentina*. The estimation of total polyphenolics of *Rauvolfia* species revealed significant diversity among the species. Variation of phenolic contents among the species was previously reported for many other species also [16, 17].

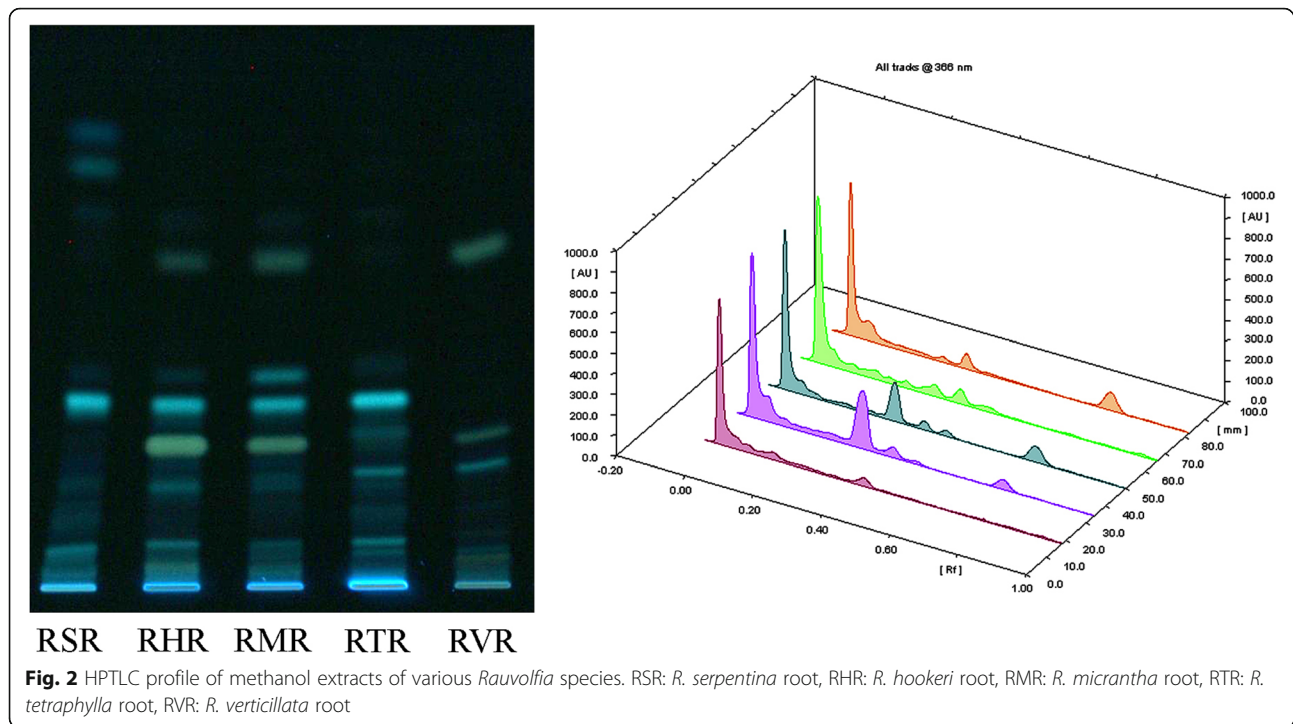
Chromatographic fingerprint analysis is found to be a cogent and realistic approach for the quality assessment and species authentication of raw drugs. It utilizes chromatographic techniques to construct specific patterns of recognition for medicinal plants. Comparative HPTLC profiles were developed for the root of various species (Fig. 1). At 254 nm, n-hexane extract showed a few bands which indicate that low polar compounds with chemical quenching is very less in *Rauvolfia* species. A common band with R_f 0.60 was observed in *R. serpentina*, *R. micrantha*, and *R. verticillata*. Compound at R_f 0.29 is common for *R. hookeri* and *R. micrantha*. At 366 nm, major bands were observed at 0.17, 0.55, and 0.75

for *R. serpentina*. Two fluorescent bands at 0.06 (blue) and 0.62 (yellow) were observed for *R. hookeri*, *R. micrantha*, and *R. verticillata*. A fluorescent band at 0.29 was found to be absent in *R. serpentina* only. Two bands at 0.77 and 0.82 were shown by *R. micrantha*, *R. tetraphylla*, and *R. verticillata*.

HPTLC profile of chloroform extracts is almost similar in their banding pattern and it is the indication of presence of similar phytoconstituents in all the selected species. Major bands were observed at R_f 0.06, 0.35, 0.41 as common bands in all the selected species. Band at R_f 0.29 is common for four species other than *R. serpentina*. A compound at R_f 0.15 was found to be absent in *R. verticillata*. A band at 0.61 is absent only for *R. tetraphylla*. On evaluating under 366 nm, a common band at 0.39 with blue fluorescence was observed for all the selected species. Compound with R_f 0.34 is absent only in *R. verticillata*. Two bands with blue fluorescence at 0.10 and 0.17 were specifically found in *R. tetraphylla*. A blue fluorescence at 0.23 was found as the common band for *R. serpentina*, *R. tetraphylla*, and *R. verticillata*. Two specific bands at 0.29 (yellow fluorescent) and 0.59 (blue fluorescence) were observed for *R. hookeri*, *R. micrantha*, and *R. verticillata*. At 0.52, a band with yellow fluorescence was shown by *R. serpentina* and *R. tetraphylla*. An intense fluorescent band at 0.75 was observed in *R. serpentina* which was not observed in any other species.

Chemical profiling of crude extracts was also carried out with methanol, water, and hydro alcohol (Figs. 2, 3, and 4). HPTLC profiles of crude extracts also showed common compounds with matching R_f values. Hydro alcoholic extracts showed bands with comparatively high resolution among the three crude extracts; this may be due to the optimum polarity of extraction medium with an aqueous and alcoholic combination. All the crude

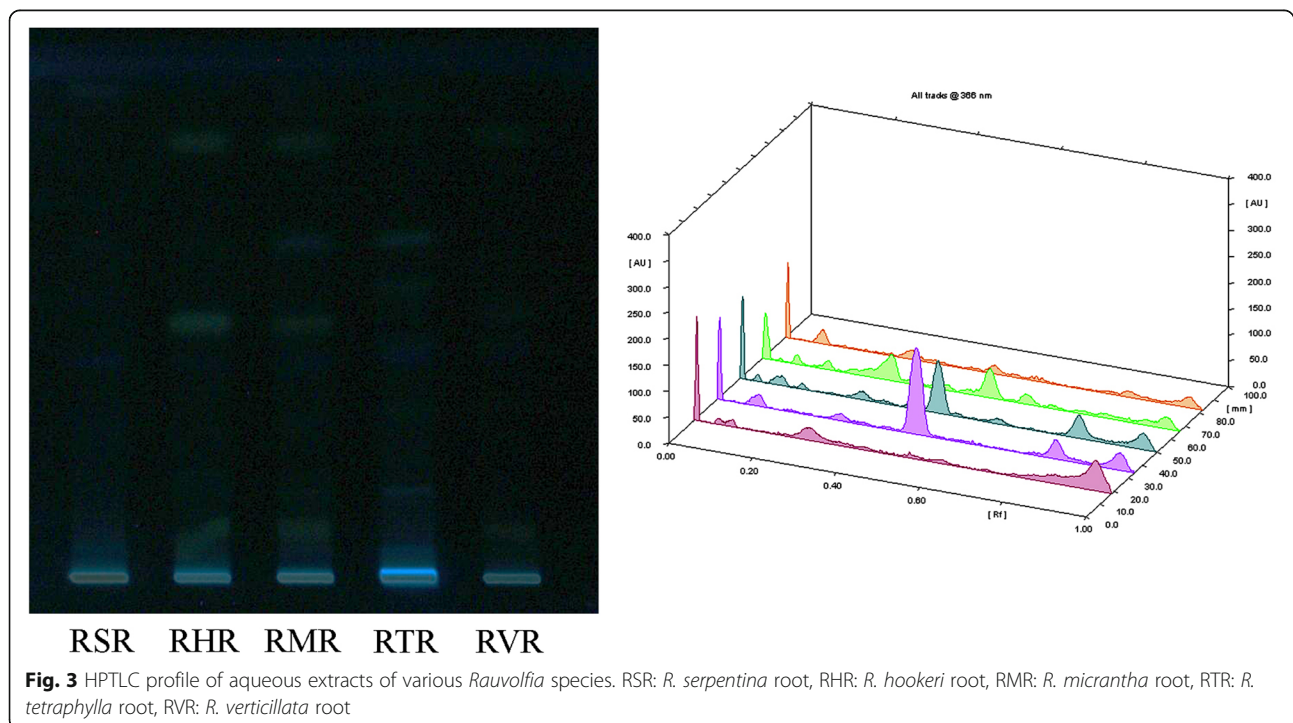


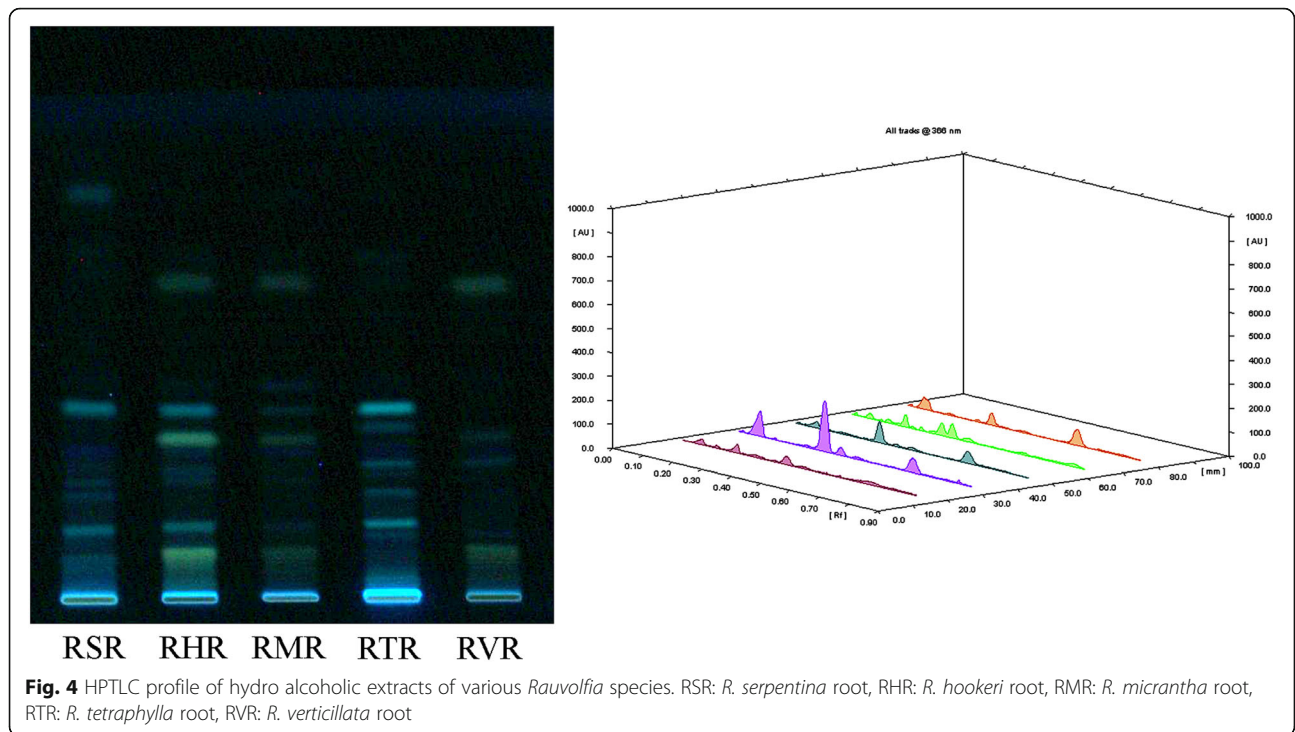


extracts also showed common bands at 254 nm and 366 nm. Scanned spectrum of different tracks confirmed the similarities of separated bands.

The developed fingerprint pattern of genuine and allied species can be used to determine not only the absence or presence of markers of interest but the ratio of

all detectable phytoconstituents as well. HPTLC profiling with spectral scanning at UV and visible range confirm the consistency of the similar R_f values of compounds separated. Although most of the selected species presented comparable chemical profiles, *R. tetraphylla* showed maximum similarity with *R. serpentina*.



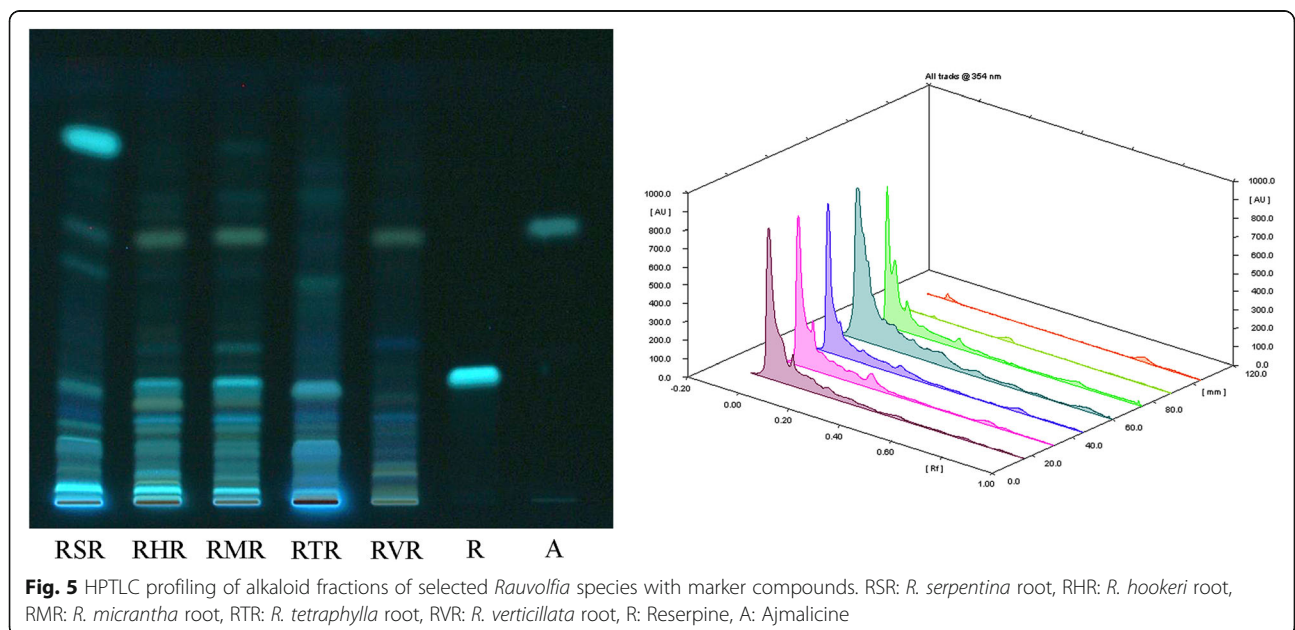


Alkaloid fractions were screened separately by HPTLC with reserpine and ajmalicine as marker compounds. The chromatogram was documented and densitometry scanning was done at 254 and 366 nm (Fig. 5).

The evaluation of chromatographic profiles showed that most of the alkaloids are present in all the selected species. The species-specific indole alkaloids, reserpine, and ajmalicine are also detected in various species. Ajmalicine is present in all the species; however, the

quantity of the same in *R. tetraphylla* is very less. Reserpine is found to be absent in *R. verticillata*.

The alkaloid fractions were subjected to LC/MS analysis for the detailed characterization. LC/MS analysis was done in ESI positive mode in order to obtain maximum ionization for alkaloids. Total ion chromatogram (TIC) obtained for MS analysis was extracted to base peak chromatogram (BPC) and integrated to record all the molecular ion peaks. The protonated molecular ions



with different [M + H] values were evaluated and further characterization was carried out by MS/MS analysis. On MS/MS analysis, molecular ions were fragmented on collision induced dissociation (CID). It was noticed that most of the alkaloids were fragmented with a specific fragmentation pattern upon CID. The tentative structure was assigned based on MS/MS fragments obtained for each [M + H] values (Table 5). The structure of alkaloids was identified by comparing the fragmentation pattern with that of previously reported ones [18, 19].

LC/MS analysis showed that most of the separated alkaloids are common for all the selected species. However, reserpine was not detected in *R. verticillata*. HPTLC and LC/MS analysis of alkaloid fractions confirmed that alkaloid profiles of most of the selected species are the same.

3.2 Pharmacological evaluation

3.2.1 Anti-hypertensive activity

Anti-hypertensive and analgesic activities of selected *Rauvolfia* species were studied in experimental animals. The anti-hypertensive activity of selected *Rauvolfia* species is presented in Table 6. A significant increase in both systolic and diastolic blood pressure was observed ($130 \pm 1.52/207 \pm 1$) in DOCA-induced rats as compared to the normal group ($p < 0.05$). The standard drug enalapril reduced the diastolic and systolic pressure into 68 ± 1 and 126 ± 1.52 respectively. Root extract of *R. serpentina* administration attenuated the blood pressure induced by DOCA-salt significantly ($p < 0.05$). Root extract of *R. tetraphylla* significantly decreased both systolic and diastolic blood pressure ($92 \pm 1/121 \pm 1$).

All the extracts showed reduction in systolic and diastolic pressure during the treatment. Root of *R. serpentina*, root extracts of *R. hookeri*, and *R. tetraphylla* reduced the diastolic pressure significantly which is comparable with that of standard drug.

3.2.2 Analgesic activity

Analgesic activity was screened by the formalin test and hot plate method. The treatment of mice with various extracts resulted in the inhibition of the formalin-induced inflammatory pain. The result showed that the standard drug reduced the paw volume immediately within 10 min whereas *R. tetraphylla* and *R. serpentina* (GP IV and GP V) were able to reduce the paw volume within a period of 20 min. This is followed by *R. hookeri* (GP III) and *R. micrantha* (GP VI). Paw reduction by *R. verticillata* (GP VII) is good when comparing to other drugs but it took 50 min and 60 min respectively to reduce the paw volume (Table 7). Root extract of *R. tetraphylla* significantly reduced the paw volume after 20 min which showed comparable analgesic activity with that of the genuine drug of *Sarpagandha*.

In hot plate method, rats treated with standard drug was able to tolerate the heat for a longer time. Almost similar result was obtained with *R. micrantha* (GP VI) followed by *R. serpentina* (GP V). All these groups work well at 60 min of treatment. *Rauvolfia hookeri* and *R. tetraphylla* showed better results respectively at 90 min (Table 8).

4 Discussion

In Ayurveda, the concept of substitution had explained when there is unavailability of any particular drug. The substitutes are of great economic importance in the herbal drug industry. The systematic identification and evaluation of substitutes for rare and endangered drugs by phytochemical and pharmacological studies is an important research area to ensure the quality of herbal products in the market.

In this study, detailed scientific investigations were carried out to find out a scientifically validated substitute for an important Ayurvedic drug which has been listed as endangered. The concept of substitution is usually

Table 5 LC/MS-MS analysis of alkaloid fractions of various *Rauvolfia* species

Sl. no.	Compound	Molecular formula	m/z [M + H]	MS/MS	Present in
1	Reserpine	C ₃₃ H ₄₀ N ₂ O ₉	609.2913	577.23, 436.16, 174.20	RSR, RHR, RMR, RTR
2	Yohimbic acid	C ₂₀ H ₂₄ N ₂ O ₃	341.1849	323.17, 192.10, 144.07	RSR, RHR, RMR, RTR, RVR
3	Reserpine acid methyl ester	C ₂₃ H ₃₀ N ₂ O ₅	415.2049	397.17, 383.15, 367.15	RSR, RHR, RMR, RTR, RVR
4	Methyl-14-hydroxy 19 methyl 16, 17 dihydro 18 oxayohimbin-16 carboxylate	C ₂₁ H ₂₄ N ₂ O ₄	369.1802	351.16, 158.06, 144.08	RSR, RHR, RMR, RTR, RVR
5	Reserpine acid	C ₂₂ H ₂₈ N ₂ O ₅	401.2022	327.20, 240.12, 221.07, 199.08, 174.09	RSR, RHR, RMR, RTR, RVR
6	Ajmalicine	C ₂₁ H ₂₄ N ₂ O ₃	353.1832	335.17, 222.12, 144.08, 158.09	RSR, RHR, RMR, RTR, RVR
7	Serpedine	C ₂₃ H ₃₀ N ₂ O ₅	415.0201	383.18, 224.13, 173.08	RSR, RHR, RMR, RTR, RVR
8	18-hydroxy yohimbine	C ₂₁ H ₂₆ N ₂ O ₄	371.1952	339.17, 240.12, 144.08	RSR, RHR, RMR, RTR, RVR
9	Yohimbine	C ₂₁ H ₂₆ N ₂ O ₃	355.2005	323.16, 337.19, 158.08, 144.08	RSR, RHR, RMR, RTR, RVR
10	Rescicine	C ₃₄ H ₄₀ N ₂ O ₉	621.0249	460.2, 383.4, 174.0	RSR, RHR, RMR, RTR, RVR
11	Deserpidine	C ₃₂ H ₂₃₈ N ₂ O ₈	579.1808	367.2, 448.1, 195.4	RSR, RHR, RMR, RTR, RVR

Table 6 Anti-hypertensive activity of various *Rauvolfia* species

Groups	Before induced with DOCA salt		After induced with DOCA salt		After treatment with plant extract	
	Diastolic	Systolic	Diastolic	Systolic	Diastolic	Systolic
Group I	57 ± 1	110 ± 1	62 ± 1	114 ± 1	65 ± 1	110 ± 1
Group II	61 ± 2 [#]	112 ± 1 [#]	113 ± 1.52 [#]	177 ± 1 [#]	130 ± 1.52 [#]	207 ± 1 [#]
Group III (enalapril)	70 ± 1	117 ± 1	106 ± 1	167 ± 1	68 ± 1*	126 ± 1.52*
Group IV (RSR)	63 ± 0.7	123 ± 0.3	93 ± 0.7*	163 ± 0.3*	81 ± 0.4*	157 ± 0.9
Group V (RHR)	67 ± 1	112 ± 2	101 ± 1	151 ± 2	78 ± 1	107 ± 1
Group VI (RMR)	59 ± 2	113 ± 0.7	102 ± 0.2	173 ± 0.7	97 ± 0.3	151 ± 0.23
Group VII (RTR)	66 ± 1	115 ± 1.5	103 ± 1*	161 ± 1.5*	92 ± 1*	121 ± 1*
Group VIII (RVR)	65 ± 1	110 ± 1	97 ± 1	160 ± 1	89 ± 0.3*	112 ± 0.43*

Values are mean ± SD, n = 6

[#]p < 0.05 when compared to GPI normal

*p < 0.05 when compared to GP-II control by ANOVA followed by Dunnett's post-test

favored when the genuine drugs are not available or available in inadequate quantity. *Rauvolfia serpentina* was assessed as a critically endangered species in 2001. Encouraging herbal industries contingent on this species to support the development of sustainable utilization is an urgent need in the present scenario. Identification of a scientifically validated substitute for this species may help the industry to ensure the therapeutic efficacy and quality of the finished products. No such studies are reported in this species. A few reports are available regarding the chemical comparison of the selected species [20].

Comparative chemical profiling of different *Rauvolfia* species using tandem mass spectroscopic analysis has been reported earlier [18] and identification of alkaloids using mass spectroscopic characterization is in agreement with the present study. No comparative pharmacological studies have been reported in these species.

HPTLC and LC/MS analysis showed that allied species are rich in indole alkaloids and most of these alkaloids are found to be common for all the species. The same is in agreement with previous reports [20, 21]. Tandem mass spectroscopic analysis led to the identification of several alkaloids present in the selected species. The result showed that the root of *R. tetraphylla* is more similar in their chemical pattern with that of *R. serpentina*. Most of the active constituents including indole alkaloids such as reserpine, ajmalicine, yohimbine, and reserpine acid are also found in *R. tetraphylla*. *Rauvolfia verticillata* showed perceptible difference due to the absence of active compound reserpine.

Pharmacological evaluation such as anti-hypertensive activity and analgesic activity also supported the results of phytochemical analysis. Hypertension is a multifaceted disease that is involved in around 40% of cardiovascular mortalities and is the result of both genetic and

Table 7 analgesic activity of various *Rauvolfia* species by formalin test

	Before treatment						After treatment					
	10 min	20 min	30 min	40 min	50 min	60 min	10 min	20 min	30 min	40 min	50 min	60 min
GP I (control)	15.0 ± 1.4	12.1 ± 1.7	5.8 ± 1.1	14.1 ± 1.7	12.0 ± 1.7	19.3 ± 2.5 [#]	10.3 ± 2.7	3.1 ± 1.9	5.5 ± 1.8	2.5 ± 1.6	5.6 ± 1.7	10.6 ± 3.7
GP II (Standard)	1.5 ± 0.5	2.5 ± 1.5	2.6 ± 1.5	2.3 ± 0.8	2.0 ± 1.2	3.1 ± 1.7	1.3 ± 1.2	3.3 ± 1.2	3.0 ± 1.4	2.1 ± 1.1*	2.6 ± 1.7*	2.1 ± 1.4*
GP III (RHR)	10.6 ± 1.7	15.6 ± 3.0	13.1 ± 3.1	15.1 ± 3.4	10.6 ± 1.7	11.0 ± 2.3	14.0 ± 2.2	5.3 ± 2.1	4.8 ± 2.0	16.3 ± 3.6	12.0 ± 2.6	3.1 ± 1.1*
GP IV (RTR)	12.6 ± 1.7	16.6 ± 4.0	11.3 ± 2.0	10.6 ± 2.1	11.5 ± 1.2	18.6 ± 1.8	12.3 ± 1.7	1.8 ± 1.1*	8.1 ± 2.7	10.5 ± 2.8	8.8 ± 2.4	3.5 ± 1.0*
GP V (RSR)	14.5 ± 3.0	14.8 ± 1.9	9.6 ± 1.6	10.8 ± 1.6	16.1 ± 4.0	10.0 ± 2.2	8.0 ± 2.0	6.0 ± 1.2	8.1 ± 1.1	6.1 ± 2.4*	12.0 ± 1.7	8.8 ± 1.8
GP VI (RMR)	12.5 ± 1.8	13.3 ± 2.4	7.6 ± 1.6	7.6 ± 2.2	7.8 ± 0.7	7.5 ± 1.8	8.3 ± 1.2	5.8 ± 2.1	3.0 ± 1.7*	1.6 ± 1.0*	1.8 ± 1.1*	1.8 ± 0.7*
GP VII (RVR)	7.5 ± 1.5	7.1 ± 1.4	8.1 ± 2.4	6.5 ± 1.5	7.5 ± 1.0	5.3 ± 1.5	2.5 ± 1.8	2.8 ± 0.7*	2.1 ± 0.7*	2.1 ± 1.7*	1.3 ± 0.8*	1.1 ± 1.1*

Values are mean ± SD, n = 6

[#]p < 0.05 when compared to GPI normal

*p < 0.05 when compared to GP-II control by ANOVA followed by Dunnett's post-test

Table 8 Analgesic activity of various *Rauvolfia* species by hot plate method

	Reaction time in seconds at time (min)			
	0 min	60 min	90 min	120 min
GP I (control)	1.17 ± 0.05	1.22 ± 0.12 [#]	1.28 ± 0.16 [#]	1.38 ± 0.19 [#]
GP II (standard)	1.46 ± 0.09	7.18 ± 0.31	6.62 ± 0.35	6.57 ± 0.62
GP III (RHR)	1.20 ± 0.07	5.61 ± 0.36	5.77 ± 0.34	5.38 ± 0.30
GP IV (RTR)	1.25 ± 0.08	5.38 ± 0.50	5.56 ± 0.42	5.35 ± 0.29
GP V (RSR)	1.29 ± 0.08	4.78 ± 0.32	4.88 ± 0.53*	4.64 ± 0.42*
GP VI (RMR)	1.30 ± 0.06	6.91 ± 0.32	6.88 ± 0.30	6.70 ± 0.34
GPVII (RVR)	1.45 ± 0.08	6.33 ± 0.33	6.28 ± 0.32	5.99 ± 0.40*

Values are mean ± SD, n = 6

[#]p < 0.05 when compared to GPI normal

*p < 0.05 when compared to GP-II control by ANOVA followed by Dunnett's post-test

environmental factors [22]. In *Ayurveda*, *Sarpagandha* has been used for the treatment of high blood pressure for centuries. In the present study, anti-hypertensive activity of allied spices of *R. serpentina* was evaluated to identify a validated substitute for the same. Among the tested species, *R. tetraphylla* showed significant anti-hypertensive activity comparing to the genuine source of *Sarpagandha* and standard drug.

Inflammation and pain are common nonspecific manifestations of many diseases. In traditional medicines, several medicinal plants have been used to treat pain and inflammation [23]. Hot plate and formalin test are considered to be a reliable animal model for the evaluation of analgesic activity. The selected species were screened for their analgesic activity, and the result showed that most of the species having significant analgesic activity. *Rauvolfia tetraphylla* showed good analgesic activity when compared to *R. serpentina*. Analgesic activity also supported the possibility of using *R. tetraphylla* as substitute for the root of *R. serpentina*.

5 Conclusion

The study demonstrated that the genuine source drug of *Sarpagandha* is chemically and biologically similar with its allied species *Rauvolfia tetraphylla*. The active chemical components, specifically, indole alkaloids, are found to be common for both of these species. Pharmacological evaluations such as anti-hypertensive activity and analgesic activity also supported the phytochemical studies. In conclusion, these studies collectively recommend the possibility of using *Rauvolfia tetraphylla* as a scientifically validated substitute for *Sarpagandha*.

Abbreviations

BCG: Bromocresol green; DOCA: Deoxycorticosterone acetate; HPTLC: High-performance thin-layer chromatography; LC-MS/MS: Liquid chromatography-mass spectroscopy

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Authors' contributions

SCT: Designed the study carried out LC/MS analysis, pharmacological evaluation and drafted the manuscript. JCK: Carried out the phytochemical analysis. JKU: Participated in the phytochemical analysis. PKM: Collected the plant materials. IB: Participated in study designing and edited the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The experimental procedures relating to the animals were authorized by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Approval No: KAHE/IAEC/2018/21-04/002).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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