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Potential of Monoterpene Indole Alkaloids Targeting Breast Cancer

Identifying substitution and adulteration of medicinal plants

National Institute of Pharmaceutical Education and Research (NIPER)
S.A.S. Nagar

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The cover page contains a figure on Ayurinformatics

EDITORIAL

Comprising 25% of global women's cancers, breast cancer is predominantly hormone receptor-positive (HR+). Standard endocrine therapy (ET) targeting the estrogen receptor reduces mortality by 40%, but resistance emerges with ER mutations in 36% of metastatic cases. In addressing this challenge, in silico methodologies, including computer-aided drug design (CADD) employing techniques such as pharmacophore modeling and molecular dynamics simulations, play a crucial role. An article in the current issue investigated monoterpene indole alkaloids of Alstonia scholaris, employing in silico and in vitro screening against MCF-7 cell lines, introducing novel molecules with unique nuclei as potential therapeutic candidates. This integrated approach, blending computational science with experimental techniques, provides efficient and cost-effective solutions for advancing breast cancer therapeutics.

The adulteration of expensive raw drugs with inferior taxa has become a routine practice, compromising the quality and safety of derived herbal products. The global upsurge in herbal medicine interest, driven by perceived safety and cultural acceptance, presents challenges, notably in developing nations. The herbal industry confronts controversies, particularly regarding certain plants use, necessitating urgent attention to identification, standardization, and quality assurance in Ayurvedic medicines. Authentication techniques, including morpho-anatomical examination, DNA barcoding, and spectroscopic methods like IR and Raman combined with chemometrics, prove crucial for detecting adulteration, offering simplicity and cost-effectiveness in ensuring herbal product integrity. The current issue underscores the identification of adulterants and substitutes in five vital plants, as documented in key Ayurvedic texts.

These articles collectively provide valuable insights, solutions, and a profound understanding of complex challenges. Once again, I wish that this year catalyzes transformative discoveries and advancements in the pharmaceutical science field, propelling us toward a future enriched with impactful contributions to pharmaceutical research.

Dr Chandraiah Godugu

Research Article

Current Research & Information on Pharmaceutical Science (CRIPS)
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Exploring the Potential of Monoterpene Indole Alkaloids from *Alstonia scholaris* for Targeting Breast Cancer through *InSilico* and *InVitro* studies

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Abstract

Chemo-resistance is a widespread issue in cancer treatment, and estrogen receptor 1 (ESR1) mutations often lead to resistance in breast cancer. Additionally, insulin like growth factor (IGF) pathway can be linked to the resistance of chemotherapy in breast cancer. So, multi-receptor-targeted molecules inhibiting ESR1 and IGF-1 could be a potential strategy to combat the drug resistance. *Alstonia scholaris,* a medicinal plant, possesses a reservoir of monoterpene indole alkaloids (MIAs) distinguished by unique ring structures and known for their cytotoxic properties. The methanolic and butanolic leaf extracts of *A. scholaris* were cytotoxic when tested against breast cancer cell line. The structures of 225 phytochemicals isolated from *A. scholaris* were docked independently against ESR1 and IGF-1 receptor and the molecules showing promising docking score were further scrutinized *insilico* for their drug likeliness and toxicity. The findings suggested that a majority of the MIAs exhibited the potential to bind to both receptors. Specifically, alstoniascholarine P and scholaricine were chosen for molecular dynamic (MD) simulations based on their scores. The MD simulations verified the stability of target ligand complexes. Scholaricine have shown moderate *invitro* cytotoxicity when tested against breast cancer cell line which warrants further investigations. In summary, this study suggests MIAs derived from *A. scholaris* exhibited drug-like characteristics, indicating potential for further exploration in the search for dual inhibitors targeting estrogen and IGF receptors.

Keywords: *Alstonia scholaris*, Scholaricine, Alstoniascholarine P, *Insilico*, ESR1 and IGF-1

Introduction

Resistance to chemotherapy poses a significant challenge in the care of cancer patients, with cancer cells developing resilience against the drugs employed in treatment, thereby diminishing the effectiveness of chemotherapeutic agents.[1] The insulin-like growth factor (IGF) pathway plays a key role in chemotherapy resistance, with IGF-1 reducing the effectiveness of doxorubicin and paclitaxel in the Michigan Cancer Foundation (MCF)-7 breast cancer cell line by promoting cell growth and inhibiting apoptosis.[2] Targeting IGF-1 action may enhance efficacy of chemotherapy in breast cancer, and the timing of IGF-1 receptor inhibition also affects chemotherapy responses. Resistance to endocrine therapy is a major factor in the resistance of cancer which is due to the mutation of the ligand-binding domain (LBD) of ESR1. IGF-1 receptor has been found to phosphorylate and activate ER on serine-167 via an S6-kinase mechanism. In addition to IGF-1 receptor inhibitor, clinical trials are evaluating combined anti-IGF-1R, anti-ER treatments in endocrine-resistant populations.[3] Natural Products are known to have affinity towards multiple targets likes phloroz in targeting sodium-glucose co-transporter SGLT 1 and SGLT 2.[4] The plant *Alstonia scholaris* have many type of alkaloids with complex ring structures such as monoterpene indole alkaloids (MIA) and the butanolic

extract of the leaves showed IC_{50} of 25.29±1.15 µg/mL against MCF-7 cell line. Here, we have applied a hybrid approach involving *in-silico* studies and *in-vitro* cytotoxicity assays to determine the dual inhibition property of MIAs.

Results and Discussion

Insilico study

The 3D protein models 3ERT and 2OJ9 were selected for ESR1 and IGF1R respectively for docking according to the Z scores (-6.3 (3ERT); -8.51 (2OJ9)) and LG scores (8.059 (3ERT); 7.442 (2OJ9)) predicted by the ProSA and ProQ tools. Using the PDBsum web server, the amino acid residues involved in the binding site formation were identified as Met343, Leu 346, Thr347, Ala350, Asp351, Glu353, Trp 383, Leu387, Arg 394, Glu419, Gly420, Met421, Leu428, Phe404, Gly521, Leu525 for 3ERT and Leu975, Gly976, Gln977, Val983, Ala1001, Met1049, Glu1050, Leu1051, Met1052, Thr1053, Arg1054, Gly1055, Met1112, Thr1127 for 2OJ9. The list of the isolated compounds from *A. scholaris* was prepared by referring the databases like Sci-finder, google scholar, Reaxys and Dictionary of Natural Products (DNP). Later, the structures of 225 compounds were screened against the active pocket regions of the ESR1 and IGF-1receptor by high throughput virtual screening (HTVS) tool having a computational algorithms (Table S1). The top 50 compounds from the HTVS screen were subjected to comprehensive docking through extra precision (XP) mode to get a semi-quantitative ranking of candidate ligands based on their ability to bind to a specific conformation of the protein receptor (Table S2). In the case of ESR1, the best five compounds in the list arranged as per XP docking score were alstoniascholarine P, echitamidine N-oxide, lagunamine, picrinine and 18-hydroxy-19,20-dihydroakuammicine while for IGF-1R, chlorogenic acid, sweroside, alstolaxepine, melosline A and scholaricine have shown better scores (Table S3). Drug likeliness parameters including physico-chemical properties of these 10 compounds were evaluated by utilizing online tools such as Lipinski RO5, Ghose (Amgen), Veber (GSK), Egan (Pharmacia) and Muegge (Bayer). It was observed that except chlorogenic acid, all compounds follow the H-bond donor criteria, while no single compound disobeyed the H-bond acceptor criteria. Log $P_{o/w}$ value for these compounds were in the acceptable range. ADMET properties were compared for these molecules and most of them showed greater than 90% intestinal absorption except for chlorogenic acid (30.9%), sweroside (33.7%) and alstolaxepine (71.33%) (Table S4). The toxicity analysis indicated that picrinine, 18-hydroxy-19, 20- ihydroakuammicine and melosline A might show hERG toxicity while alstolaxepine and melosline A might be causing genotoxicity (AMES mutagenesis). Considering all the above factors, alstoniascholarine P and scholaricine were selected for further studies.

To check the potential of dual inhibition alstoniascholarine P and scholaricine were docked against IGF-1R and ESR1 and the scores were -8.459 and -8.268 kcal/mol respectively. The binding affinity were equivalent or close to the score of native ligands. Additionally, molecular simulations were carried out for both compounds against both targets. The protein root mean square deviations (RMSD) were compared with 4-hydroxy tamoxifen as a reference ligand and observed that during 40 to 80 ns holo1 (Fig.1A: ESR1-4-hydroxy-tamoxifen) was showing fluctuating from ~0.65 to ~3.0 Å, while in holo2 (Fig.1B: ESR1-alstoniascholarine P), it was changing from ~0.8 to ~2.4 Å and holo3 (Fig.1C: ESR1- scholaricine) showed ~2.0 to ~3.0 Å. The RMSD value was in the range of ~2.5 to ~3.5 Å in the apo state. Similarly, the RMSD graph of Holo4 (Fig.1D: IGF-1R - 4-hydroxy-tamoxifen) shows fluctuation between ~1.8 to ~4.2 Å, holo5 (Fig.1E: IGF-1R-alstonias-cholarine P) with fluctuation between ~1.4 to ~2.7 Å, and holo6 (Fig.1E: IGF-1R- scholaricine) from ~1.2 to ~3.6 Å during the run of 40-80 ns (Fig.1.). Therefore, molecular dynamic studies indicated that alstoniascholarine P and scholaricine are making stable binding with 3ERT and IGF-1R.

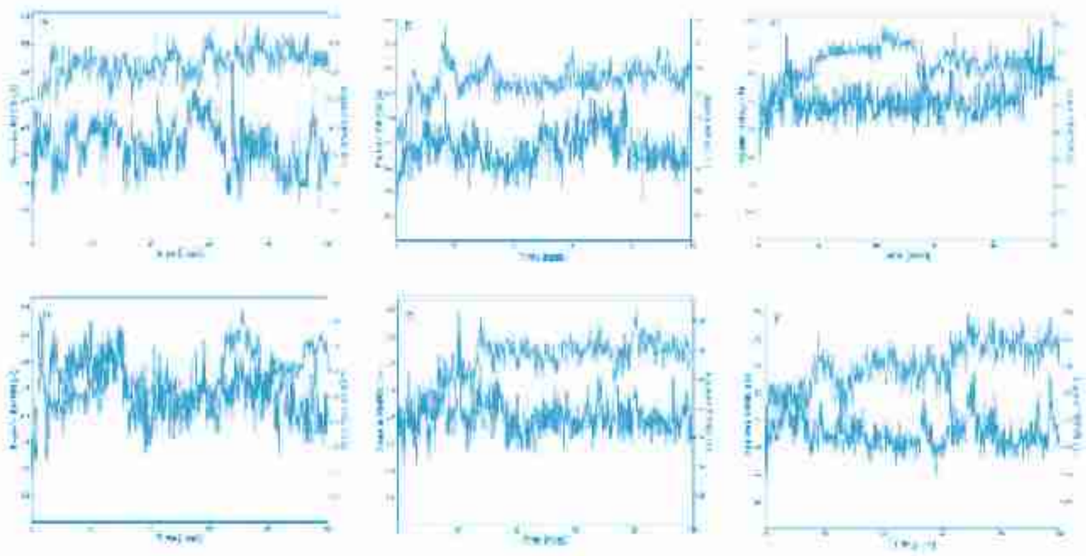

Fig. 1 RMSD plots of receptor-ligand complexes in MD simulations over a period of 100 ns A) ESR1-4-hydroxy-tamoxifen; B) ESR1-alstonias cholarine P; C) ESR1-scholaricine D)IGF-1R-4-hydroxy-tamoxifen; E) IGF-1R-alstoniascholar- ine P; F) IGF - 1R - scholaricine.

The RMSD results were further confirmed by utilising root mean square fluctuation (RMSF) to change the residues (Fig.S3). RMSF is basically used to assess the flexibility or dynamic behaviour of atoms or residues in a molecular system, such as a protein or a complex. The mobility of different residues was observed in both states through RMSF plots and it was observed that these residues were Leu466, Thr465, Ser464, and Ser463; Glu1069, Asn1070, Asn1071, Pro1072, Val1073 and Leu1074 involved in elbow loop formation in ESR1 and IGF-1R respectively (Fig. S6). Radius of gyration (rGyr) was calculated to analyse the overall compactness of protein and ligand complexes (Fig. S1). The data suggests the tested compounds can make stable complex with the targets comparable to the standard drug and the MD simulation study indicated that both compounds have ability to stabilize ESR1 as holo2 and holo3 showed one H-bond interaction after 100 ns simulation experiment. The H-bond interaction was constant for all the tested holo forms; however, the higher number of consistent interactions were observed in alstoniascholarine P against IGF-1R (holo5) (Fig. S2).

Extraction, isolation and invitro cytotoxicity study of MIAs

For isolation of the selected alkaloids from *A. scholaris,* an alkaloid rich extract was obtained from the dried plant material of *A. scholaris* using water: methanol (1:1) and n-butanol in series. The butanolic extract when subjected to chromatographic purification resulted into isolation of scholaricine. However, we were unsuccessful in isolating alstonia - scholarine P in its pure form, where the non-indole nitrogen of scholaricine is attached to oxygen via a coordinate bond. Scholaricine was obtained as white powder for which the ESI-HRMS showed a $[M+H]^+$ peak at *m/z* 357.1818, which analysed for $C_{20}H_{24}N_2O_4$. The key chemical shifts of scholaricine such as the presence of a substituted indole ring (δ_H 6.99, 6.95, 6.90PPM), one methoxy group showing singlet at δ_H 3.89, and one methyl doublet at δ_H 1.1PPM were observed in ^{1}H NMR confirming its structure (Fig.2 , Table 1). Further, ^{13}C NMR data were in agreement with scholaricine (Fig.S4-S5).[5] The purified compound was evaluated for cytotoxicity against MCF-7 cell line and the observed IC_{50} was 18.13 µM with doxorubic in used as a positive control with an IC_{50} value 0.53 ± 0.04 µM.

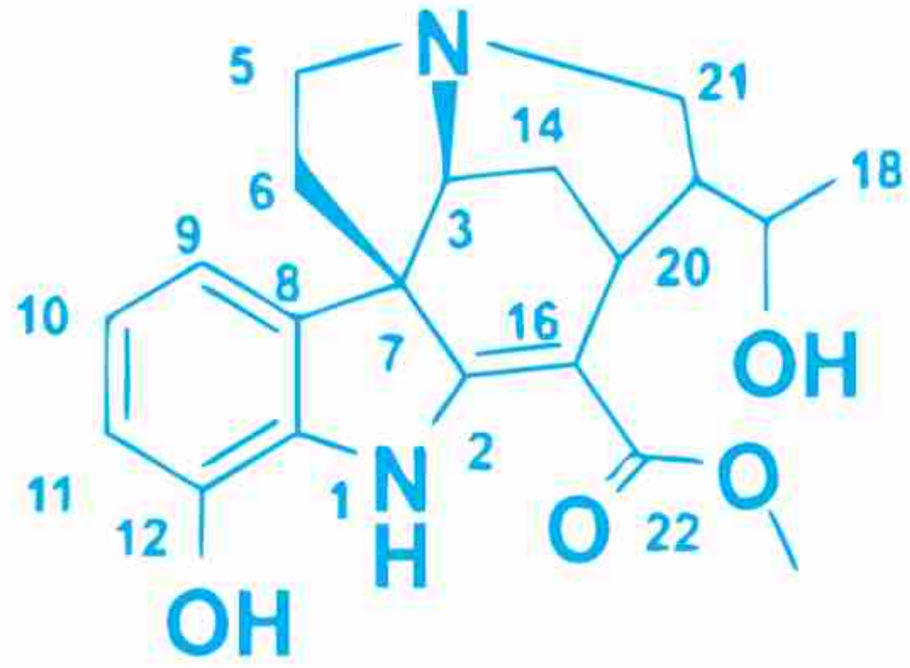

Fig. 2. Structure of scholaricine

Table 1. Observed ^{1}H and ^{13}C NMR chemical shifts of scholaricine.

No.	δ^H (ppm)	δ^C (ppm)
2	4.62 (1H)	168.75
3	-	60.55
5	3.12, 3.67 (2H)	52.07
6	2.15,2.80 (2H)	40.58
7	-	55.01
8	-	131.09
9	6.78 (1H)	111.26
10	6.81 (1H)	116.88
11	6.76 (1H)	123.53
12	-	141.89
13	-	133.57
14	1.50, 2.15 (2H)	29.34
15	3.46 (1H)	27.30
16	-	96.49
18	1.18 (3H)	19.61
19	3.27 (1H)	67.62
20	1.97 (1H)	43.94
21	2.38, 3.52 (2H)	47.31
22	-	168.71
OCH_3	3.89 (3H)	52.50

Experimental

Extraction and isolation of scholaricine

A. scholaris leaves (Voucher no. NIPER-A 07-2019) were collected from Gandhinagar, Gujarat, India. The plant sample was authenticated by Prof. A. Shukla, M.G. Science Institute, Navrangpura, Ahmedabad, Gujarat, India. Scholaricine was isolated from the hydro alcoholic extract (1:1) later partitioned using n-butanol and an alkaloid enriched fraction of 55g was obtained. The column chromatography of the butanolic fraction resulted in 16 fractions. Scholaricine (4mg) was isolated from fraction 1. The compound was isolated using preparative HPLC using Kinetex C18 -; 250 x 4.60 mm, 5µ; 15% acetonitrile and 85% 0.1% formic acid at 1 mL/min flow rate.

In vitro cytotoxicity study

The MCF-7 breast cancer cells were obtained from National Centre for Cell Science, Pune; low glucose DMEM (Dulbecco's Modified Eagle Medium), trypsin, and EDTA (Ethylene Diamine Tetra Acetic Acid) were procured from HiMedia (Mumbai, India); penicillin, streptomycin, and FBS (Fetal Bovine Serum) were obtained from Gibco (Mumbai, India). Alamar blue assay followed as per protocol.[6]

The cells were treated with methanol and butanol extract at four different concentrations: 1, 5, 20, 30 and 50 µg/mL. The study was performed in three replicates. At 510 and 590 nm, the optical density was observed. The following equation was used to compute the cytotoxicity value:

% cytotoxicity = (control-blank)-(test-blank)/(control-blank) × 100

Insilico studies

Molecular Docking study

To validate drug-target conjugation, the prepared 225 ligands/compounds were docked with minimized protein structures by using the Glide tool (version 21.1) based on Minte carlo Simulation & Anyalysis (MCSA). Each ligand binds with the target, the generated scores were recorded. First, all the generated ligands screened through HTVS mode, and further top 50 compounds were docked in XP mode. The target was kept rigid, while ligands were flexible to rotate and find to most probable binding poses which finally result in different scores.

Drug-likeness filter and ADMET study

SwissADME tool was used to access five different rule-based filters like Lipinski (Pfizer), Ghose (Amgen), Veber (GSK), Egan (Pharmacia) and Muegge (Bayer) . pkCSM software was used to predict ADMET properties like %Human intestinal absorption, skin permeability (log Kp), BBB permeability (log BB), steady-state volume of distribution (VDss), metabolism (CYP2D6 and CYP3A4 inhibition), total clearance, and oral rat acute toxicity data[7].

Other parameters of toxicity like HERG inhibition, hepatoxicity, carcinogenicity and Ames mutagenesis were evaluated with the admet SAR tool.[8]

MD simulation

Desmond suit (version 2021-1) MD simulation package was used to analyses the Apo and Holo states to understand the dynamic behavior, binding mode, and specificity of these inhibitors.[9] The orthorhombic box (10×10×10 Å) boundary was built up to describe the shape and size of the repeating unit. By introducing adequate counter Na^+ /Cl^- ions, the system was neutralized. After constructing the solvated system including the protein-ligand complex into the TIP3P solvent model, the system was subjected to NPT ensemble simulations at 300 K temperature and 1.01325 bar pressure for 100 ns. The structural changes and dynamic behavior of the protein were investigated using the RMSD and RMSF plots, radius of gyration and H-bond interaction. The existence of intermolecular interactions were depicted through BIOVIA Discovery Studio Visualizer 2019 San Diego, CA, USA.

Conclusion

The plant *A. scholaris* holds a great importance as a medicinal herb in India which is used in folk and traditional medicine to address various ailments. Its ripe fruits treat syphilis, insanity, epilepsy, and serve as a tonic, antiperiodic, and anthelminthic. The milky juice heals ulcers, and the bark serves as a bitter tonic and febrifuge. It is effective for treating malaria, diarrhea, dysentery, and preventing liver damage from various toxins.[10,11] However, the unique indole alkaloids found in abundance from the plant have not been explored to the full potential. In our study, we have embarked on a combined approach with *insilico*

screening method involving the HTVS and XP screening followed drug likeness filtering, RMSD and RMSF simulations along with *invitro* cytotoxicity study. Our findings point to the promising role of scholaricine, belonging to the akuammicine type MIAs, as potential molecule in inhibiting ESR1 and IGF-1R. The translational value of these findings holds immense promise as many anticancer drugs have the drawback of chemo-resistance, multi-target inhibition property may be one of the solutions. This finding opens the door to new realm of possibilities, such as structure activity relationship studies, in-depth mechanistic studies and *invivo* evaluations. These strategies not only unveil the untapped potential of natural products derived molecules but also offer a starting point for expediting their journey through the drug discovery pipeline.

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Conflict of Interest statement

No potential conflict of interest was reported by the authors.

References

1. Phi, L. T. H., Sari, I. N., Yang, Y. G., Lee, S. H., Jun, N., Kim, K. S. & Kwon, H. Y. (2018). Cancer stem cells (CSCs) in drug resistance and their therapeutic implications in cancer treatment. *Stem cells international*.

2. Clemmons, D. R. (2007). Modifying IGF1 activity: an approach to treat endocrine disorders, atherosclerosis and cancer. *Nat. Rev. Drug Discov.* 6, 821–833.

3. Becker, M. A., Ibrahim, Y. H., Cui, X., Lee, A. V., and Yee, D. (2011). The IGF pathway regulates ERa through a S6K1-dependent mechanism in breast cancercells. *Mol. Endocrinol.* 25, 3516–3528.

4. David-Silva, A., Esteves, J. V., Morais, M. R. P., Freitas, H. S., Zorn, T. M., Correa-Giannella, M. L., & Machado, U. F. (2020). Dual SGLT1/SGLT2 inhibitor phlorizin ameliorates non-alcoholic fatty liver disease and hepatic glucose production in type 2 diabetic mice. *Diabetes, Metabolic Syndrome and Obesity*, 739-751.

5. Atta-Ur-Rahman; A. Asif; M. Ghazala; M. Fatima; J. Alvi. K.A. (1985) Scholaricine, an alkaloid from alstonia scholaris. *Phytochemistry,* 24(11), 2771–2773.

6. Pandey K., Chaitrali S., Khemraj B., and Abhijeet S. Kate. " (2020) Pharmaceutical perspective on bioactives from Alstonia scholaris: ethnomedicinal knowledge, phytochemistry, clinical status, patent space, and future directions. *Phytochemistry reviews*, 19: 191-233.

7. Pires, Douglas EV, Tom L. Blundell, and David B. Ascher. (2015) "pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures." *Journal of medicinal chemistry*, 58, no. 9: 4066-4072.

8. Yang, Hongbin, Chaofeng Lou, Lixia Sun, Jie Li, Yingchun Cai, Zhuang Wang, Weihua Li, Guixia Liu, and Yun Tang.(2019)"admetSAR 2.0: web-service for prediction and optimization of chemical ADMET properties." *Bioinformatics*, 35, no. 6 1067-1069.

9. Schrödinger Release 2021 - 1: Desmond Molecular Dynamics System, D. E. *Shaw Research, New York.*

10. Jahan, S., Gupta, U., Chaudhary, R., & Goyal, P. K. (2009). Prophylactic use of *Alstonia scholaris* (Sapthaparna) against gamma irradiation. *Pharmacol Online*, *1*, 160-75.`

11. Gupta, U. Chaudhary, R. Goyal, P. K. (2010) Post - treat- ment effects of *Alstonia scholaris* extract against radiation-induced biochemical alterations in Swiss albino mice, *Iran. J. Radiat. Res.,* 8 (3): 169-177

Review Article

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Identifying Substitution and Adulteration of Some Common Medicinal Plants - Part I

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Abstract

The global surge in interest for herbal medicines, driven by perceived safety and cultural acceptance, has led to a reliance on these remedies, particularly in developing countries. However, the herbal industry faces significant challenges, including controversies surrounding the use of certain plants. Adulteration and substitution of herbal products for commercial gain are pressing issues, demanding urgent attention in the identification, standardization and quality assurance of Ayurvedic medicines.

Various authentication techniques, including morpho-anatomical examination, palynology, organoleptic assessment, DNA barcoding, chromatographic and spectral analysis are discussed. Spectroscopic methods, especially IR and Raman spectroscopy combined with chemometrics emerge as useful tools for detecting adulteration, offering simplicity and cost-effectiveness. This review highlights the identification of adulterants and substitutes in five crucial plants documented in key Ayurvedic texts, underlining the need for ongoing advancements in authentication techniques for herbal products.

Introduction

There is a growing global interest in herbal medicines due to their widespread acceptance and perceived safety. Medicinal plants serve as a valuable resource for Ayurvedic and other traditional systems, as well as modern medicine.[1] In developing countries, approximately 80% of the rural population relies on herbal remedies at the primary healthcare level.[2,3] However, the use of many plant species in the herbal industry has become a subject of concern, with several factors contributing to this controversy, including the limited availability of certain plants, inadequate knowledge, and the coexistence of various knowledge systems. The use of Sanskrit-based polynomial nomenclature, diverse interpretations within different communities and the existence of vernacular equivalents results in issues of controversy, adulteration, and substitution in herbal medicine.

Adulteration involves the deliberate debasement of herbal products for commercial gain, which may entail deterioration, blending with other substances, and other miscellaneous reasons. Substitution refers to the replacement of original medicinal plants with equivalent alternatives.[4] *Charaka* and *Sushruta*, prominent Ayurvedic classics, do not explicitly enumerate *Pratinidhi Dravyas*.[5,6] However, Acharya Vagbhata offers insights into how substitute plants (*Pratinidhi*) are chosen, emphasizing the consideration of shared properties such as taste (*Rasa*), characteristics (*Guna*), potency (*Virya*), post-digestive effect (*Vipaka*), and, most importantly, therapeutic action (*Karma*).[7] Currently, adulteration and substitution of herbal drugs present significant challenges within the herbal industry and Ayurvedic practices. Hence, there is an urgent need to establish reliable methodologies for the accurate identification and standardization of herbal medicines.[8] Various methods are employed to ensure the authenticity of raw materials in the herbal industry. Among these, commonly utilized techniques for authentication and differentiation between medicinal plants and their adulterants or substitutes include morpho-anatomical examination, palynology (study of pollen), organoleptic assessment (sensory evaluation), DNA barcoding, chromatographic analysis (HPLC, HPLC-MS, DARTTOF-MS, and GC-MS), and various

spectral techniques (FT-IR, NIR, Raman, UV and NMR spectroscopy).[4,9,10] There is an urgency of addressing economically motivated adulteration and the need for continuous advancements in authentication techniques for herbal products. Spectroscopic methods, notably IR and Raman spectroscopy combined with chemometrics, are acknowledged for their pivotal role in successfully identifying adulteration in herbs and spices. Renowned for their simplicity and cost-effectiveness, these techniques prove indispensable in addressing diverse challenges, including taxonomic research, quality control, and the identification of counterfeit products.[9,10] Furthermore, the incorporation of DNA barcoding, especially when integrated with metabolomics, transcriptomics, and proteomics, emerges as a promising authentication tool. The collaborative efforts between regulatory bodies and research institutions are crucial for establishing DNA barcoding protocols, enhancing herbal pharma covigilance.[11,12] This review aims to emphasize the identification of adulterants/substitutes in selected five crucial plants that play significant roles in both traditional medicine and the global market.[13] These plants are documented in The Ayurvedic Pharm acopoeia of India, Bhavaprakash Nighantu, Yogratnakar, and Bhaishajya-Ratnawali with their adulterants/substitutes.[14-17] This review article fills a critical gap in the existing literature on the authentication of some common ayurvedic medicinal plants. Through an extensive literature study, it was discerned that detailed information on certain crucial aspects is conspicuously absent in previous reviews. Specifically, previous reviews landscape on spices and herbs, while comprehensive in their own right, often exclude information pertaining to five key medicinal plants. We aim to review plants that are medicinally important and are of high commercial value. This review is first in the series, covering these five plants, we plan to review other plants in our future articles.

Table 1: Botanical name of plant, its major adulterant and methods of detection

Sr. No.	Botanical name	Plant part	Adulterant	Methods of detection
1.	*Berberis aristata* DC.	*Roots*	*Berberis aristata* DC.	Pharmacognostic differentiation, HPTLC, DART-MS, HPLC-ESI-QTOF-MS/MS,
2.	*Nardostachys jatamansi* DC.	Roots and rhizomes	*Selinum vaginatum* C.B.Clarke	Pharmacognostic differentiation, HPTLC, HPLC, DNA Barcoding
3.	*Crocus sativus* L.	Stigma	*Carthamus tinctorius L.*	Pharmacognostic differentiation, DNA Barcoding, HPLC/PDA/MS, FT-IR
4.	*Cyanthillium cinereum* Less.	Entire plant	*Emilia sonchifolia* (L.) DC.	Pharmacognostic differentiation, DNA Barcoding, HPTLC/GC-MS
5.	*Inula racemosa* Hook	Roots	*Saussurea lappa* Clark	Pharmacognostic differentiation, TLC, qNMR

Berberis aristata DC.

Berberis aristata DC. (Berberidaceae) is a rare and endangered species.[18,19] It holds significant importance in traditional Ayurvedic texts like the *Charaka* and *Susruta Samhitas*, where it is extensively discussed and documented as one of the most crucial herbs.[20-22] The annual estimated consumption is 500 metric tonnes.[23]

Substitute

Berberis asiatica Roxb. ex. DC. (Berberidaceae) frequently serves as a widely used alternative.[24] The *Berberis* genus is quite extensive, comprising approximately 12 genera and roughly 600 species found worldwide. In India alone, there have been reports of about 77 species of *Berberis*.[20] However, identifying and classifying species within the *Berberis* genus presents taxonomic challenges. This difficulty arises because these plants display considerable morphological variations influenced by natural hybridization and environmental factors. Moreover, the features used for identification, such as flowers,

leaves, stems, and berries, can overlap and change with the season and the age of the plant, making it challenging to identify them in the field.[24,25]

Ethnopharmacology, Marketed formulations and Therapeutic uses

Traditionally, *B. aristata* has gained recognition for qualities including the ability to reduce toxins and excess fats (*Lekhaniya*), anti-hemorrhoidal properties (*Arshoghna*), a role in purifying lactating mother's milk (*Stanyasodhana*), capacity to promote wound healing (*Ropana*), ability to induce sweating (*Svedala*), rejuvenative effects (*Rasayana*), anti-itch properties (*Kandughna*), and utility in treating skin disorders.[26] In Raithal, Uttarkashi and *Bhotiya* communities in Himalayan ranges, India, Rasaut is a widely used medicine for eye issues made from *B. aristata* roots.[27-29] In the Kumaon region of India, the root bark decoction of *B. aristata* and *B. asiatica* is employed to treat eye problems and boils.[27,30] Tibetan people use *B. aristata* root decoction for piles, gastric issues, and related ailments, referring to the plant as Kershuen.[27,31] In the Dehradun district of Uttarakhand, India, the local residents employ *B. aristata* as a substance to poison fish.[27,32] Tribals in Uttarakhand, also use *B. aristata* for snake and scorpion bites.[27,33] In Himachal Pradesh, India, the *Malani* tribal communities employ it as a remedy to skin ailments, jaundice, hemorrhoids, and malaria.[26,34] In the Garhwal Himalaya region, it is used as a psychomedicine.[26,35] Tribals living in Sikkim and Darjeeling, India, utilize the plant extract for its anti-diabetic properties.[26,36]

B. aristata is used to manage a wide spectrum of health conditions, including allergies, metabolic disorders, various eye diseases, osteoporosis, skin diseases, menorrhagia, fever, diarrhoea, cholera, jaundice, malaria, ear and urinary tract infections. It possesses medicinal properties such as being antibacterial, antifungal, anti-inflammatory, analgesic, anti-scorbutic, and anti-hepatopathic.[26,27]

Significant formulations such as *Bhrngarja Taila, Ashvagandhadyarista, Khadirarista, Jatyadi Taila,* and *Triphala Ghrta* are recommended for various therapeutic uses, including *Kandu, Medoroga, Mukharoga, Vrana, Amatisara, Urustambha, Kapharoga, Karnaroga, Netraroga,* and *Meha,* with a recommended dose of 5-10 ml of the drug in *Kvatha* form.[14]

Phytochemistry

The primary phytoconstituents found in *B. aristata* are alkaloids. The roots of this plant contain berberine (2.23%), palmatine, and a protoberberine alkaloid called karachine along with compounds like oxyberberine, oxyacanthine, aromoline, berberine chloride and berbamine.[37,38] Another alkaloid known as taxilamine has also been identified in this plant.[39] The flowers of *B. aristata* contain various polyphenolic flavonoids and acids such as rutin, quercetin, meratin, caffeic acid and chlorogenic acid.[40] Additionally, alkaloids like pseudoberberine chloride, pakistanine, 1-*O*-methylpakistanine and pseudopalmatine chloride have been isolated from the plant bark.[21,26,4]

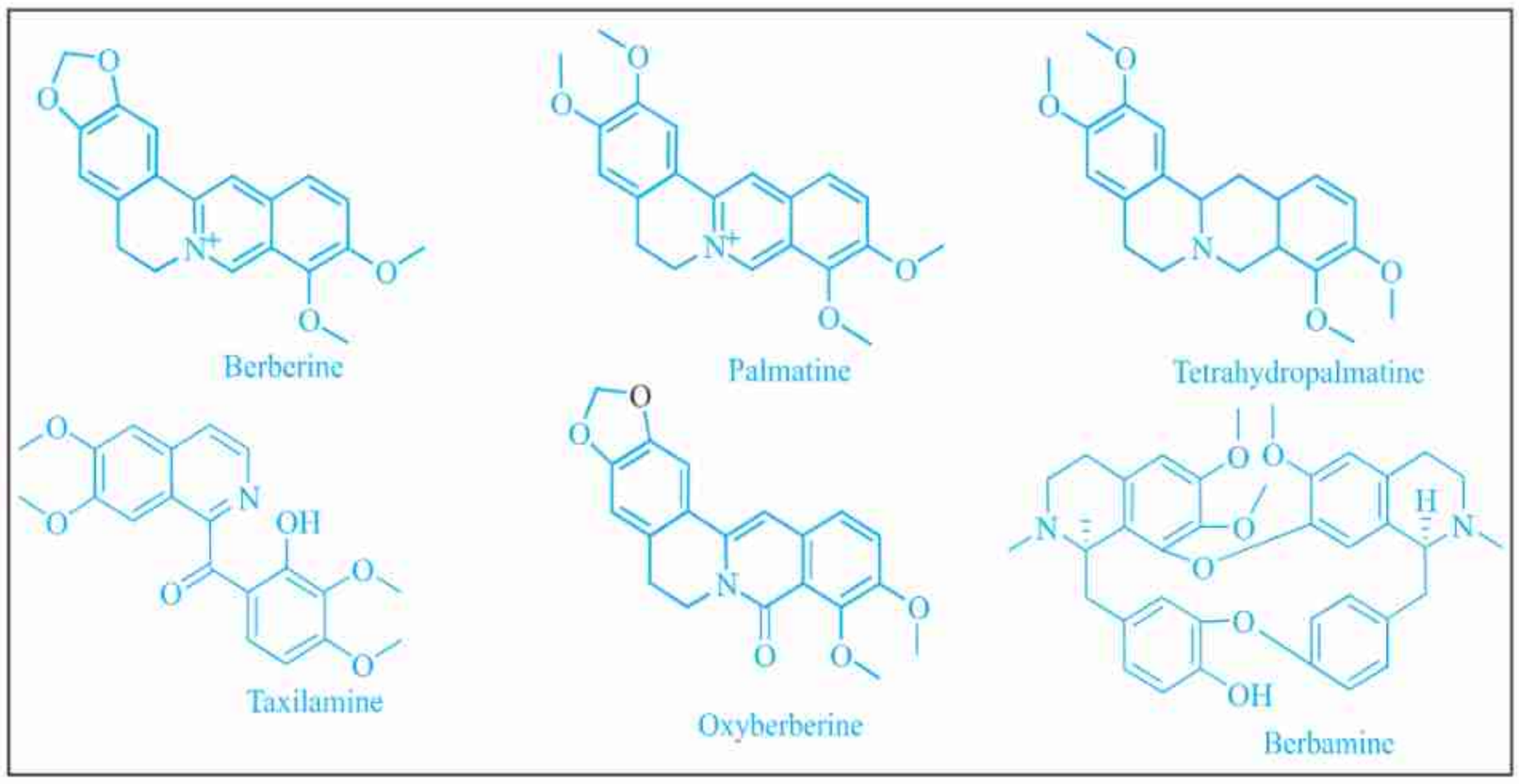


Figure 1 : Major Phytoconstituents in *Berberis species*

Comparative methods

Pharmacognostic differentiation

Anatomical examination reveals distinctive characteristics in the root drug, including pericyclic fiber patches, pitted sclereids, as well as cells containing berberine and heterocyclic medullary rays.[21,24]

Table 2 : Comparative identification of *B. aristata* and *B. asiatica*[21,24]

Features	B. *aristata*	B. *aristica*
Botanical identification		
Growth Habit	Large deciduous shrub (1.8-3.6 m)	Small evergreen shrub (1.2-1.8 m)
Macroscopic identification		
Roots	Hard and greyish brown	Thick, woody, yellowish brown, and knotty
Odour	Phenolic odour	odourless
Microscopic identification		
Growth Habit	Large deciduous shrub (1.8-3.6 m)	Small evergreen shrub (1.2-1.8 m)
Macroscopic identification		
Cortical region	Cork cells, cortex with tannins, starch grains, rhomboidal crystals	Cork cells, cortex with tannins, pericyclic fibers, and possible yellow-coloured alkaloids
Vascular tissues	Polyarchy xylem	8-10 celled wide secondary xylem, sieve tubes, companion cells, phloem fibers in secondary
Medullary rays	Pitted medullary rays filled with alkaloidal content only	Medullary rays contain both starch and alkaloidal content

High Performance Thin Layer Liquid Chromatography

Comparative analysis revealed differences in berberine content between root and stem bark samples, with root samples exhibiting higher berberine levels than the bark. The root samples consistently showed higher berberine content, with *B. asiatica* having the highest amount of berberine, and this difference was statistically significant ($p<0.05$). In *B. asiatica*, the berberine content was 4.3% in the root and 3.0% in the stem bark and *B. aristata* contained berberine, with 3.8% in the root and 2.6% in the stem bark.[42] There can be several factors leading to content variation of phytochemicals in plants, therefore it is important that the presence or absence of some unique chemical marker is used to ascertain adulteration or substitution in medicinal plants.[42]

Direct analysis in real-time mass spectrometry

The application of Principal Component Analysis (PCA) to the DART MS data resulted in the creation of score plots that clearly demonstrated unique groupings and distinctions between the various species and plant components under examination. PCA was successful in pinpointing the specific peaks that act as markers to differentiate *B. aristata* from *B. asiatica*. In the PCA analysis, data within the *m/z* range of 100-750 were considered, focusing on molecular ion species with peak intensities exceeding 5%. The selected *m/z* values represented either $[M^+$ or $[M^+H]^+$ ion species, characterized by defined isotopic peak patterns, enabling a robust analytical approach. Notably, PCA revealed distinct phytochemical differences among various plant species, including *B.*

aristata. Eleven key peaks at *m/z* 209, 279, 340, 352, 370, 609, 610, 621, 623, 640, and 641 were pivotal in discriminating roots, contributing significantly to 70% of the overall variance. These peaks exhibited varying cumulative contributions across all roots, with *B. asiatica* displaying the highest and *B. aristata* the lowest. Similar discriminatory patterns were observed in stems, where the first two principal components explained 79% of the variance, effectively distinguishing between *B. aristata* and *B. asiatica*. The fingerprint of stem, characterized by peaks at *m/z* 336, 339, 341, 342, 356, 370, 397, 429, 607, played a significant role in this discrimination. PCA applied to leaf data showcased distinct clustering, with nine peaks at *m/z* 210, 271, 339, 342, 343, 429, 609, 610, and 697 contributing to 70% of the variance. Further partwise differentiation within *B. aristata* was achieved through eight marker peaks at *m/z* 322, 336, 343, 355, 357, 370, 623, and 641. The chemometric tool, PCA, efficiently determined the number of principal components necessary for distinguishing *B. aristata* from substituted plants and allowed for the differentiation between plant parts.[43]

HPLC-ESI-QTOF-MS/MS

The study encompassed several species including *B. aristata*, *B. asiatica*, *B. chitria*, *Berberis jaeschkeana*, *B. koehneana*, *B. lyceum*, *B. petiolaris*, and *B. pseudoumbellata*. A combination of reversed-phase high-performance liquid chromatography combined with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (HPLC-ESI-QTOF-MS/MS) was employed to explore the distribution and differentiation of these compounds. High-resolution mass spectrometry (HR-MS) and collision-induced dissociation (CID) mass spectrometry experiments gave determine molecular mass and fragmentation patterns. In total, 59 compounds were provisionally identified, including 3 acids, 25 alkaloids, and 12 flavonoids. Seventeen reference standards were used to validate these identifications. In the assessment of variability among eight Berberis species and for efficient data organization, Principal Component Analysis (PCA) was employed. PCA, An unsupervised clustering method, was utilized to maximize the retention of variance in multi-dimensional data while reducing its dimensionality. The resulting data were presented in a two-dimensional plot (score plot), with coordinate axes representing the directions of the two largest variations. The HPLC-ESI-QTOF-MS data from various parts of Berberis species were subjected to PCA. From the initial 59 compounds, specific peaks were excluded to optimize the PCA results. Eigen value analysis revealed 29 peaks contributing to 70% variance in PC1 vs PC2 for leaf data, with covariances of 40% and 30%. Similarly, for stem data, 39 peaks exhibited 66% variance in PC1 vs PC2, with covariances of 39% and 27%. For root data, 38 peaks contributed to 62% variance in PC1 vs PC2, with covariances of 33% and 28%. The score plot for leaf data demonstrated distinct clustering according to species, mirroring a similar trend in the stem and root plots. This suggests that the application of HPLC-ESI-QTOF-MS coupled with PCA is a suitable methodology for species identification.[44]

DNA barcoding

DNA markers were generated through the process of amplifying and sequencing the spacer region, which included ITS1, 5.8S rRNA, and ITS2, using genomic DNA as the source material. The boundaries of the ITS1, 5.8S, and ITS2 sequences were established by analysing the submitted sequence data accessible on GenBank (http://www.ncbi.nlm.nih.gov/). Sequences were aligned employing CLUSTALW (http:// www.ebi.ac.uk.clustalw/), and homology percentages were calculated. Species-specific DNA primers were designed using NCBI primer blast tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and synthesized. PCR reactions were performed with these primers, targeting the ITS region, using optimized conditions: initial denaturation, followed by 35 cycles of denaturation, annealing, and extension, concluding with a final extension. This approach aimed to selectively amplify and compare ITS regions among different Berberis species. For *B. aristata*, the DNA marker designated as AR1F has a sequence of TCTCGCTCTGATAAATACAACTCG and AR1R has a sequence C G T C A A C A G G C A A CACGAC, the resulting PCR product is 405 base pairs in size. For *B. asiatica*, the DNA marker designated as AS1F has a sequence of G T A C A A G GTTTCCGTAGGTGAACT and AS1R has a sequence GTGCCTCAGCCTAGTGGTTTG, the

resulting PCR product is 401 base pairs in size. The utility of these markers has been validated, demonstrating their effectiveness and reliability in verifying the identity of both *B. aristata* and *B. asiatica*. These DNA markers serve a crucial role as molecular pharmacognostic tools, facilitating the quality evaluation of the unprocessed herbal materials.[45]

Nardostachys jatamansi DC.

Nardostachys jatamansi DC. is a perennial herb found in the Alpine Himalayas and belongs to the Valerianaceae family. This medicinal plant has been esteemed for its healing properties for generations, having been used in Ayurvedic medicine in India, as well as in ancient Greek and Arab traditions, and even in ancient Egypt and Rome.[46,47] The annual estimated consumption is 200 metric tonnes.[22]

Adulterant

Selinum vaginatum C. B. Clarke, a significant medicinal and aromatic plant, is a member of the Apiaceae family.[48] The roots and rhizomes of *S. vaginatum* bear a striking resemblance and are a precise match for a commercially used drug, both in their macroscopic and microscopic characteristics. Consequently, it is employed as an undisclosed adulterant in the commercial drug.[49]

Ethnopharmacology, Marketed formulations and Therapeutic uses

N. jatamansi

Traditionally, *N. jatamansi* has gained recognition for its functions as a brain tonic (*Medhya*), rejuvenates the mind (*Rasayana*), aids in promoting sleep (*Nidhrajnana*), alleviates mental ailments (*Manasrogaghna*), aids digestion (*Pachana*), cough and breathing difficulties (*Kasawasahara*), helps in skin diseases and itching (*Kushtaghna*), relieves burning sensations (*Dahaprasha-mana*), enhances complexion (*Varnya*), and stimulates hair growth (*Roma sanjanana*).[50]

The rhizomes of this plant find diverse applications in different medicinal systems. In Ayurvedic medicine, they serve as a bitter tonic, stimulant, and antispasmodic. They are used to address conditions such as epilepsy and hysteria. In the Unani system of Medicine, *N. jatamansi* is employed as a hepatoprotective, cardiotonic, diuretic, and analgesic.[51]

Plants belonging to the genus Nardostachys have been extensively utilized either alone or in combination with other herbal or mineral remedies to address various ailments by indigenous communities across multiple countries. These include the Tibetan and various ethnic groups in China. The underground parts, encompassing roots and rhizomes, of Nardostachys hold significant therapeutic value and are administered in powdered form, decoctions, perfumes, incense, macerations, or infusions to alleviate a spectrum of conditions. These conditions range from mental disorders such as insomnia, epilepsy, convulsions, and depression, to cardiovascular issues like heart palpitations and blood disorders, as well as gastrointestinal discomforts such as dyspepsia, intestinal pain, along with skin problems like melasma, lentigines, toothaches, and foot swelling. In China, the recommended therapeutic dosage of *N. jatamansi* DC. roots and rhizomes is approximately 3-6 g, with external applications advised in moderation, including gargling with infusions, or applying powder to affected areas. Traditional usage of Nardostachys in China, Korea, and Thailand primarily revolves around addressing gastrointestinal and dermatological issues, whereas in India, its traditional uses are predominantly targeted towards mental and cardiovascular health. Modern pharmacological investigations since the 1950s have unveiled a plethora of contemporary medical applications for this genus, including sedative, analgesic, anti-neuroinflammatory, anticonvulsant, antidepressant, neuroprotective, cardioprotective, anti-inflammatory, anti-hypertensive, anti-proliferative, antibacterial, antifungal, and antioxidant properties, among others.[52-62]

It is included in formulations such as Abana, Mentat, Anxocare (Himalaya Drug Company), and *Dasmularishta*, *Mahamarichadi tail* (Dabur).[53] The crucial formulation, *Jatamamsyarka*, is utilized for therapeutic purposes, addressing conditions such as *Daha, Kustha, Visarpa, Manasaroga,* and *Anidra*, with a recommended dosage of 2-3 g of the drug in powder form or 5-10 g for decoction.[14]

S. vaginatum

A comprehensive investigation into the ethnomedicinal practices of Uttarakhand revealed intriguing uses of *Selinum vaginatum* within the indigenous *Bhotia* tribe of Mana village. Their distinctive approach involved using the plant roots or the entire plant, ignited to produce smoke, which was then administered to cows to enhance lactation. Meanwhile, in the Nanda Devi biosphere reserve, *S. vaginatum* emerged as a remedy for mental disorders, particularly in the form of powdered treatment for conditions like epilepsy and hysteria. Local communities have shared a traditional medicinal recipe which incorporates *S. vaginatum* root powder for duration of six months, aiming to alleviate conditions such as epilepsy, convulsions, and hysteria. Additionally, leaves of the plant were utilized for various medicinal purposes, including managing blood dysentery, colic, urinary complaints, diabetes, and enhancing lactation while treating menstrual problems. In high-altitude regions of Churah subdivision, a Gurjar tribe incorporated all parts of *S. vaginatum* into incense stick production. The Lahaul valley inhabitants, residing in a proposed Cold Desert Biosphere Reserve, attributed *S. vaginatum* to treating skin diseases, hysteria, dysmenorrhea, and liquor preparation. The plant, designated as Critically Endangered, also played a role in the Amchi System of Medicine in the Lahaul Valley, addressing ailments such as asthma, cough, and serving as an analgesic and antibacterial agent. Further research highlighted the significance of *S. vaginatum* in the Parbati valley, where locals regarded it as Bhutjata or Bhutkeshi, using it for making ornaments, fodder, and medicinal purposes to address mental disorders. Essential oil extracted from the roots proved valuable as a hypotensive, sedative, analgesic, and incense stick ingredient. From the Urgam Valley to the *Jaad Bhotiya* Community in Uttarakhand, *S. vaginatum* roots found diverse applications, from acting as a coolant to treating skin diseases and swelling muscles. The Kugti wildlife sanctuary inhabitants employed the rhizome for its perceived ability to ward off evil spirits. Similarly, in the Manali region, the plant's roots served as a nervine sedative. The Dudu valley-Jammu and Bhaderwah Hills showcased the roots of *S. vaginatum* being utilized as a carminative for fever and worms. In the Hariyali Devi landscape of Uttarakhand, the plant gained popularity for its application in addressing painful toothaches, and locals believed that having the entire plant in households could ward off evil spirits. These diverse ethnomedicinal uses of *Selinum vaginatum* underscore its cultural and therapeutic significance across the Himalayan regions.[63-68]

Phytochemistry

N. jatamansi

N. jatamansi is rich in both volatile and non-volatile constituents. The volatile compounds consist mainly of sesquiterpenes, while the non-volatile extracts contain sesquiterpenes, coumarins, neolignans, lignans, and alkaloids.[69] The roots are the source of its essential oil, notably rich in sesquiterpenes and coumarins.[70] The principal sesquiterpene present is jatamansone, also known as valeranone.[71] Other sesquiterpenes found in the plant include nardin, α-patchoulene, angelicin, elemol, jatamansin, jatamansinol, jatamansone, oroselol, patchouli alcohol, valeranal, valeranone, nardostachnol, seychellene, seychelane, and nardostachone.[72-74] The rhizomes of the plant yield a variety of components, such as essential oil, resin, sugars and starch, and gum. The main phytoconstituents include jatamansic acid, malliene, calarenol, jatamansin, heptacosanyl pentanoate and pyranocoumarin.[74-76] Furthermore, an alkaloid known as actidine has also been reported together with nardol.[46,62,77]

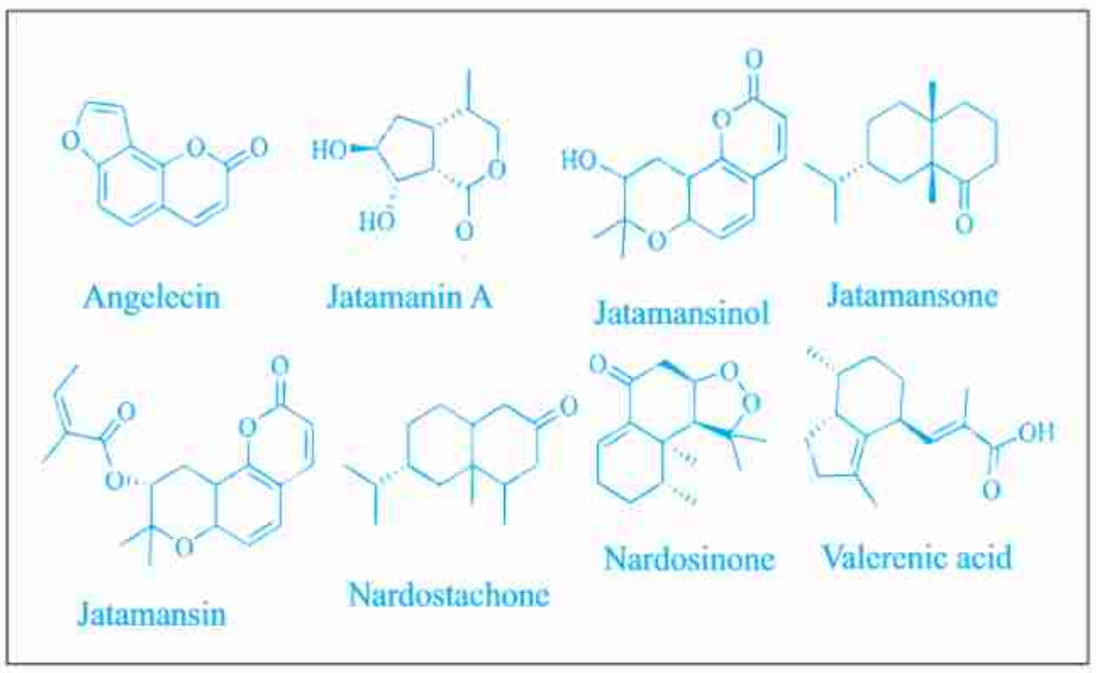


Figure 2 : Major Phytoconstituents in *N. jatamansi*

S. vaginatum

The essential oil composition of *S. vaginatum* from two geographically distant locations, Rohtang (Himachal Pradesh) and Tungnath (Uttarakhand) was examined using GC-FID and GC-MS techniques. This analysis identified a total of 28 compounds, with only 12 compounds shared between both populations. Additionally, the volatile oil from the underground parts of *S. vaginatum* was composed of 37 constituents, making up more than 95% of the total volatile content, as determined by GC/FID and GC/MS analysis.[48,78] Vaginatin was a noteworthy component in the oil, a sesquiterpene extracted from the roots of *S. vaginatum*.[48,79] In *S. vaginatum*, a novel coumarin compound named selinidin with diuretic properties was identified.[80] Selinidin and vaginidin were also isolated from *S. vaginatum* alongside known coumarin compounds, angelicin, oroselol, lomatin and selinitin.[81-83] Additionally, the methanolic extract of *S. vaginatum* revealed the presence of a hydroxyl-cinnamic acid derivative.[84] The rhizome of *S. vaginatum* was found to contain a substantial amount of valerenic acid.[63]

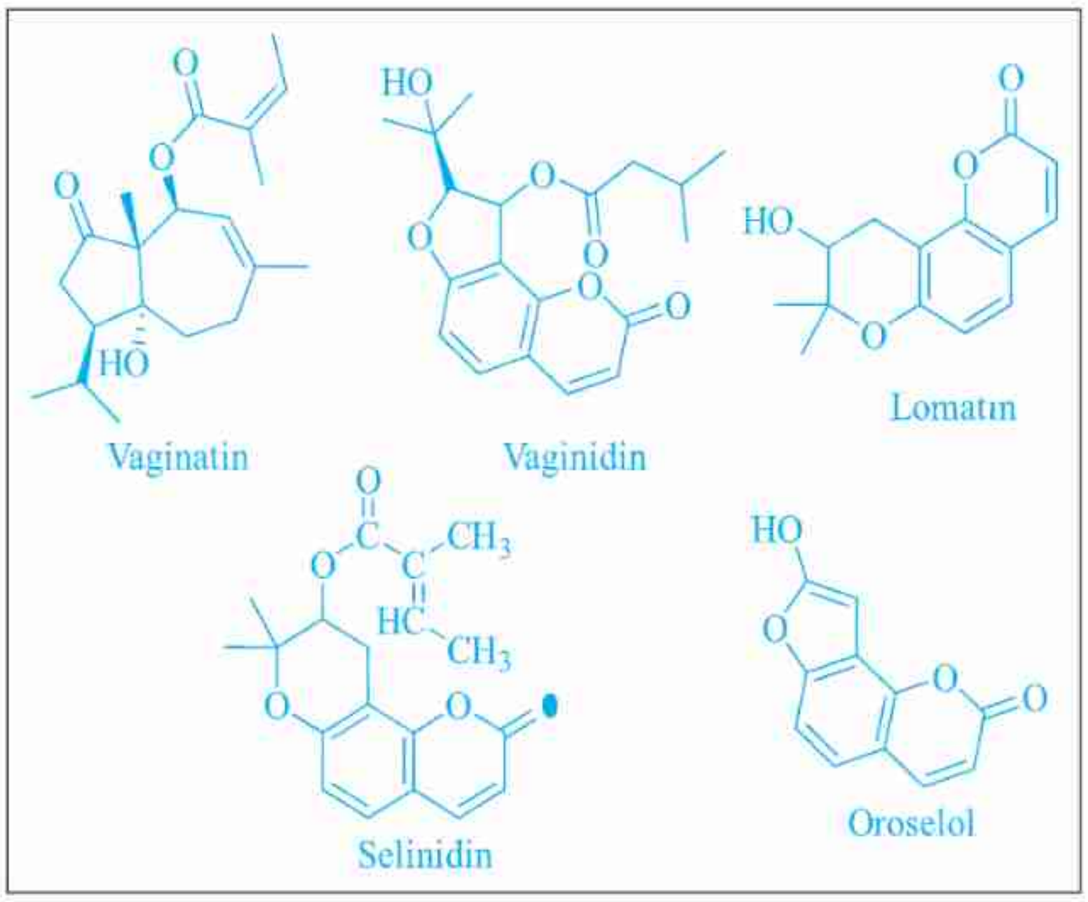


Figure 3: Major phytoconstituents in *S. vaginatum*

Comparative methods

Pharmacognostic differentiation

Notable differences exist both at the macroscopic and microscopic levels. Macroscopically, these distinctions manifest in the size of the rhizome, coloration, the quantity and thickness of hairs. At the microscopic level, distinctions are evident in the number of layers within the cortex and cork regions, the abundance of starch grains, the characteristics of medullary rays, the presence of schizogenous canals, and the presence or absence of pith.[46,48,85-87]

Table 3 : Comparative identification of N. jatamansi and S. vaginatum

Features	*N. jatamansi*	*S. vaginatum*
Botanical identification		
Growth Habit	Perennial herb, 10-60 cm tall	Small evergreen shrub (1.2-1.8 m)
Macroscopic identification		
Rhizome	2-10 cm, reddish-brown, fibrous remnants	10-15 cm, tufted hairs
Microscopic identification		
Cork	2 to 5 layers of cells containing oil granules	4 to 8 layers of cells filled with a brownish substance
Schizogenous canal	Present	Abundantly present
Secretory canals	Absent	Epithelial cells line the ground tissue of the rhizome
Cambium ring	Clearly defined and unbroken	Indistinct
Starch grains	Abundantly present in groups	Less in numbers
Medullary rays	Bi to multiserrate	Uni to multiserrate
Pith	Present parenchymatous	Absent
Parenchymatous cells	Absence of aggregation of yellowish acicular crystals	Aggregation of yellowish acicular crystals

High Performance Thin Layer Liquid Chromatography

An established TLC method was utilized for assessing and quantifying the valerenic acid content in the methanolic extract of *N. jatamansi* (NJ) and *S. vaginatum* (SV). According to this method, the free valerenic acid content in the root samples was determined to be 0.9151 for SV and 0.3136 for NJ.[63]

High-Performance Liquid Chromatography

Phenolic compounds, including chlorogenic acid, ferulic acid, protocatechuic acid, and syringic acid, were both qualitatively and quantitatively determined through HPLC analysis in the methanol extracts of *N. jatamansi* and *S. vaginatum*. Significant levels of phenolic content were detected in the methanol extracts of both *N. jatamansi* and *S. vaginatum*. Specifically, the methanol extracts obtained from *N. jatamansi* roots contained 39.54 mg GAE/g (gallic acid equivalents per gram), which was approximately 1.7 times higher than the total phenolic content observed in the methanol extract derived from *S. vaginatum* roots, amounting to 22.74 mg GAE/g.[84]

Crocus sativus L.

Saffron is derived from the dried crimson stigmas of *C. sativus* L., an autumn-flowering herbaceous plant that falls under the Iridaceae family. It is predominantly grown in countries including Iran, India, Afghanistan, Greece, Morocco, Spain, and Italy.[88] Saffron is believed to have been initially recorded in an Assyrian botanical document from the 7th century BC during the reign of Ashurbanipal. Subsequently, historical records spanning 4,000 years have revealed its utilization in the treatment of around 90 different medical conditions.[89,90] The annual estimated consumption is 5 metric tonnes.[22]

Adulterant

Carthamus tinctorius L., commonly known as safflower or sometimes referred to as false saffron, is a member of the Asteraceae plant family.[91]

Due to high cost of saffron it is frequently susceptible to adulteration, leading to the utilization of more affordable substitutes that can be classified into three primary categories. Firstly, there are materials that imitate the appearance of saffron, including the pale, slim styles of crocus, as well as stamens and strips from the saffron crocus corolla. Additionally, adulteration may involve the rejuvenation of depleted saffron by introducing dyes to revive its color and visual appeal. Lastly, unscrupulous practices include introducing foreign substances to saffron with the aim of increasing its weight and, consequently, profits. Instances of such additional materials encompass ligulate florets from marigold (*Calendula officinalis*) and *Arnica montana*, stigma of *Crocus cartwrightianus, Crocus kosaninii, Crocus kotschyanus* and *Crocus speciosus,* fruit or fruit extract of *Gardenia jasminoides,* sometimes coloured with methyl orange and referred to as feminell or Chinese safflower, ligulate florets of safflower (*Carthamus tinctorius*), the slender stems and roots of specific monocotyledon plants that have been artificially coloured, and even the stigmas of *Zea mays* Linn, known as corn silk.[89,92]

Ethnopharmacology, Marketed formulations and Therapeutic uses

C. sativus

C. sativus, notably the most renowned species, has been extensively documented for its significant historical uses. The dried stigma, referred to as saffron, played multifaceted roles as a medicinal substance, dye, perfume, and condiment in ancient civilizations such as those of India, Egypt, Greece, Persia, and Rome. Within Islamic Traditional Medicine, saffron earned widespread recognition as a nerve tonic and aphrodisiac. It found applications in treating conditions like dysmenorrhea and premature ejaculation. In Iran, "Kal mas" or "Zaferan," (stigma), is specifically employed for heart strengthening. Indian Traditional Medicine recognizes the saffron stigma, commonly referred to as "Kesar," as a nerve sedative and aphrodisiac, often used to enhance immunity. Traditional Chinese Medicine recommends the topical application of saffron stigma for addressing nervous system disorders, alleviating asthma, pertussis, and inflammation. In Iraq, the stigma and style, known as "Zeferan," have been utilized to treat insomnia, migraine, and stimulate metabolism. In Spain, popularly labeled as "Azafran," it is employed for relieving toothaches. Additionally, in Italy, saffron decoction, known as "Zafferano," is suggested as a digestive and sedative preparation, while the infusion

serves as a mouth rinse.[93-98]

Modern pharmacological research has uncovered a range of beneficial properties associated with saffron, such as antidepressant, anti-inflammatory, anti-tumour, and memory-enhancing effects.[99-102] Saffron extract demonstrates chemoprotective properties, shielding against oxidative stress induced by genotoxins in mice.[103-106] Studies have reported anticonvulsant effects in mouse models for both PTZ and maximal electroshock (MES) seizures, suggesting potential applications in neuropharmacology.[89,107]

It is a component of formulations such as Tentex forte, and J.P. Nikhar oil (Jamuna Pharma).[53]

C. tinctorius

Examining the historical context, safflower seeds, packets, and floral garlands were frequently discovered in the vicinity of ancient Egyptian mummies. In various regions of Iran, safflower is commonly consumed in its raw form. The use of safflower dye extended to European cuisines, serving both as a flavoring and coloring agent. Particularly noteworthy was the pivotal role of safflower dyes in the carpet-weaving industries of Europe, the Middle East, and the Indian subcontinent. This specialized application aligns with its binomial name, "tinctorius" that typically have historical association with dyeing. In Thailand, the aqueous extract of safflower flowers has been extensively utilized as hair color. In Indian traditional medicine, safflower is commonly employed for addressing conditions such as scabies, arthritis, mastalgia, amenorrhea, gastric tumors, wounds, and various ailments, both internal and external, as per Chinese folklore. Iranian traditional medicine recognizes safflower for its efficacy in treating issues like skin patches, baldness, phlegm, and colic. Persian folk medicine has historically utilized *C. tinctorius* for managing diabetes, fever, and dropsy. Moreover, certain plants from the Compositae family have been traditionally known for their abortion-promoting properties. In traditional medicine, water extracts of safflower find application in alleviating painful menstruation, serving as a sedative, acting as a laxative for constipation, and possessing anti-inflammatory properties. The dried florets of *C. tinctorius* (Carthami flos) have gained popularity for their widespread use in treating various heart and gynecologic diseases.[91,108-112]

Phytochemistry

C. sativus

Saffron is characterized by specific components that give it its unique properties. These include crocin (33%), responsible for its color, picrocrocin (24%), which imparts bitterness, and safranal (13%), responsible for its aroma and scent.[57] Saffron is composed of over 150 volatile compounds contributing to its aroma. In addition to these, saffron contains non-volatile active components, many of which are carotenoids, such as, zeaxanthin, lycopene, α- and β-carotenes.[113] Further studies have identified various flavonoids and bioflavonoids.[89,114-118]

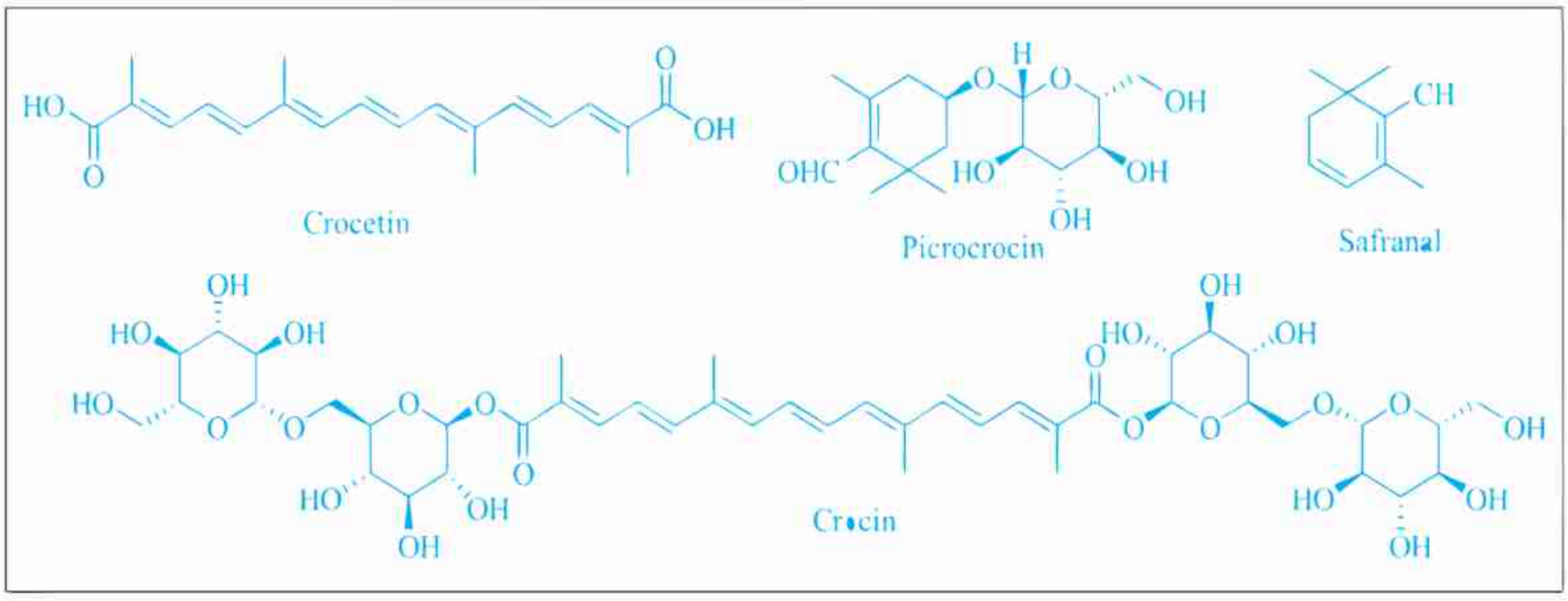


Figure 4 : Major Phytoconstituents in *C. sativus*

C. tinctorius

C. tinctorius, is rich in diverse compounds. It contains over 200 substances, including flavonoids, phenylethanoid glycosides, fatty acids, and steroids. The seed oil composition resembles that of olive oil, with high levels of linoleic (63%-72%) and oleic acids (16%-25%).[119] In safflower flowers, triterpene alcohols like heliaol and flavonoid glycosides such as carthamin are predominant.[120,121] Luteolin and its glucopyranosides are found in both flowers and leaves.[122,123] Safflower also yields unique compounds like tinctormine, hydroxysafflor yellow A, safflor yellow B, safflomin C, and nicotiflorin.[124,125] Additionally, the safflower essential oil comprises components like caryophyllene, *p*-allyltoluene, 1-acetoxytetralin and heneicosane.[91,108,126]

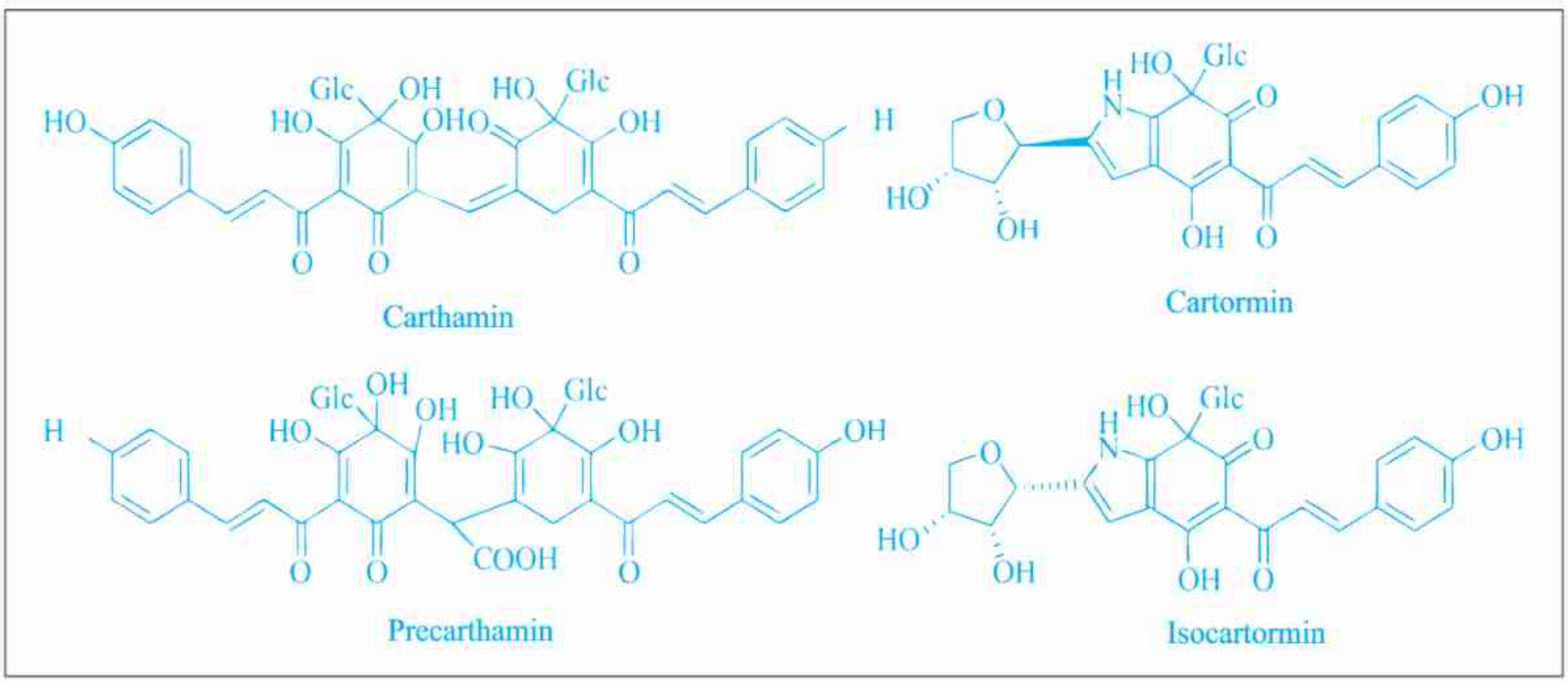


Figure 5 : Major Phytoconstituents in *C. tinctorius*

Comparative methods

Saffron and safflower are distinct plants with different growth habits, flower characteristics and parts of interest. Saffron is renowned for its red stigmas used as a spice, while safflower is cultivated for its oil-rich seeds.[89,118,127,128]

Table 4 : Comparative identification of *C. sativus* and *C. tinctorius*

Features	*C. sativus*	*C. tinctorius*
Botanical identification		
Growth Habit	Small plant, 10-25 cm in height	Larger plant, 30-150 cm in height
Propagation	Propagated through bulb or corm division	Grown from seeds
Macroscopic identification		
Flowers	Lily-like, purple tepals, intense red stigma	Composite flowers, yellow, orange, or red, lasting 3-4 days
Organoleptic characters	Bright orange stigma, strong aromatic odor	Flowers in yellow, orange, or red colors

DNA barcoding

A technique known as Barcoding Melting Curve Analysis (Bar-MCA) was employed to identify adulterants. This method utilizes DNA barcoding region trnH-psbA. By optimizing DNA concentrations and annealing temperatures during amplification, distinct peaks were observed at specific temperatures for saffron (at 81.92°C) and the adulterant *C. tinctorius* (at 80.10°C). Notably, the melting curves created for saffron and its adulterants exhibit noticeable differences in peak locations or shapes.[129]

HPLC/PDA/MS

The HPLC/PDA/MS specificity enabled the clear and unambiguous identification of marker molecules specific to the adulterant, distinguished through their absorbance and mass values.[130]

FT-IR

FT-IR spectroscopy and chemometric techniques were employed to differentiate saffron from other plant-derived materials to detect potential adulteration. Specific spectral features, such as hydroxyl and carbonyl groups were used to distinguish between different substances. Adulterant identification was achieved by analyzing distinct spectral regions. The band at 1706 cm^{-1} primarily results from the stretching of the C=O bonds in -COOR groups was observed mainly in saffron. However, in safflower, the band associated with the stretching of C=C bonds or conjugated C=O shifted to around 1631 cm^{-1}. The PLS-DA models exhibited strong performance in effectively distinguishing between pure saffron and adulterated samples, demonstrating both high sensitivity and specificity rates. Variable selection methods were employed to enhance model performance, and limits of detection were determined for various adulterants. Analytical sensitivity was assessed to estimate the minimum concentration difference that can be detected. This approach showed promise for detecting adulteration in saffron samples at low levels.[131]

Cyanthillium cinereum Less.

Cyanthillium cinereum, previously known as *Vernonia cinerea*, is herbaceous plant from the Asteraceae family that can be found extensively in tropical regions, particularly in Southeast Asia.[132,133]

Adulterant

Emilia sonchifolia (L.) DC. from the Asteraceae family is an annual, partly upright herb that grows abundantly in India, commonly considered a weed in cultivation and wastelands.[135] It shares resemblance in terms of its flower and fruit features with *C. cinereum*. Furthermore, these two plants are frequently found in the same environment. Inadequate harvesting methods and the absence of standardized quality control have resulted in contamination by *E. sonchifolia*.[132,135]

Ethnopharmacology, Marketed formulations and Therapeutic uses

C. cinereum

With significant medicinal value recognized across diverse traditional practices and acknowledged in Ayurveda, this plant offers a wide range of therapeutic applications. Its uses include decoctions or infusions of the entire plant for treating fever, addressing urinary bladder spasms, and alleviating strangury. In combination with quinine, it is utilized for malaria treatment attributed to sesquiterpene lactones with antimalarial activity. The therapeutic potential extends to addressing asthma, cancer, diarrhoea, etc. Its seeds serve as sources for alexipharmic and anthelmintic drugs, offering alternative treatments for leprosy and skin diseases. The stem/bark paste is applied for wound healing; flowers are used for conjunctivitis and arthritis. Additionally, the root infusion acts as an antidote to venoms and bites from snakes or scorpions. This rich history in traditional medicine underscores its diverse pharmacological attributes, including pain relief, anti-inflammatory effects, antibacterial qualities, and antioxidant potential. It also demonstrates ameliorative properties, diuretic and antidiuretic effects, and the ability to control mosquito larvae.[137-144]

Key formulations, namely Candrakala Rasa and *Alamottadi Kashayam*, are recommended for therapeutic purposes, addressing conditions like *Jvara, Vismajvara, Sidhma, Visphota, Bhutabadha, Grahabadha, Sphotaka, Pradara,* and *Slipada*. The suggested dosage is 10-20 ml in *Swarasa* form and 5-10 g in powder form (external use).[14]

E. sonchifolia

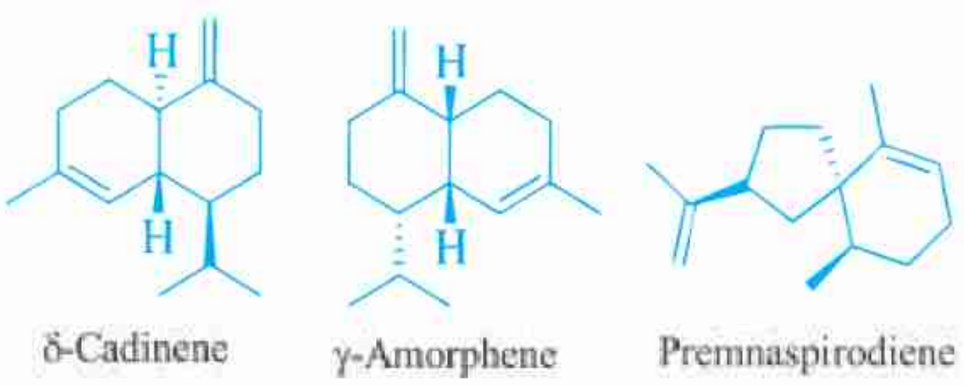
In traditional medicine, the juice extracted from the

leaves of E. sonchifolia is used to treat a variety of ailments of eyes, wounds, sore ears, and bowel complaints.[145] Chinese traditional medicine employs the leaves to address fever and dysentery.[146] African folk medicine uses leaf tea for dysentery treatment.[147] Moreover, people chew the flower heads and hold them in their mouths for around 10 minutes to prevent tooth decay.[148] This plant is recognized for its astringent, depurative, diuretic, expectorant, and febrifuge.[149] Furthermore, a wide range of biological activities associated with *E. sonchifolia* include cytotoxic and antitumor effects, anti-inflammatory properties, antinociceptive attributes, modulatory effects, antiviral activity, and benefits for erythropoiesis and liver protection.[150-154]

Phytochemistry

C. cinereum

A chemical analysis of *C. cinereum* revealed the presence of several classes of natural compounds. Notably, hirsutinolide-sesquiterpene lactones are the differentiating compounds. The primary components of the essential oil obtained were *β*-caryophyllene, *δ*-cadinene, *γ*-amorphene, *cis*-*β*-guaiene, premnaspirodiene, and 9-epi-*β*-caryophyllene. This oil is rich in sesquiterpene hydrocarbons accounting for 81.2% of its composition.[133,144]



Vercinolides (A-H)	R_1	R_2
Vercinolide A	A	Ac
Vercinolide B	A	Me
Vercinolide C	B	Ac
Vercinolide D	B	Me
Vercinolide E	B	H
Vercinolide F	B	Ac
Vercinolide G	B	Me
Vercinolide H	A	Ac

Figure 6 : Major Phytoconstituents in *C. cinereum*

E. sonchifolia

The non-volatile compounds found in the ethanolic extract include flavonoids such as rhamnetin, isorhamnetin, quercetin, luteolin, falavonoids such as tricin-*7-O-β*-D-glucopyranoside, 5,2',6'-trihydroxy-7,8-dimethoxyflavone-2'-*O-β*-D-glucopyranoside, and organic acids such as succinic acid, fumaric acid, *p*-hydroxybenzoic acid, 4-hydroxy isophthalic acid, 3,4-dihydroxycinnamic acid, and other compounds such as esculetin, isowedelolactone, and uracil.[155] Additionally a number of pyrrolizidine alkaloids are reported including senkirkine, doronine, senecionine, seneciphylline, integerrimine, otosenine, neosenkirkine, and doronine.[135,156,157]

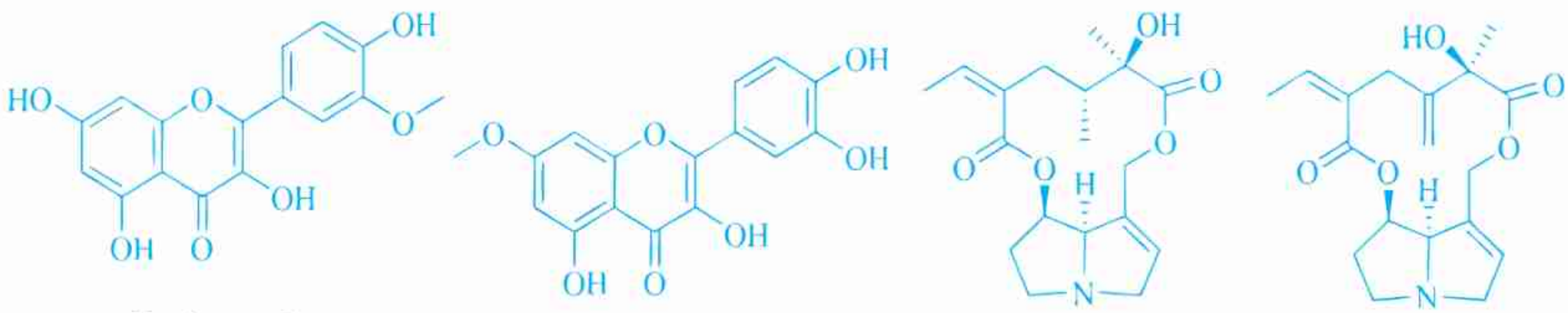


Figure 7 : Major Phytoconstituents in *E. sonchifolia*

Comparative methods

Pharmacognostic differentiation

C. cinereum and *E. sonchifolia* are herbaceous plants with slender stems that fall within the Asteraceae family. They both produce flower capitula with either purple or white florets, and the flowers of *C. cinereum* bear a striking resemblance to those of *E. sonchifolia*. Nevertheless, unique characteristics become evident when examining them on a macroscopic level.[132]

Table 5 : Comparative identification of *C. cinereum* and *E. sonchifolia*

Features	*C. cinereum*	*C. sonchifolia*
Macroscopic identification		
Leaves	Sserrated edges, tapering or narrowed bases	Auriculate bases, triangular or pinnate-lobed lower leaves, arrow-shaped upper leaves wrapping around the stem
Microscopic identification		
Pappi	More than three rows of elongated cells	2 to 3 rows of unicellular trichomes
Trichomes	Greater length, T-shaped and glandular trichomes	Lesser length, multicellular trichomes in a single row (uniseriate)
Cortex	2-4 layers of collenchyma cells	1-3 layers of collenchyma cells

DNA barcoding

A genetic analysis was conducted to distinguish between *C. cinereum* and *E. sonchifolia*. The study utilized four DNA barcode regions and found that specimens of the same species had identical DNA sequences, with no intraspecific divergence. However, variations in sequence length and insertions/deletions (indels) were observed. The highest sequence variation was in the ITS region, making it a useful tool for differentiation. This genetic analysis is valuable for accurate identification of these plant species.[132]

HPTLC/GC-MS

HPTLC profiles were generated for the ethanolic extracts of *C. cinereum* and *E. sonchifolia*. The patterns appeared similar, but a distinct band at Rf of 0.48 was found only in *C. cinereum*. GC-MS analysis revealed four triterpenoid compounds (β-amyrin, taraxasterol, lupeol, and betulin) exclusively in *C. cinereum*. This chemical difference can be used to distinguish between the two plant species.[132]

Inula racemosa Hook.

Inula racemosa Hook is a perennial herb, critically endangered and can be found across the Himalayan region. It is known as "Puskarmool" belonging to family Asteraceae in Ayurvedic medicine.[158,159] The annual estimated consumption is 3 metric tonnes per annum.[160,161]

Substitute

Saussurea lappa Clark. also known as *Saussurea costus*, belonging to the Asteraceae family.[162] As a result of excessive exploitation, this species has also reached a critical endangerment status in its natural habitat. It is noted that *I. racemosa* is substituted with *S. lappa* and vice versa.[163] Extensive research has explored the chemical properties of these constituents.

Ethnopharmacology, Marketed formulations and Therapeutic uses

I. racemosa

Puskarmool, a vital herb in Ayurvedic medicine, acts as an expectorant and bronchodilator, treating skin diseases and tuberculosis.[164] Traditional Indian Medicine has long utilized it for ailments related to "*Pranavaha Srotas Vikara*," as mentioned in ancient texts like Bhav Prakasha Nighantu and *Charaka Samhita*. This herb, known as "*Pushkara Dravya*" and "*Madhura Gandha*" in the Vedas, is recommended for various conditions such as inflammation, cough, mental disorders, cardiac problems, anemia, and bronchial asthma. [165] Its effectiveness in balancing (*Vata-Kapha Dosha*) is attributed to its pungent and bitter taste. It has a pungent taste after digestion (*Katu Vipaka*) and its ability to treat hiccups (*hikka*), respiratory issues (*Shwasa*), fever (*Jvara)*, and chest pain are documented (*Charaka Samhita)*.[166]

The primary active ingredient is derived from the roots of *I. racemosa* as many polyherbal formulations, particularly for treating spleen-related inflammatory conditions and cardiac ailments. [167] In Traditional Tibetan Medicine (TTM), *I. racemosa* is employed to treat acute intestinal inflammation, bacillary dysentery, relieve shoulder pain and neck, address liver issues, prevent abortions, and alleviate abdominal swelling and bronchial asthma.[168,169] In Pakistan, the roots of *I. racemosa* are commonly used as a substitute for *S. lappa* in treating chronic bronchitis, asthma, and pulmonary disorders. Additionally, in Ladakh, indigenous communities utilize the plant's roots for various purposes, including as an antiseptic, anti-parasitic, diuretic, blood pressure-lowering agent, and to promote peristaltic movements.

It is a component of formulations such as *Khadiradi Gutika* (Dabur India limited), *Divya mukta vati* (Patanjali Ayurved limited), Pushkarmool tablets (Bioayurveda), Pushkarmool Herbal Powder (Heilen Biopharm), Breathe free capsules (Organic India), Pushkarmool powder (Herbs Forever, USA), Epilac tablet (Ayursun Pharma), Pushkarmool churna (Tansukh Herbals Pvt. Ltd.), Pushkarmool capsules (Chakrapani Ayurveda), Cardiwin DS tablets (Ayurvedant).[158]

Saussurea lappa

S. lappa, commonly known as kuth root or costus, is widely acknowledged for its effectiveness in managing over 43 different diseases. Ayurvedic practitioners make use of *S. lappa* roots and stalks due to their diverse therapeutic properties, including being antiulcer, anti-inflammatory, antifungal, antihelmintic, antidiabetic, and antihepatotoxic. In Ayurveda, it finds application in treating a spectrum of conditions like flatulence, cold, pruritus, epilepsy, itching, headache, leukoderma, and gout.

In the realm of Traditional Tibetan Medicine, *S. lappa* holds a significant place in more than seventy formulations. It contributes to addressing issues like lung inflammation and chest congestion, often combined with ingredients like hippophae to combat lung inflammation. Moreover, it is acknowledged for its capacity to stimulate the brain and address disorders associated with the liver, blood, and kidneys. *S. lappa* is recommended for diverse conditions such as leprosy, tuberculosis, hiccups, malaria, intestinal carcinogenesis, and edema.[170-182]

Phytochemistry

I. racemosa

I. racemosa produces significant quantities of sesquiterpene lactones, including alantolactone (0.7%) and isoalantolantlactone (0.4%) as the major constituents.[183] Alloalantolactone, dihydroisoalantolactone, dihydroalantolactone, inunolide, dihydroinunolide, *neo*-alantolactone, isoalloalantolactone, inunal, isoinunal, alantodiene and soalantodieine are some of the other sesquiterpene lactones.[184] Daucosterol, β-sitosterol and D- mannitol are also present in roots. Aerial parts of *I. racemosa* possess various other sesquiterpene lactones, including 1-desoxy-8-epi-ivangustin, 8-

epiisoivangustin, propionyloxycostunolide, 9β-(2-methylbutaryloxyl) costunolide, 4β-5α-epoxy-10α, 4β, 5α-epoxy-4,5-*cis*-inunolide. Six known eudesmane-12,8-olides were also identified,[185] including septuplinolide, macrophyllilactone E, isoalantolactone, and 11,13-dihydroivalin.[186]

lappa

It roots contain diverse active compounds: sesquiterpenes, flavonoids, lignans, phytosterols, alkaloids, terpenes, anthraquinones, and more. Among these, sesquiterpenes and flavonoids are key drivers of the varied pharmacological effects are categorized into guaiane, eudesmane, and germacre groups based on their carbocyclic structure. [187] The guaiane class comprises different sesquiterpenes, including 3-

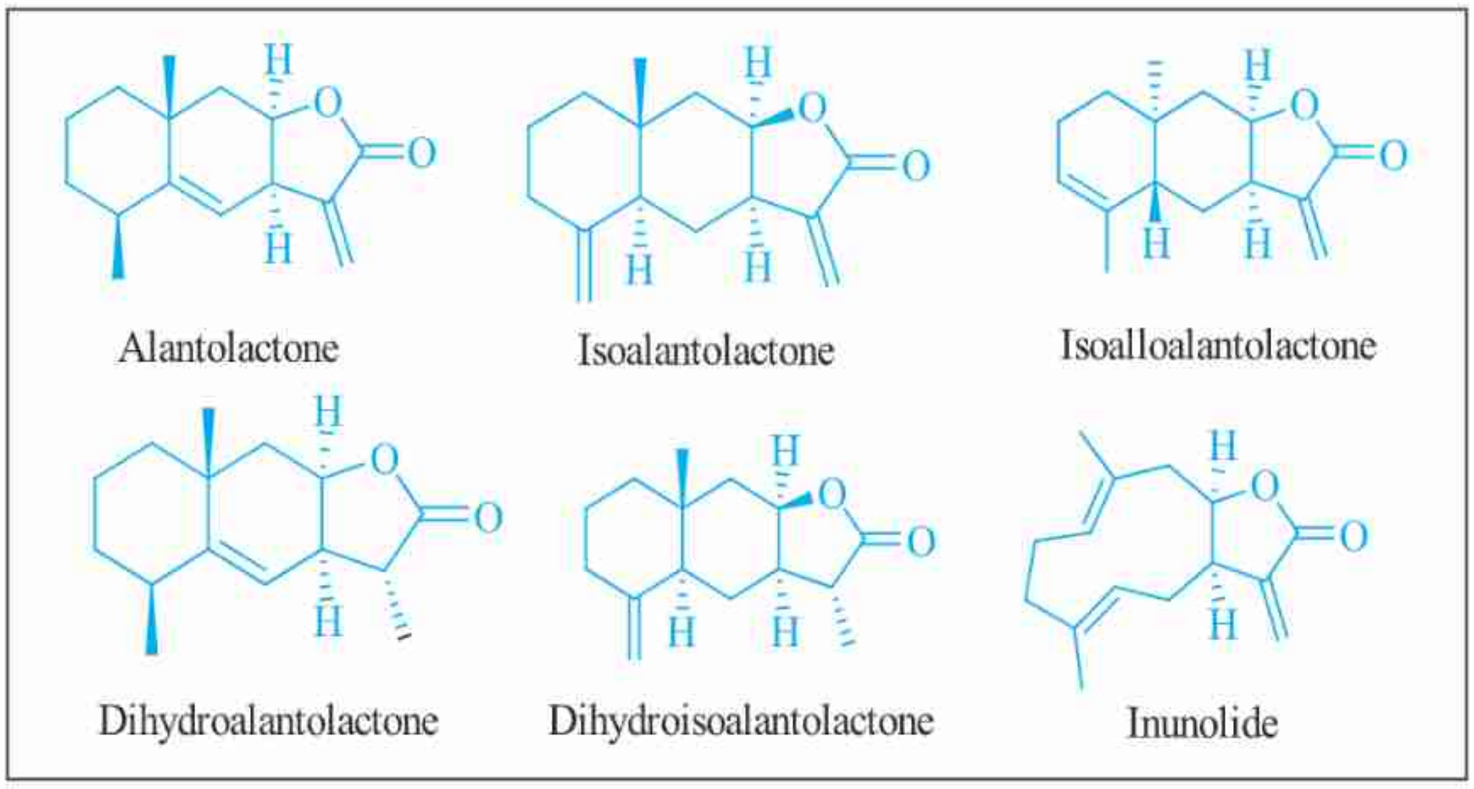


Figure 8: Major phytoconstituents in *I. racemosa*

Epizaluzanin C, cynaropicrin, zaluzanin C (0.0023%), 11β,13-Dihydro-3-epizaluzanin C, 11β,13-Dihydro-3-epizaluzanin C, isodehydrocostuslactone, dehydrocostuslactone, and 4β-methoxy dehydro costuslactone. [188,189] The various compounds in eudesmane group are saussureal, β-cyclocostunolide, saussureamine D, saussureamine E, santamarine.[190] Several germacranes were isolated like *S. lappa* include dihydrocostunolide, costunolide, methoxydihydrocostunolide, germacrene.[191] Among these, dehydrocostus lactone and costunolide are the major constituents.

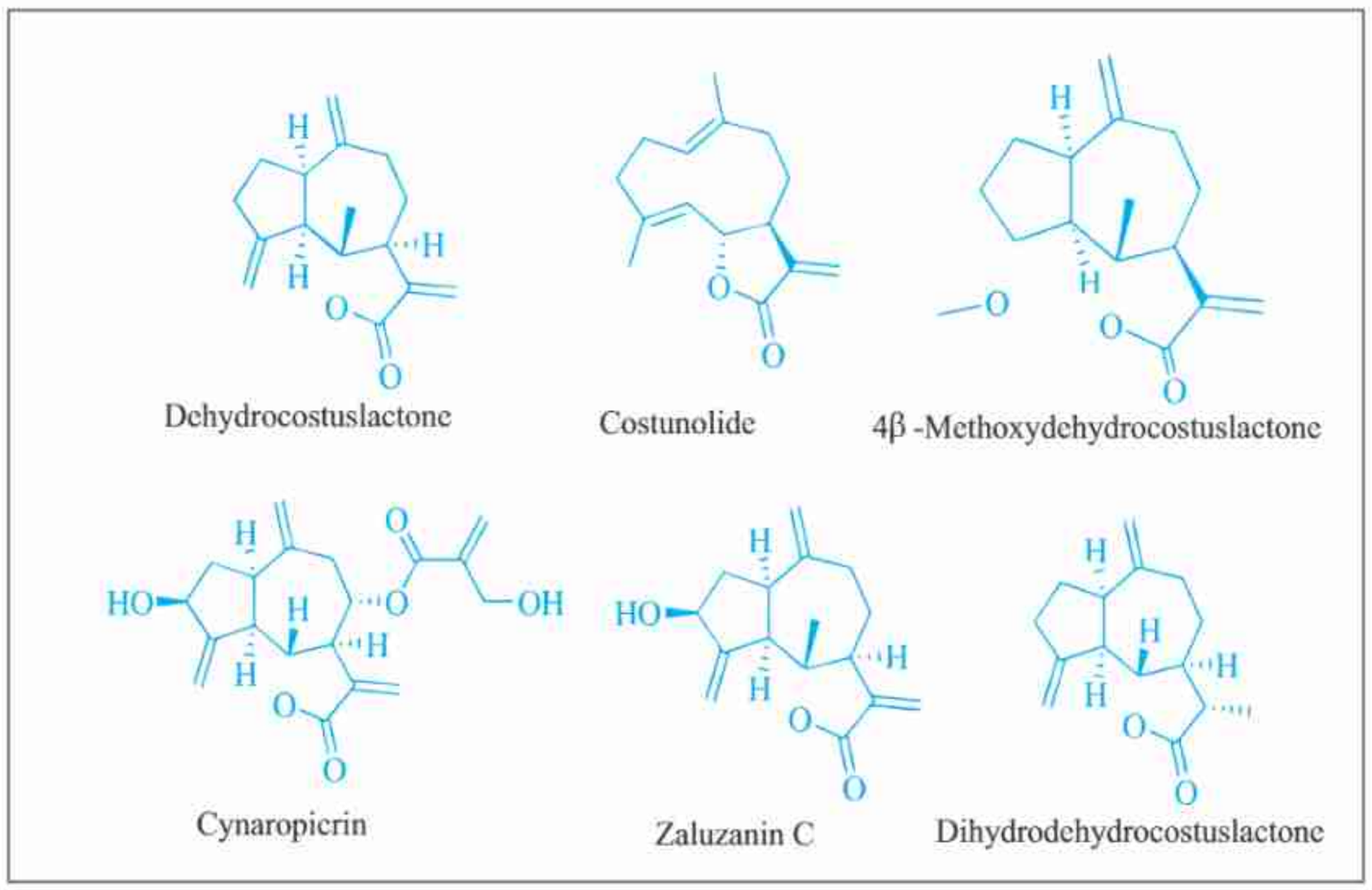


Figure 9 : Major Phytoconstituents in *S. lappa*

Comparative methods

Pharmacognostic differentiation

To distinguish between the two plants, specific characteristics are described below:

Table 6 : Comparative identification of *I. racemosa and lappa,* [158,162,187]

Features	*Inula racemosa*	*Saussurea lappa*
Botanical identification		
Growth Habit	Grooved stem, 1-5 feet tall, at elevations of 5000 to 14000 ft	Tall, reaching 1-2 m at elevations between 2500 to 3000 m
Microscopic identification		
Roots	Rough, branched, irregularly fusiform, short, brownish grey to yellowish bark	Short, smooth, with a dirty grey to yellow colour externally
Root Microscopic identification		
Diameter	1-2 cm	5 cm
Xylem	Scarce fibers in small patches attached to vessel groups in the central part of the xylem	Well-developed fibers arranged concentrically around vessels
Vascular cambium	Phelloderm typically consists of 2-4 layers.	Phelloderm typically consists of 3-6 layers.
Rhytidomes	Prominent with deep vertical cracks	Faint with slender vertical cracks
Vessela	Horizontally slit-like pits, and a few have round bordered pits	Vessels primarily feature scalariform to reticulate thickenings
Cortex	Radial cracks visible in aging roots	Solid, devoid of radial cracks

Thin-layer chromatography

A unique thin-layer chromatography test was developed to differentiate between *Saussurea lappa* and *Inula racemosa*. The treatment of chromatogram (silica gel) with sulfuric acid produces green and purple spots with *S. lappa* shows, and brown spots with *Inula racemosa*.[192]

qNMR

Quantitative Nuclear Magnetic Resonance (qNMR) was utilized to analyze primary sesquiterpene lactones in medicinal plants *I. racemosa* and *S. lappa*. The method quantified alantolactone and isoalantolactone as major compounds in *Inula racemosa*, and costunolide and dehydrocostuslactone as major compounds in *S. lappa*. The qNMR results matched those obtained from HPLC and HPTLC analyses. All these four sesquiterpene lactones contained-methylene–lactone moiety where the-methylene protons of all four compounds showed distinctive signals in their ^{1}HNMR spectra. In all three cases, These differences noticed in the chemical shifts of α-methylene protons, such as δ 6.22/5.64 (doublet, J = 1.7 Hz) for alantolactone and δ 6.14/5.60 (doublet, J = 0.7 Hz) for isoalantolactone in *I. racemosa*, and δ 5.54/6.28 (doublet, J = 3.4 Hz) for costunolide and δ 5.51/6.24 (doublet, J = 3.3 Hz) for dehydrocostuslactone in *S. lappa* can serve as key markers for plant differentiation. Quantitative analysis via the MEKC method (Micellar Electrokinetic Chromatography) in HPTLC reveals variations: *I. racemosa* shows 0.98-1.01% alantolactone and 1.20-1.29% isoalantolactone, while *S. lappa* displays 0.07-1.00% costunolide and 0.04-1.21% dehydrocostuslactone. These differences provide a basis for plant

identification, and anydeviations in chemical profiles may indicate potential substituents emphasizing the need for authenticity verification through complementary tests and reference databases.[193]

Conclusions

Medicinal plants display considerable morphological variations influenced by natural hybridization and environmental factors. Moreover, the features used for identification, such as flowers, leaves, stems, and berries, can overlap and change with the season and the age of the plant, making it challenging to identify them in the field. Evaluations based on the visual aspects and sensory properties of plant material, combined with microscopy, remain effective for rapidly detecting only major alterations in medicinal plants. DNA-based methods hold potential as reliable tools for verifying authenticity, particularly in discerning closely related species and varieties. This is particularly valuable when traditional taxonomic methods, like microscopy, face difficulties in differentiation.

Traditional herbal fingerprint analysis relies on classical chromatographic methods, which offer comprehensive data on plant metabolites but are time-consuming and involve meticulous sample preparation. To meet the need for quicker and more cost-effective analysis, emerging methods are being developed to complement or replace classical techniques. Spectroscopy, recognized for its straightforward sample preparation, high reproducibility, and ability to simultaneously detect various plant metabolites, is gaining prominence as a rapid and affordable analytical technique. Although spectroscopic methods alone cannot provide detailed information on a sample's chemical composition or specific markers, the integration of spectral data with chemometrics analysis proves instrumental in extracting crucial information for authenticity testing and the identification of undesired components in herbal raw materials.

References

1. Dixit V K 2011 Controversial ayurvedic herbs. J. Adv. Pharm. Technol. Res. 2, 78.
2. Hamilton A C 2004 Medicinal plants, conservation and livelihoods. Biodivers. Conserv.13, 1477-517.
3. Chen S L, Yu H, Luo H M, Wu Q, Li C F and Steinmetz A 2016 Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chinese Medicine. 11, 1-0.
4. Shaheen S, Ramzan S, Khan F and Ahmad M (2019) Adulteration in herbal drugs: A burning issue, Springer International Publishing, NY, US.PPI-118.
5. Agnivesha, Charaka, Dridhabala (2005) Charakasamhita, Vimana Sthana, Rogabhishjitiya Vimanam, 8/149, edited by Vaidya Jadavaji Trikamaji Acharya. Chaukhamba Surbharati Prakashan, Varanasi, P254.
6. Joshi P R, Patel, B R and Shukla V J 2012 An overview of the causes of current practices in Pratinidhi Dravyas (substitution of drugs) in Ayurveda including newer techniques for their evaluation. *Ayu*. 33, 481.
7. Vagbhata A H (2007) Sutrasthana 15/46, edited by Pt. Hari Sadashiva Shastri Paradakara. Chaukhamba Surbharati Prakashan, Varanasi.
8. Keshari P 2021 Controversy, adulteration and substitution: Burning problems in ayurveda practices. Int. J. Ayurvedic med. 5, 2505-2516.
9. Kucharska-Ambrożej K, Karpinska J 2020 The application of spectroscopic techniques in combination with chemometrics for detection adulteration of some herbs and spices. Microchem. J. 153, 104278.
10. Osman A G, Raman V, Haider S, Ali Z, Chittiboyina A G, Khan I A 2019 Overview of analytical tools for the identification of adulterants in commonly traded herbs and spices. J. AOAC Int. 102, 376-85.
11. Ganie S H, Upadhyay P, Das S and Sharma M P 2015 Authentication of medicinal plants by DNA markers. Plant Gene. 4, 83-99.
12. Mahima K, Sunil Kumar K N, Rakhesh K V, Rajeswaran P S, Sharma A and Sathishkumar R 2022 Advancements and future prospective of DNA barcodes in the herbal drug industry. Front. Pharmacol. 13, 947512.
13. Sharma A, Shanker C, Tyagi L K, Singh M and Rao C V 2008 Herbal medicine for market potential in India: an overview. Acad J Plant Sci. 1, 26-36.
14. Anonymous (1989) The Ayurvedic Pharma-copoeia of India, Part-I, vol.-I, II and III, first Englished., Government of India, Ministry of Health and Family Welfare, Department of Health, New Delhi.
15. Mishra B (2002) Bhavaprakash Nighantu, Vidyotini, Chaukhamba Sanskrit Sansthan Varanasi 10, 181-183.
16. Anonymous (2015) Yogratnakar, Vidyotini Hindi commentary, Shri Lakshmipati Shastri Commentator, Brahmashankar Shastri Editor, P- 171.
17. Shastri A. (2005) Baishajya Ratnavali, Chaukhambha Sanskrit Sansthan, Varanasi.
18. IUCN (2023) The IUCN red list of threatened species. Version 2022-2.https://www.iucnr edlist.org. Accessed on

21.11.202

19. Gowthami R, Sharma N, Pandey R and Agrawal A 2021 Status and consolidated list of threatened medicinal plants of India. Genet. Resour. Crop Evol. 68, 2235-63.
20. Chander V, Aswal J S, Dobhal R and Uniyal D P 2017 A review on Pharmacological potential of Berberine; an active component of Himalayan *Berberis aristata*. J. Phytopharmacol. 6, 53-58.
21. Srivastava S K, Khatoon S, Rawat A K, Mehrotra S and Pushpangadan P 2001 Pharmacognostic evaluation of the root of *B. aristata* DC. Pharm. Biol. 7, 102-106.
22. Shenoy P K R and Yoganarasimhan S N 2009 Evaluation of antibacterial activity of Elanir kujambu-an Ayurvedic eye formulation. Indian J Tradit Know 8, 272-274.
23. Sharma A, Shanker C, Tyagi L K, Singh M, Rao C V 2008 Herbal medicine for market potential in India: an overview. Acad J Plant Sci. 1, 26-36.
24. Srivastava S K, Singh Rawat A K and Mehrotra S 2004 Pharmacognostic evaluation of the root of *Berberis asiatica*. Pharm. Biol. 42, 467-473.
25. Belwal T, Bisht A, Devkota H P, Ullah H, Khan H, Pandey A, Bhatt I D and Echeverría J 2020 Phytopharmacology and clinical updates of Berberis species against diabetes and other metabolic diseases. Front. Pharmacol. 11, 41, 1-27.
26. Potdar D, Hirwani R R and Dhulap S 2012 Phyto-chemical and pharmacological applications of *Berberis aristata*. Fitoterapia. 83, 817-30.
27. Srivastava S, Srivastava M, Misra A, Pandey G and Rawat A 2015 A review on biological and chemical diversity in Berberis (Berberidaceae). EXCLI J. 14, 247.
28. Kirtikar K R and Basu B D (1935) Indian medicinal plants, Vol. III, Lalit Mohan Basu Publication, Allahabad.
29. Uniyal M R 1964 Medicinal plants of the Bhagirathi valley lying in the Uttarkashi forest division U.P. Indian For. 94, 407-20.
30. Shah N C and Joshi M C 1971 An ethnobotanical study of the Kumaon region of India. Eco Bot. 25, 414-22.
31. Chauhan N S, Uniyal M R and Sannad B N 1978 A preliminary study of the indigenous drug used at Tibetan medical centre; Dharamshala (H.P.). Nagarjuna. 22, 190-3.
32. Jain N and Suri R K 1980, Insecticidal, insect repellent and pesticidal plants of Dehradun, Nagarjuna. 23, 177-81.
33. Mittre V 1981 Wild plants in Indian folk life - A historical perspective. In: Jain SK (ed): Glimpses of Indian ethnobotany, Oxford & IBH Publ. Co., New Delhi, PP 37-58.
34. Sharma P K, Chauhan N S and Lal B 2005 Studies on plant associated indigenous knowledge among the Malanis of Kullu district, Himachal pradesh. Indian J. Tradit. Knowl. 4, 403-8.
35. Tiwari J K, Ballabha R and Tiwari P 2010 Ethnopaediatrics in Garhwal Himalaya, Uttarakhand, India. (Psychomed Med), New York. Sci J. 3, 123-6.
36. Chhetri D R, Parajuli P and Subba G C 2005 Antidiabetic plants used by Sikkim and Darjeeling Himalayan tribes, India. J. Ethnopharmacol. 99, 199-202.
37. Blasko G, Murugesan N, Freyer A J, Shamma M, Ansari A and Atta-ur-Rahman 1982 Karachine: an unusual protoberberine alkaloid. J Am Chem Soc. 104, 2039-41.
38. Atta-ur-Rahman and Ansari A 1983 Alkaloids of *Berberis aristata* - isolation of aromoline and oxyberberine. J Chem Soc Pak. 5, 283-4.
39. Blasko G, Murugesan N, Freyer AJ, Shamma M, Ansari A and Atta-ur-Rahman 1982 Taxilamine, a pseudobenzylisoquinoline alkaloid. Heterocycles. 19, 257-9.
40. Sivakumar R and Nair A G 1991 Polyphenolic constituents of the flowers of *Berberis aristata*. J Indian Chem Soc. 68, 531-2.
41. Saied S, Batool S and Naz S 2007 Phytochemical studies of *Berberis aristata*. J Basic Appl Sci. 3, 1-3.
42. Garhwal S 2010 Analysis of berberine content using HPTLC fingerprinting of root and Phytochemical and pharmacological studies in genus Berberis bark of three Himalayan Berberis species. Asian J. Biotechnol. 2, 239-245.
43. Bajpai V, Singh A, Arya K R, Srivastava M and Kumar B 2015 Rapid screening for the adulterants of *Berberis aristata* using direct analysis in real-time mass spectrometry and principal component analysis for discrimination. Food Additives and Contaminants: Part A. 32, 799-807.
44. Singh A, Bajpai V, Kumar S, Kumar B, Srivastava M, Arya K R and Sharma K R 2016 Distribution and discrimination study of bioactive compounds from Berberis species using HPLC-ESI-QTOF-MS/MS with principle component analysis. Nat. Prod. Commun. 11, 1934578X1601101209.
45. Balasubramani S P, Goraya G S and Venkata- subramanian P 2011 Development of ITS sequence-based markers to distinguish *Berberis aristata* DC. from *B. lycium* Royle and *B. asiatica* Roxb. 3 Biotech. 1, 11-9.
46. Bhatt M and Kothiyal P 2015 A review article on phytochemistry and pharmacological profiles of *N. jatamansi* DC-medicinal herb. J. pharmacogn. phytochem. 3, 102-6.
47. Kumar V P, Chauhan N S and Rajani M 2006 Search for antibacterial and antifungal agents from selected Indian medicinal plants. J. Ethnopharma- col. 107, 182-188.
48. Srivastava R P, Dixit P, Singh L, Verma P C and Saxena G 2018 Status of Selinum spp. L. a Himalayan medicinal

Plant in India: a review of its pharmacology, Phytochemistry and traditional uses. Curr. Pharm. Biotechnol. 19, 1122-34.

49. Srirama R, Santhosh Kumar J U, Seethapathy G S, Newmaster S G, Ragupathy S, Ganeshaiah K N, Uma Shaanker R and Ravikanth G 2017 Species adulteration in the herbal trade: causes, consequences and mitigation. J. Drug Safety. 40, 651-61.

50. Pandey V N 1991 Medico-ethano botanical exploration in Sikkim Himalaya; Central Council for Research in Ayurveda and Siddha. 1, 137-189.

51. Ali S, Ansari K A, Jafri M A, Kabeer H and Diwakar G 2000 *N. jatamansi* protects against liver damage by induced by thioacetamide in rats. J Ethonopharmacol. 71, 359- 363.

52. Kokate C K, Prohit A P and Gokhale S B (2010) Pharmacognosy, Edn 46, Vol 1st ,2nd, Nirmal Prakshan, Pune.

53. Shah B N, (2009). Textbook of Pharmacognosy and Phytochemistry, Edn 1, published by Elseveir, Noida, India.

54. Subashini R, Ganapragasam A, Yogeeta S and Devaki T 2007 Protective effect of *N. jtamansi* (Rhizomes) on mitochondrial Respiration and Lysosomal hydrolases during doxorubicin induce mycocardial injury in rats. J Health Science. 53, 67-72.

55. Bhattacharyya SK, Bhattacharyya D 1982 Effect of restraint stress on rat brain serotonin. J Bio Sci. 4, 269-274.

56. Sur TK and Bahttacharyya D 1997* Indian J Pharmacol. 29, 318-321.

57. Evans WC (2008) Trease and Evans Pharmacognosy, Edn 15, published by Elseveir, Noida, India.

58. Bagchi A, Oshima Y and Hikino H 1991 Neolignans and lignans of *N. jatamansi* roots. Planta Med. 57, 96-97.

59. Nadkarni A K and Nadkrani K M 1994 Indian Materia Medica, Popular Book Depot, Bombay. 7,946-948.

60. Sukhdev 1997 Ethnotherapeutics and Modern drug development. Potential of Ayurveda Current Science. 73, 920.

61. Chopra I C and Jamwal K S 1954 Pharmacolog- ical action of some common essential oil bearing plants used in indigenous Medicine; Part 2nd ; Pharmacological action of *Alpinia galangal*, *Pistacia integerrima*, *Piper betel* and *N. jatamansi*. Indian J Med Res. 42, 385-388.

62. Singh T G 2020 Phytochemical activities and pharmacology of herbal drug: *Nardostachys jatamansi*. Plant Arch. 20, 3842-8.

63. Srivastava A, Tiwari S S, Srivastava S and Rawat A K 2010 HPTLC method for quantification of valerenic acid in Ayurvedic drug jatamansi and its substitutes. J. Liq. Chr. Related. Tech. 33, 1679-88.

64. Saraswat N, Sachan N and Chandra P 2020 A Detailed Review on the rarely found Himalayan herb *Selinum vaginatum*: Its Active constituents, pharmacological uses, traditional and potential benefits. Pharmacophore. 11, 40-52.

65. Chauhan R S, Nautiyal M C, Figueredo G, Chalard P 2012 Volatile composition of underground parts of *Selinum vaginatum* and possible uses. Chem. Nat. Compd. 48, 901-2.

66. Pandey M M, Katara A, Pandey G, Rastogi S and Rawat A K 2013 An important Indian traditional drug of Ayurveda jatamansi and its substitute Bhootkeshi: Chemical profiling and antimicrobial activity. Evi. Based Com. Alter. Med. 142517.

67. Sharma P K, Chauhan N S and Lal B 2004 Observations on the traditional phytotherapy among the inhabitants of Parvati valley in western Himalaya, India. J. Ethnopharmacol. 92, 167-76.

68. Nand K and Suneet N 2018 Ethnobotanical uses of wild medicinal plants by the local community in the Asi Ganga sub-basin, Western Himalaya. J. Complement. Med. Res. 9, 34-46.

69. Sharma N, Sharma A R, Patel B D and Shrestha K 2016 Investigation on phytochemical, antimicrobial activity and essential oil consti-tuents of *Nardostachys jatamansi* DC. in different regions of Nepal. J. Coast. Life Med. 4, 56-60.

70. Chatterjee A, Basak B, Datta U, Banerji J, Neum- an A and Prange T 2005 Studies on the chemical constituents of *Nardostachys jatamansi* DC (Valerianaceae). Indian J. Chem. 44, 430-433.

71. Rucker G, Tautges J, Wenzl H and Graf E 1978 Isolation and pharmacodynamic activity of the sesquiterpene valeranone from *Nardostachys jatamansi* DC. Arzneimittel-forschung. 28, 7-13.

72. Shabhag S N, Mesta C K, Maheshwari M L and Bhattacharya S C 1965 Terpenoids LXXV: Constituent of jatamansi and synthesis of (±) Dihydrosamidin and Visnadin from jatamansin. Tetrahedron. 21, 3591-3595.

73. Kapoor L D (2001) CRC Handbook of Ayurvedic Medicinal Plants, CRC Press, Boca Raton, Florida.

74. Bose B C, Vijayvarngiya R and Bhatnagar J N 1957 *Nardostachys jatamansi* DC: a phytochemical study of its active constituents. Indian J Med Science. 11, 799-802.

75. Hörster H, Rücker G and Tautges J 1977 Valeranon- gehalt in den unterirdischen teilen von *Nardostachys jatamansi* and *Valeriana officinalis*. Phytochem. 16, 1070-1.

76. Ali M (2008) Pharmacognosy and Phytochemi- stry, Vol. 1st, CBS Publisher and Distributors, New Delhi.

77. Venkateshwar R G, Annamalai T and Mukhopadhyay T 2008 Nardal: a new sesquiterpene aldehyde form the plant of *Nardostachys jatamansi*. Indian J Chem. 47, 163-165.

78. Chauhan R S, Nautiyal M C and Tava A 2014 Variability in the essential oil composition of *Selinum vaginatum* CB Clarke. (Apiaceae) in north-west Himalaya, India. J. Essent. Oil-Bear. Plants. 17, 906-10.

79. Mesta C K, Paknikar S K and Bhattacharyya S C 1968 The structure of vaginatin: a new sesquiterpene from the root extractive of *Selinum vaginatum*. Chem. Commun. 584, 584-585.

80. Seshadri T R and Sood M S 1964 Constitution of Selinidin: A new coumarin from *Selinum vaginatum* Clarke. Tetrahedron Lett. 5, 3367-3373.

81. Seshadri T R, Handa K L and Sood M S 1966 Chemical components of the roots of *Selinum vaginatum*-1. Coumarins of the petroleum ether extract. Tetrahedron. 23, 1883-1891.

82. Seshadri T R and Sood M S 1967 Chemical comparison of the roots of *Selinum vaginatum* and *Nardostachys jatamansi*. Phytochem. 6, 445-446.

83. Seshadri T R and Sood M S 1967 Constitution of Selinone, A new flavanone from *Selinum vaginatum* Clarke. Tetrahedron Lett. 8, 853-855.

84. Pandey M M, Katara A, Pandey G, Rastogi S and Rawat A K 2013 An important Indian traditional drug of ayurveda jatamansi and its substitute bhootkeshi: chemical profiling and antioxidant activity. Evi. Based Com. Alter. Med. 142517.

85. Mehra P N, Jolly S S 1963 The identity and pharmacognosy of the adulterant of *Nardostachys jatamansi* DC. Planta Med. 11, 8-15.

86. Singh V, Dubey P, Srivastava S and Rawat A K 2011 Botanical standardization of the Jatamansi, their substitute and adulterant species. Indian J. Tradit. Knowl. 10, 599-603.

87. Rahman H, Shaik H A, Madhavi P, Eswaraiah M C 2011 A review: Pharmacognostics and pharmacological profiles of *Nardastachys jatamansi* DC. Elixir Pharm. 39, 5017-20.

88. Cardone L, Castronuovo D, Perniola M, Cicco N and Candido V 2020 Saffron (*Crocus sativus* L.), the king of spices: An overview. Sci. Hortic. 272, 109560.

89. Srivastava R, Ahmed H, Dixit R K and Saraf S A 2010 *Crocus sativus* L.: a comprehensive review. Pharmacogn Rev. 4, 200.

90. Bolhassani A 2018 Bioactive components of saffron and their pharmacological properties. Stud. Nat. Prod. Chem. 58, 289-311.

91. Delshad E, Yousefi M, Sasannezhad P, Rakhshandeh H and Ayati Z 2018 Medical uses of *Carthamus tinctorius* L. (Safflower): a comprehensive review from traditional medicine to modern medicine. Electron. Physician. 10, 6672.

92. Girme A, Mirgal A, Darji B, Gafner S and Hingorani L (2022) Adulteration of saffron and saffron extracts. Botanical Adulterants Prevention Bulletin, Austin.

93. Baumann B B 1960 The botanical aspects of ancient Egyptian embalming and burial. Econ Bot. 14, 84-104.

94. Maimonides M (1974) On the causes of sympt -oms (12th century). University of California Press, California.

95. Encyclopaedia Britannica (1974) Vol. 9. Encyclopaedia Britannica Inc., Chicago.

96. Gainer J W and Chisolm G M 1974 Oxygen diffusion and atherosclerosis. Athersclerosis. 19, 135-8.

97. Bhat J V and Broker R 1953 Riboflavin and thiamine content of saffron, *Crocus sativus* Linn. Nature. 172, 544.

98. Gainer J L (1977) Increasing fermentation yields. U.S. Pat. 4038144.

99. Hosseinzadeh H, Karimi G H and Niapoor M 2004 Antidepressant effects of *Crocus sativus* stigma extracts and its constituents, crocin and safranal, in mice. J.Med. Plants. 3, 48-58.

100. Abdullaev F J 1993 Biological effects of saffron. Biofactors. 4, 83-6.

101. Zhang Y X, Sugiura M, Saito H, Shoyama Y 1994 Acute effects of *Crocus sativus* L. on passive avoidance performance in mice. Biol Pharmacol Bull. 17, 217-21.

102. Abe K, Sugiura M, Ymaguchi S, Shoyama Y and Saito H 1999 Saffron extract prevents acetaldehyde-induced inhibition of long-term potentiation in the rat dentate gyrus in vivo. Brain Res. 851, 287-9.

103. Abdullaev Jafarova F, Caballero-Ortega H, Riverón-Negrete L, Pere Pereda-Miranda R, Rivera-Luna R, Manuel Hernández J, *et al.* 2002 In vitro evaluation of chemoprotective potential of saffron. Rev Inves Clin. 54, 430-6.

104. Nair S C, Kurumboor S K and Hasegawa J H 1995 Saffron chemoprotective in biology and medicine: a review. Cancer Biother & Radio pharm. 10, 257-64.

105. Premkumar K, Abraham S K, Santhiya S T, Gopinath P M and Ramesh A 2001 Inhibition of genotoxicity by saffron (*Crocus sativus* L.) in mice. Drug Chem Toxicol. 24, 421-8.

106. Premkumar K, Abraham S K, Santhiya S T and Ramesh A 2003 Protective effects of saffron (*Crocin sativus* L.) on genotoxins-induced oxidative stress in swiss albino mice. Phytother Res. 17, 614-7.

107. Hosseinzadeh H and Khosravan V 2002 Anticonvulsant effects of aqueous and ethanolic extracts of *Crocus sativus* L. stigma in mice. Arch Irn Med. 5, 44-7.

108. Asgarpanah J and Kazemivash N 2013 Phytochemistry, pharmacology and medicinal properties of *Carthamus tinctorius* L. Chin. J. Integr. Med. 19, 153-9.

109. Wang G and Li Y 1985 Clinical application of safflower (*Carthamus tinctorius*). Tradit Chin Med Sci J. 1, 42-3.

110. Zhou F R, Zhao M B and Tu P F 2009 Simultaneous determination of four nucleosides in *Carthamus tinctorius* L. and Safflower injection using high performance liquid chromatography. J Chin Pharm Sci. 18, 326-30.

111. Punjanon T, Arpornsuwan T and Klinkusoom N 2004 The pharmacological properties of safflower (*Carthamus tinctorius* L). Bull Health Sci Technol. 7, 51-63.

112. Madaan N, Mudgal V, Mishra S, Srivastava A K and Singh R B 2011 Studies on biochemical role of accumulation of heavy metals in Safflower. Open Nutraceuticals J. 4, 199-204.

113. Liakopulou-Kyriakides M and Kyriakides D A 2002 *Crocus sativus*-Biological active Constituents. Stud Nat Prod Chem. 26, 293-312.

114. Calixto J B, Beirith A, substances from plants. Phytother Res. 14, 401-18.

115. Forestieri A M, Torento A and Tripodo M M 1994 Biological effects of hesperidin, a citrus flavonoid (Note-I) :anti-inflammatory and analgesic activity. Farmaco (Societa chimica italiana). 40, 709-12.

116. Ramesh M, Rao Y N, Rao A V, Prabhakar M C, Rao C S, Muralidhar N, *et al.* 1998 Antinociceptive and anti-inflammatory activity of a flavonoid isolated from *Carraluma attenuata*. J. Ethnopharmacol. 62, 63-6.

117. Fatehi M, Rashidabady T and Fatehi-Hassanabad Z 2003 Effects of *Crocus sativus* petal's extract on rat blood pressure and on response induced by electrical field stimulation in the rat isolated vas deferens and guinea-pig ileum. J. Ethnopharmac- ol. 84, 199-203.

118. Mzabri I, Addi M and Berrichi A 2019 Traditional and modern uses of saffron (*Crocus sativus*). Cosmetics. 6, 63.

119. Kim S K, Cha J Y, Jeong S J, Chung C H, Choi Y R and Cho Y S 2000 Properties of the chemical composition of safflower (*Carthamus tinctorius* L.) sprout. Korean J Life Sci. 10,1068-73.

120. Akihisa T, Yasukaw K, Oinuma H, Kasahara Y, Yamanouchi S, Takido M, *et al.* 1996 Triterpene alcohols from the flowers of compositae and their anti-inflammatory effects. Phytochem. 43, 1255-1260.

121. Shirwaikar A, Khan S, Kamariya Y H, Patel B D and Gajera F P 2010 Medicinal plants for the management of post-menopausal osteoporosis: a review. Open Bone J. 2, 1-13.

122. Xiao P G and Liu C X 2005 Pharmacology, pharmacokinetics and toxicology of Chinese traditional medicine for stroke therapy. Asian J Drug Metabol Pharmacokin. 5, 83-124.

123. Lee J Y, Chang E J, Kim H J, Park J H and Choi S W 2002 Antioxidative flavonoids from leaves of *Carthamus tinctorius*. Arch Pharm Res. 25, 313-319.

124. Meselhy M R, Kadota S, Momose Y, Hatakeyama N, Kusai A, Hattori M, *et al.* 1993 Two new quinochalcone yellow pigments from *Carthamus tictorius* and calcium antagonistic activity of tictormine. Chem Pharm Bull. 41, 1796-1802.

125. Huang J L, Fu S T, Jiang Y Y, Cao Y B, Guo M L, Wang Y, *et al.* 2007 Protective effects of Nicotiflorin on reducing memory dysfunction, energy metabolism failure and oxidative stress in multi-infarct dementia model rats. Pharm Biochem Behav. 86, 741-748.

126. Shao J F, Wang Y B, Chen Q, Liu Z Q, Liu Q, Cai W G, *et al.* 2011 A daily variation in essential oil composition of flower of different accessions from *Carthamus tinctorius* L. in Sichuan province of China. J Med Plant Res. 5, 3042-3051.

127. Al-Snafi A E 2016 The pharmacology of *Crocus sativus*-A review. IOSR J. Pharm. 6, 8-38.

128. Lamichhane G Devkota H P, Sai K and Poudel P (2022) *Carthamus tinctorius* L.: Traditional Uses, Phytochemistry, and Pharmacological Activities. In: Devkota, H.P., Aftab, T. (eds) Medicinal Plants of the Asteraceae Family. Springer, Singapore.

129. Jiang C, Cao L, Yuan Y, Chen M, Jin Y and Huang L 2014 Barcoding melting curve analysis for rapid, sensitive, and discriminating authentica- tion of saffron (*Crocus sativus* L.) from its adulterants. Biomed Res. Int. 2014.

130. Sabatino L, Scordino M, Gargano M, Belligno A, Traulo P and Gagliano G 2011 HPLC/PDA/ESI-MS evaluation of saffron (*Crocus sativus* L.) adulteration. Nat. Prod. Commun. 6, 1934578X1100601220.

131. Petrakis E A and Polissiou M G 2017 Assessing saffron (*Crocus sativus* L.) adulteration with plant-derived adulterants by diffuse reflectance infrared fsourier transform spectroscopy coupled with chemometrics. Talanta. 162, 558-66.

132. Thongkhao K, Pongkittiphan V, Phadungcharoen T, Tungphatthong C, Urumarudappa SK, Pengsuparp T, Sutanthavibul N, Wiwatcharak- ornkul W, Kengtong S and Sukrong S 2020 Differentiation of *Cyanthillium cinereum*, a smoking cessation herb, from its adulterant *Emilia sonchifolia* using macroscopic and microscopic examination, HPTLC profiles and DNA barcodes. Sci. Rep. 10, 14753.

133. Zhang M, Yang X, Wei Y, Wall M, Songsak T, Wongwiwatthananukit S and Chang L C 2019 Bioactive sesquiterpene lactones isolated from the whole plants of *Vernonia cinerea*. J. Nat. Prod. 82, 2124-31.

134. Jakupovic J, Banerjee S, Castro V, Bohlmann F, Schuster A, Msonthi J D, *et al.* 1968 Poskeanolide, a seco-germacranolide and other sesquiterpene lactones from Vernonia species. Phytochemistry. 25, 1359-64.

135. Joshi R K 2018 Volatile constituents of *Emilia sonchifolia* from India. Nat. Prod. Commun. 13, 1934578X1801301030.

136. Haines H H (2006) The Botany of Bihar and Orissa. Shiva Offset Press, Dehradun, India.

137. Iwalewa, E O, Iwalewa O J and Adeboye J O 2003 Analgesic, antipyretic, anti-inflammatory effects of *Vernonia cinerea* less leaf. J. Ethnopharmacol. 86, 229-234.

138. Kumar PP and Kuttan G 2009 *Vernonia cinerea* L. scavenges free radicals and regulates nitric oxide and proinflammatory cytokines profile in Carrageenan induced paw edema model. Immunopharmacol and Immunotoxicol. 31, 94-102.

139. Youn U J, Park E J, *et al.* 2012 Anti-inflammatory sesquiterpene lactones from the flower of *Vernonia cinerea.* Bioorganic Med. Chem. Lett. 22, 5559-5562.

140. Gokilaveni C, Nishadh A and Selvi V 2006 Ameliorative role of *Vernonia cinerea* in carbon tetrachloride induced hepatic dysfunction in rats. Anc. Sci. Life. 25, 1-5.

141. Adeboye J O, Asije W and Awe S O 1997 Diuretic and antidiuretic activity of the leaf extracts of *Vernonia cinerea.* Phytother Res. 11, 454-456.

142. Rizvi S M, Biswas D, Arif J M and Zeeshan M 2011 In-vitro antibacterial and antioxidant potential of leaf and flower extracts of *Vernonia cinerea* and their phytochemical constituents. Int. J. Pharm. Sci. Rev. Res. 9, 164-169.

143. Arivoli S, Tennyson S and Martin J J 2011 Larvicidal efficacy of *Vernonia cinerea* (L.) (Asteraceae) leaf extracts against the filarial vector Culex quinquefasciatus Say (Diptera: Culicidae). J. Biopestic. 4, 37–42.

144. Joshi R K 2014 Sesquiterpene rich essential oil of *Vernonia cinerea* Less. from India. S. Afr. J. Bot. 95, 129-30.

145. Chopra R N, Nayar S L and Chopra I C (1986) Glossary of Indian Medicinal Plants (Including the Supplement). Council of Scientific and Industrial Research, New Delhi.

146. Matos F A, Machado M L, Alencar J W, Matos M O and Craveiro A A 1991 Plants used in traditional medicine of China and Brazil. Memorias do Instituto Oswaldo Cruz. 86, 13-16.

147. Duke J A and Ayensu E S (1985) Medicinal Plant of China. Reference Publications, Inc.

148. Manandhar N P (2002) Plant and People of Nepal. Timber Press, Oregon.

149. Shylesh B S and Padikkala J 2000 In vitro cytotoxic and antitumor property of *Emilia sonchifolia* (L.) DC in mice. J. Ethnopharmacol. 73, 495-500.

150. Essien G E, Nwidu L L and Nwafor P A 2009 Anti-inflammatory and analgesic potential of methanolic extract of *Emilia Sonchifolia* (Compositae) leaves in rodents. Afr. J. Biomed. Res. 12, 199-207.

151. Couto V M, Vilela F C, Dias D F, Dos S H, Soncini R, Nascimento C G and Giusti-Paiva A 2011 Antinociceptive effect of extract of *Emilia sonchifolia* in mice. J. Ethnopharmacol. 134, 348-353.

152. Lija Y, Biju P G, Reeni A, Cibin T R, Sahasranamam V and Abraham A 2006 Modulation of selenite cataract by the flavonoid fraction of *Emilia sonchifolia* in experimental animal models. Phytother Res. 20, 1091-1095.

153. Yadava R N, Raj M 2012 Antiviral activity of a new flavone glycoside from *Emilia sonchifolia* DC. Indian J. Chem. 51, 635-638.

154. Edagha I A, Davies K G, Akpan B C, Mbadugha C C and Udoiso W U 2014 Ethanolic extract of *Emilia sonchifolia* leaves possess erythropoietic and hepatoprotective effect in mice infected with Plasmodium Berghei. Maced. J. Med. Sci. 15, 11-17.

155. Shen S M, Shen L G, Lei Q F, Si J Y, Liu C M and Lu H 2012 Chemical constituents contained in aerial parts of *Emilia sonchifolia*. China Journal of Chinese Materia Medica. 37, 3249-3251.

156. Cheng D, Roder E 1986 Pyrrolizidine alkaloids from *Emilia sonchifolia* Planta Med. 6, 484-486.

157. Hsieh C H, Chen H W, Lee C C, He B J , Yang Y C 2015 Hepatotoxic pyrrolizidine alkaloids in *Emilia sonchifolia* from Taiwan. J. Food Compos. Anal. 42, 1-7.

158. Rathore S, Raj Y, Debnath P, Kumar M, and Kumar R 2022 Ethnopharmacology, phytochemistry, agrotechnology, and conservation of *Inula racemosa* Hook f. A critically endangered medicinal plant of the western Himalaya. J. Ethnopharmacol. 283, 114613.

159. Saxena S, Bhardwaj A K, Kumar V, Patel M K, Kumar R and Chaurasia O P 2018 Sustainable utilisation of medicinal plants of Ladakh and Lahaul-Spiti of trans-Himalaya. Def. Life Sci. J. 3, 120-125.

160. Anonim A (2018) Agro techniques of Selected Medicinal Plants. TERI Press, New Delhi.

161. Rana C S, Negi Y S, Sawant L P and Raturi P P 2022 Commercial cultivation and sustainability of Pushkarmool (*Inula racemosa*), a case study from Keylong, Lahaul & Spiti, Himachal Pradesh, India. J. Mountain Res. 17, 25-33.

162. Zahara K, Tabassum S, Sabir S, Arshad M, Qureshi R, Amjad M S and Chaudhari S K 2014 A review of therapeutic potential of *Saussurea lappa*-An endangered plant from Himalaya. Asian Pac. J. Trop. Med. 1, 60-69.

163. Singh P, Sharma B M 1972 Pharmacognostic study of root of *Inula racemosa* Hook Quart. J. Crude Drug Res. 12, 1929-1936.

164. Khurana P, Singh A, and Saroch V 2015 Role of

Pushkarmool in the asthma management: a conceptual study. J. Tradit. Nat. Med. 1, 10-12.

165. Das A, Shakya A, Ghosh S K, Singh U P and Bhat H R 2020 A review of phytochemical and pharmacological studies of inula species. Curr. Bioact. Comp. 16, 557-567.

166. Arumugam P, Murugan M, and Thangaraj N 2012 Evaluation of anti-inflammatory and analgesic effects of aqueous extract

obtained from root powder of *Inula racemosa* Hook. f. J. Med. Plant Res. 6, 2801–2806.

167. Seca A M, Grigore A, Pinto D C, and Silva A M 2014 The genus Inula and their metabolites: From ethnopharmacological to medicinal uses. J. Ethnopharmacol. 154, 286-310.

168. Gautam H, and Asrani R K 2018. Phytochemical and pharmacological review of an ethno medicinal plant: *Saussurea lappa*. Vet Res Int. 6, 1-9.

169. Khurana P, Singh A and Saroch V 2015 Role of Pushkarmool in the asthma management: a conceptual study. J. Tradit. Nat. Med. 2015 1, 10-2.

170. Madhuri K, Elango K and Ponnusankar S 2012 *Saussurea lappa* (Kuth root): review of its traditional uses, phytochemistry and pharmacology. Orient Pharm Exp Med. 12, 1-9.

171. Sutar N, Garai R, Sharma U S, Singh N and Roy S D 2011 Antiulcerogenic activity of *Saussurea lappa* root. Int J Pharm Life Sci. 2, 516- 520.

172. Sunkara Y, Robinson A, Babu K S, Naidu V G, Vishnuvardhan M V, Ramakrishna S, Madhavendra S S and Rao J M 2010 Anti-inflammatory and cytotoxic activity of chloroform extract of roots of *Saussurea lappa* Clarke. J Pharm Res. 3, 1775-8.

173. Barrero A F, Oltra J E, Álvarez M, Raslan D S, Saúde D A, and Akssira M 2000 New sources and antifungal activity of sesquiterpene lactones. Fitoterapia. 71, 60-4.

174. Seki K, Hashimoto A, Kobayashi H, Kawahara Y. Yamahara 1991 Motility inhibitory effect on Anchusan and Jintan and its active components in Anisakis type larvae. Yakuri to Chiryo. 19, 265-289.

175. Upadhyay O P, Singh R H and Dutta S K 1996 Studies on antidiabetic medicinal plants used in Indian folklore. Aryavaidyan. 9, 159-167.

176. Yaeesh S, Jamal Q, Shah A J and Gilani A H 2012 Antihepatotoxic activity of *Saussurea lappa* extract on D-galactosamine and lipopolysacchari de-induced hepatitis in mice. Phytother Res. S229-S232.

177. Koul S C 1941 Some wildflowers of Kashmir and their indigenous use. J Bombay Nat Hist Soc. 42, 452-454.

178. Awasthi P, Sood I and Syal S 2008 Isolating diesel-degrading bacteria from air. Curr. Sci. 94, 178-180.

179. Tsarong T J (1986) Handbook of traditional Tibetan drugs: their nomenclature, composition, use, and dosage. Tibetan Medical Publications.

180. Lee M G, Lee K T, Chi S G and Park J H 2001 Costunolide induces apoptosis by ROS-mediated mitochondrial permeability and cytochrome C release. Biol Pharm Bull. 24, 303-306.

181. Malik A H, Khuroo A A, Dar G H and Khan Z S 2011 Ethnomedicinal uses of some plants in the Kashmir Himalaya. Indian J. Tradit. Knowl. 10362-366.

182. Gautam H, and Asrani R K, 2018. Phytochemical and pharmacological review of an ethno medicinal plant: *Saussurea Lappa*. Vet Res Int. 6, 1-9.

183. Arora R, Maheshwari M, Chandel K and Gupta R 1980 Mano (*Inula racemosa*): Little known aromatic plant of Lahaul valley. India. Econ. Bot. 34, 175-180

184. Raghavan R, Ravindranath K R, Trivedi G K, Paknikar S K 1969 Bhattacharyya S C. Inunolide a new sesquiterpene lactone from *Inula racemosa* root. Indian J. Chem.

185. Fu B, Su B N, Takaishi Y, Honda G, Ito M, Takeda Y, Kodzhimatov O K and Ashurmetov O 2001 A bis-sesquiterpene and sesquiterpenolides from *Inula macrophylla*. Phytochemistry. 58, 1121-8.

186. Firdous Q, Bhat M F and Hussain M M 2018 Ethnopharmacology, phytochemistry and biological activity of *Inula racemosa* Hook. F: a review. *Int J Res Ayurveda Pharm*. 9, 95-102.

187. Hassan R and Masoodi M H 2020 *Saussurea lappa*: A comprehensive review on its pharmacological activity and phytochemistry. Currt. Trad. Med. 1, 613-23.

188. Sun C M, Syu W J, Don M J, Lu J J and Lee G H 2003 Cytotoxic sesquiterpene lactones from the root of *Saussurea lappa*. J Nat Prod. 66, 1175-80.

189. Singh I P, Talwar K K, Arora J K, Chhabra B R and Kalsi P S 1992 A biologically active guaianolide from *Saussurea lappa*. Phytochemistry. 31, 2529-31.

190. Talwar K, Singh I and Kalsi P 1992 A sesquiterpenoid with plant growth regulatory activity from *Saussure lappa*. Phytochemistry. 31, 336-8.

191. Hui Y, Jinlun X and Handong S 1997 Study on chemical constituents of *Saussurea lappa*. Plant Diversity. 19, 85-91.

192. Arora R K, Maheshwari M L, Chandel K P and Gupta R 1980 Mano (*Inula racemosa*): little known aromatic plant of Lahaul valley, India. Economic Botany. 1175-80.

193. Choudhary A, Sharma R J, and Singh I P 2016 Determination of major sesquiterpene lactones in essential oil of *Inula racemosa* and *Saussurea lappa* using qNMR. J. Essent. Oil -Bear. Plants. 19, 20-31.

CRIPS Digest

New broad-spectrum and potent antibacterial agents with dual-targeting mechanism: Promoting FtsZ polymerization and disrupting bacterial membranes

The rapid emerging cases of multidrug-resistant bacteria (or superbugs) have posed a threat to humankind. The situation is currently becoming alarming because of fewer new antibacterial agents coming to market. Filamentous temperature-sensitive protein Z (FtsZ), that takes part in cell division is now being seen as a new target to combat the bacterial drug resistance mechanism. Focusing on this approach, five series of 1-methyl-2,5-diphenylpyridin-1-ium derivatives as shown in Fig. 1 were synthesized with modification in styrene position. The chemistry generated 48 compounds starting from 5-bromo-2- chloro-3-methylpyridine. The basis of this novel chemical framework came after obtaining a hit compound PubChem ID 123258599 after pharmacophore virtual screening for FtsZ protein. Various structural modifications were carried out on PubChem ID 123258599 considering the FtsZ protein 3D conformation (Fig. 1).

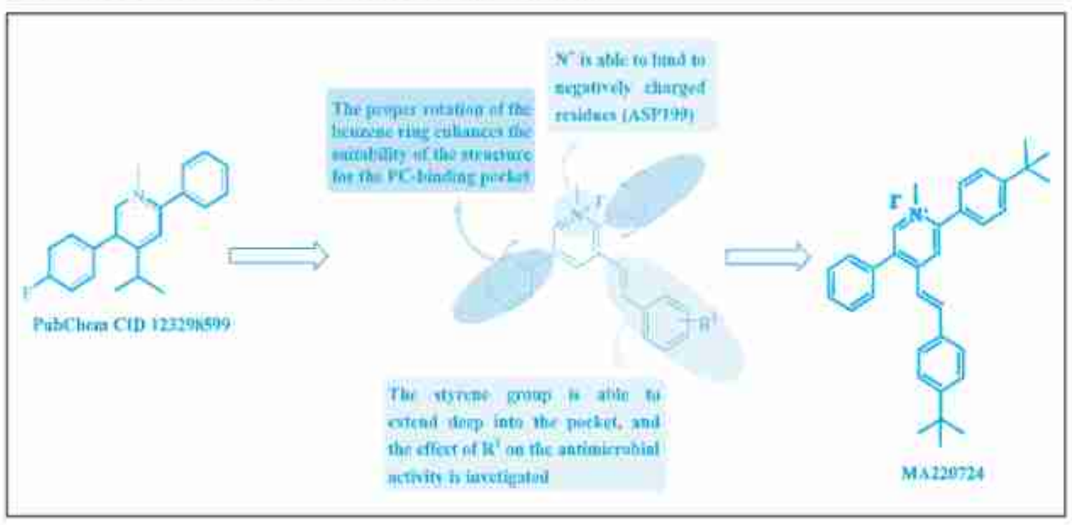


Fig. 1. Target compound design method as novel structural antibacterial agents with dual-targeting mechanism.

Compounds MA220607 and MA220724 exhibited potent broad-spectrum antibacterial activity against both Gram-negative and Gram-positive bacteria (including drug-resistant strains). Both showed dual mechanism of action of antibacterial activity, one by facilitating FtsZ polymerization and other by perturbing the bacterial membranes. MA220607 exhibited MIC value of 0.062-2 μg/mL for gram-positive bacteria and for gram-negative bacteria it was 0.5–4 μg/mL. On the other hand, MA220724 showed MIC value of 0.25-2 μg/mL and 0.5–4 μg/mL against gram-positive and gram-negative bacteria respectively. The two compounds were subjected to many biological tests- (i) Estimation of minimum bactericidal concentration, (ii) Evaluation of potential resistance development, (iii) Bactericidal time kill kinetics, (iv) Biofilm inhibition analysis, (v) Effects on the cell morphology of B. subtilis, (vi) Stimulation of FtsZ polymerization dynamics, (vii) FtsZ protofilaments observation, (viii) Depolarization of cytoplasmic membrane, (ix) Permeabilization testing, (x) Chemical analysis to explore disrupting bacterial membrane, (xi) Hemolytic toxicity, (xii) Efficacy in mouse infection model etc. The CADD study included molecular docking, binding free energy calculations, and Conformational analysis. Docking results showed that MA220607 bound stably to of FtsZ protein PC-binding pocket with ionic interaction between Asp199 and pyridine moiety of MA220607. Overall, MA220607 was discovered as a potent broad spectrum antibacterial agent against Gram-negative and Gram-positive bacteria together with various clinically common drug-resistant bacteria. Eur. J. Med. Chem. 263 (2024) 115930

NNO Pincer-Supported Pd(II)-Catalyzed Reductive N-Alkylation of Challenging Nitroarenes with Alcohols via Borrowing Hydrogen Strategy

Carbon-carbon (C-C) and carbon-nitrogen (C-N) bond formations are fundamental processes in organic chemistry, playing pivotal roles in the synthesis of numerous pharmaceuticals, agrochemicals, and materials (Figure 1). Pincer complexes have emerged as powerful tools for promoting various synthetic transformations. Pincer complexes typically consist of a tridentate ligand coordinated to a transition metal center, providing a highly stable and well-defined coordination environment for catalysis. While transition metals catalyzed cross-coupling reactions have been widely used for this purpose, they often involve organohalide reagents and harsh conditions, leading to waste production. To address this, "borrowing hydrogen" (BH) or hydrogen auto-transfer (HA) reactions have emerged as elegant and sustainable strategies. These reactions utilize alcohols as both alkylating agents and hydrogen donors, producing water as the side-product. Alcohol, a readily available and inexpensive starting material, has gathered attention as a versatile precursor for C-C and C-N bond formations. Its ability to serve as both a nucleophile and an electrophile makes it an attractive substrate for catalytic transformations. At the start of the 1980s, Watanabe and Grigg independently presented pioneering reports of BHP for the N-alkylation of amines by alcohols in the presence of homogeneous catalysts.

Recently Sundaram *et. al.* developed a protocol for sustainable catalytic synthesis of selective mono-alkylated amines from nitroarenes and alcohols by new Pd(II)-NNO pincer-type complexes involving borrowing hydrogen process. This work involves the catalyst synthesis and characterisation using IR, NMR, HRMS, and X-ray single crystal diffraction. A wide variety of primary and secondary alcohols have been successfully coupled with a series of nitroarenes under mild reaction conditions with 1 mol % Pd(II) of catalyst loading and H_2O as eco-friendly byproducts. The 26 different derivatives amines have been synthesized. Control experiments including deuterium labelling confirmed the formation of intermediates aniline, aldehyde and imine, that the indicate mechanism is following the borrowing hydrogen process.[1] Sekar, P. K.; Rengan, R.; Sundarraman B.; *J. Org. Chem.*, 2024, 11, Pg No.

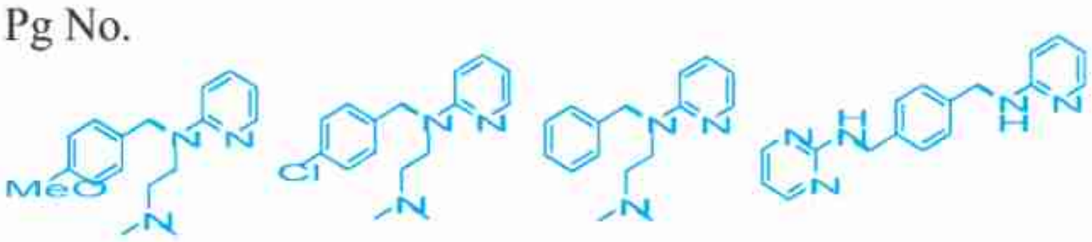


Figure 1. Importance of N-alkylation: C-N-containing drugs.

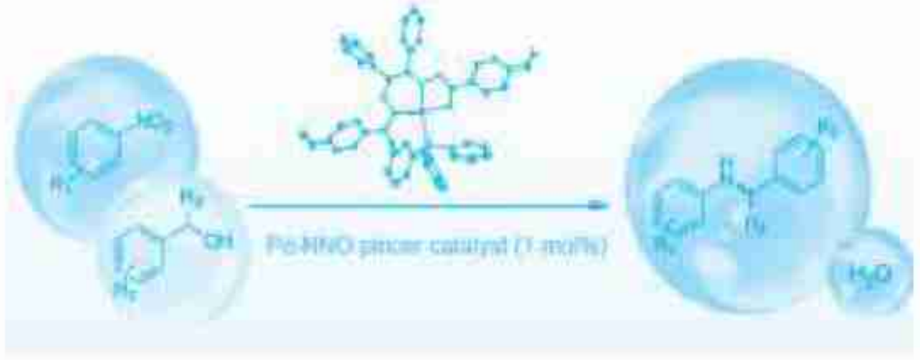


Figure Reductive N-alkylation of nitroarenes with benzyl alcohol using Pd(II) pincer complex.

A REPORT : AYURINFORMATICS SYMPOSIUM 2024

The Ayurinformatics symposium was jointly organized by National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar, All India Institute of Ayurveda (AIIA), New Delhi and Shri Dhanwantry Ayurvedic College and Hospital (SDACH), Chandigarh. The two day symposium was held on 15th–16th March 2024.

Purpose: The major aim of the symposium was to bring together all eminent researchers as well as budding minds in the fields of Ayurveda (Traditional System of Indian Medicine) and Pharmacoinformatics.

Background: Ayurveda is an ancient science and information technology is a modern science. Ayurinformatics is an emerging topic integrating information technology with ayurveda. Several varieties of research efforts are in progress in Ayurinformatics, this includes chemo-informatics of ayurvedic formulations, database development in molecular modelling of phytoconstituents in ayurvedic formulations, network pharmacology efforts, Ayurgenomics in gene prediction and much more.

Summary of Sessions: Eminent speakers and scientists from Ayurveda and Modern Information Technology fields gave lectures on the emerging topics in Ayurinformatics. The welcome address was given by the Director, NIPER S.A.S. Nagar followed by remarks shared by Prof. Tanuja Nesari, Director, AIIA and the inaugural ceremony was addressed by Prof. P. K. Mukherjee, Director, IBSD as a chief guest.

On the 1st day, at the beginning of the symposium the importance of Ayurveda drugs combined with AI (Artificial Intelligence) enabling personalized healthcare was delivered by Dr. Bala Pesala, Founder and CEO Ayur.AI. The session was continued by Dr. Ganesh Bagler on the topic expressing the importance of Ayurveda Spices combined with Informatics in making food computable. In between the 1st and 2nd technical sessions, the poster session were held, out of 3 awards, the first prize was awarded to a postgraduate student Shraddha Chugh. The 2nd round of technical sessions was continued by Dr. Anandaraman Sharma on topic '*In Silico* studies and Panchakarma'.

On the 2nd day, in 3rd technical session, Prof. Santosh Bhujbal highlighted the 'HPTLC Bioautography: Effective Therapeutic Standardization Tool for Herbal Formulations'. The session was followed by Dr. Prashant Gupta on the topic 'Ayurinformatics – A roadmap to research work'. A visit to Heritage Centre and Laboratories, NIPER, S.A.S. Nagar was held in between the 3rd and 4th technical sessions, where enthusiasts explored the ancient Ayurveda Medicine literatures and scriptures. In the 4th and the final technical session, Dr. Ramadasu Maganti, Principal, SDACH gave a lecture on 'Isolation of Newer Molecules from Ayurvedic Formulations for UTI'. The symposium was concluded by the session of Prof. P. V. Bharatam (presented by Mr. Akash Kendre) explaining the recent pioneering efforts, major goals and emerging trends of Ayurinformatics in a crux.

This first comprehensive Ayurinformatics symposium not only provided the best platform to discuss research work with experts but also it opened opportunities to collaborate with other scientists from all across India.

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