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Comparative and functional screening of three species traditionally used as antidepressants: *Valeriana officinalis* L., *Valeriana jatamansi* Jones ex Roxb. and *Nardostachys jatamansi* (D.Don) DC.

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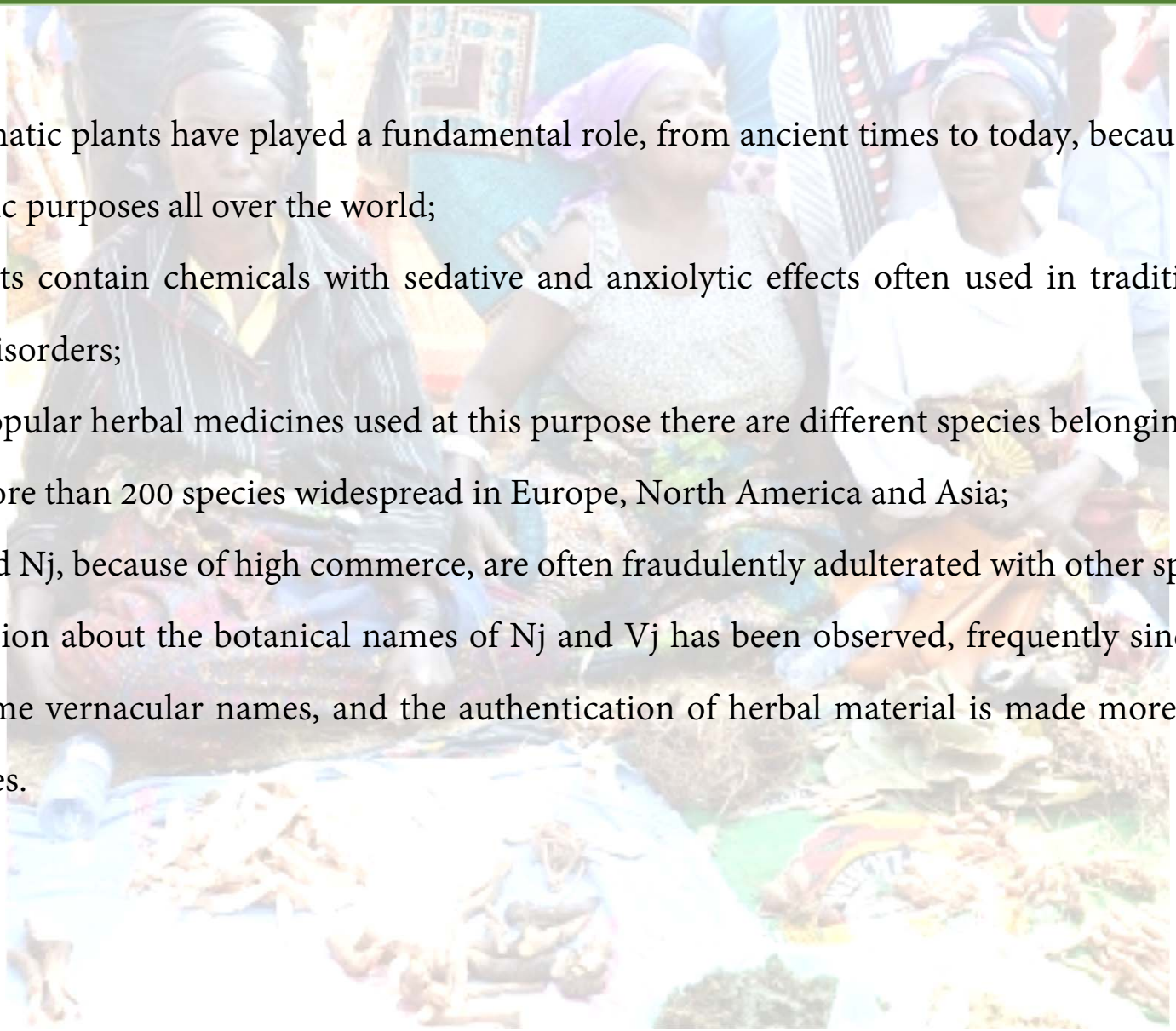
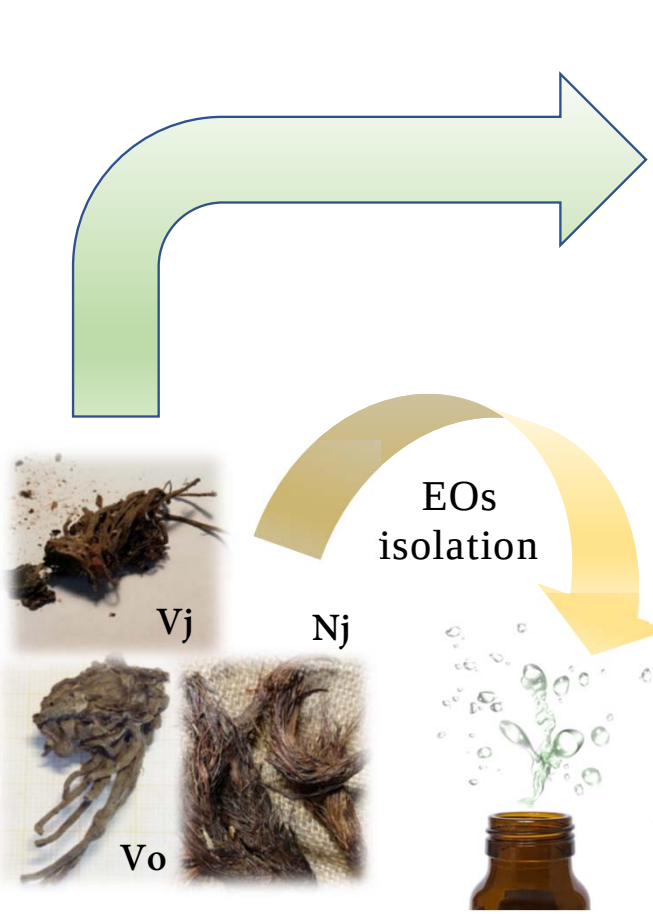
plants MDPI

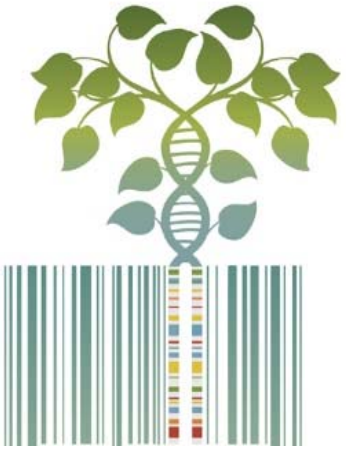


- Medicinal and aromatic plants have played a fundamental role, from ancient times to today, because they have been used for many therapeutic purposes all over the world;
- Some of these plants contain chemicals with sedative and anxiolytic effects often used in traditional medicine for the treatment of CNS disorders;
- Among the most popular herbal medicines used at this purpose there are different species belonging to the Caprifoliaceae family including more than 200 species widespread in Europe, North America and Asia;
- The roots of Vo and Nj, because of high commerce, are often fraudulently adulterated with other species;
- In addition, confusion about the botanical names of Nj and Vj has been observed, frequently since both the species are known with the same vernacular names, and the authentication of herbal material is made more difficult by the use of dried roots/rhizomes.

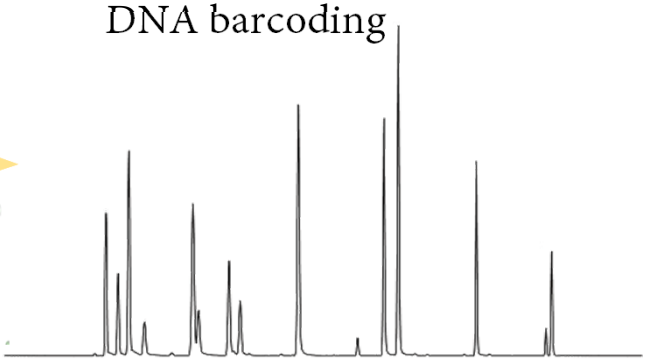
The aim of study was to systematically investigate the genetic and botanical features of these plants by DNA barcoding and micromorphological analyses and subsequently, to characterize and compare the chemical composition and neuroactive effects of EOs isolated by steam distillation from root/rhizome of Vo, Vj and Nj.



EOs isolation

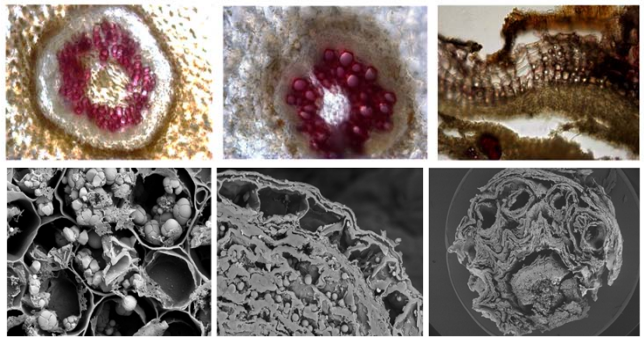


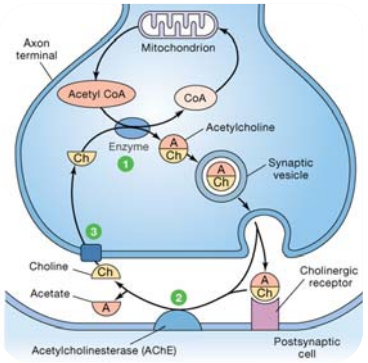
DNA barcoding



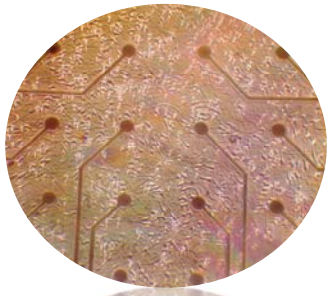
GC-MS analysis

Micro-morphological analyses





Acetylcholinesterase inhibition assay



Microelectrode array-based system

DNA barcoding studies

In the table are shown declared species, resulted species, origin, collection year and accession numbers deposited in the EMBL Nucleotide Sequence Database (www.ebi.ac.uk/embl).

Declared Species	Resulted species	Origin	Collection Year	Accession Number
V. officinalis	*V. officinalis*	China	2019	LR861814
V. jatamansi	*V. jatamansi*	Darchula District, Nepal	2019	LR861815
N. jatamansi	*N. jatamansi*	Darchula District, Nepal	2019	LR861816

Each barcode sequence was taxonomically assigned by using BLASTn analysis to the plant species with the nearest matches (maximum identity >99% and query coverage of 100%).

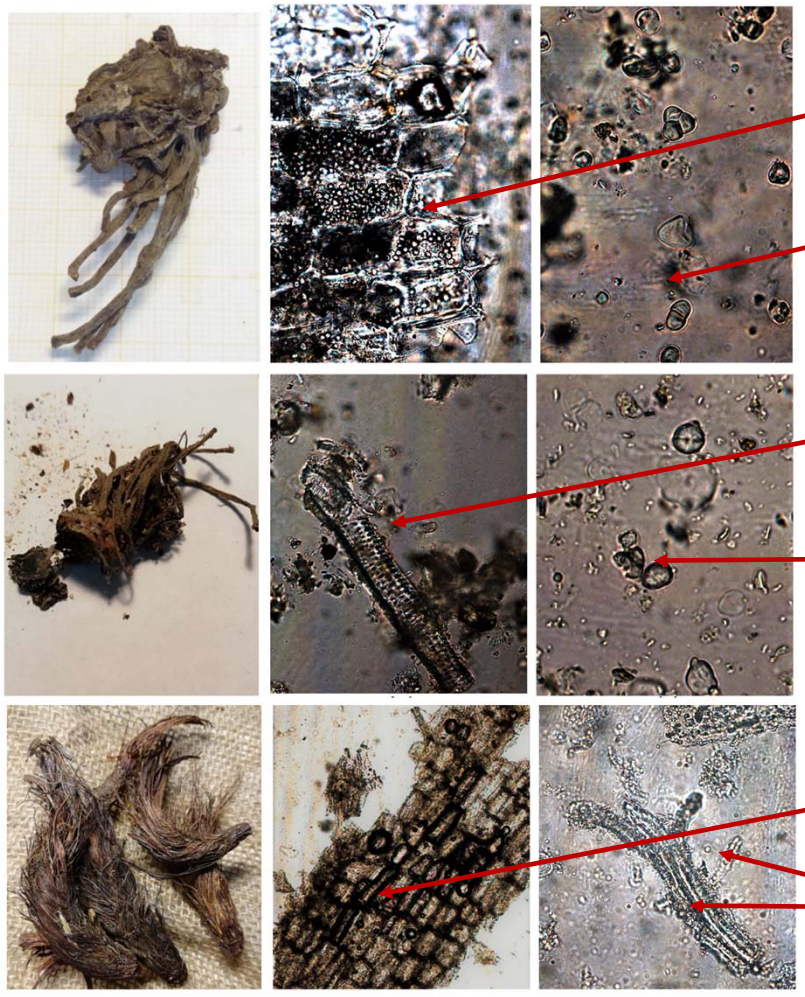
All the samples returned 100% maximum identity

Macro-micro morphological studies

V. officinalis

V. jatamansi

N. jatamansi



Parenchymatous cells with starch grains (20×)

Starch grains (40×)

Scalariform vessels (20×)

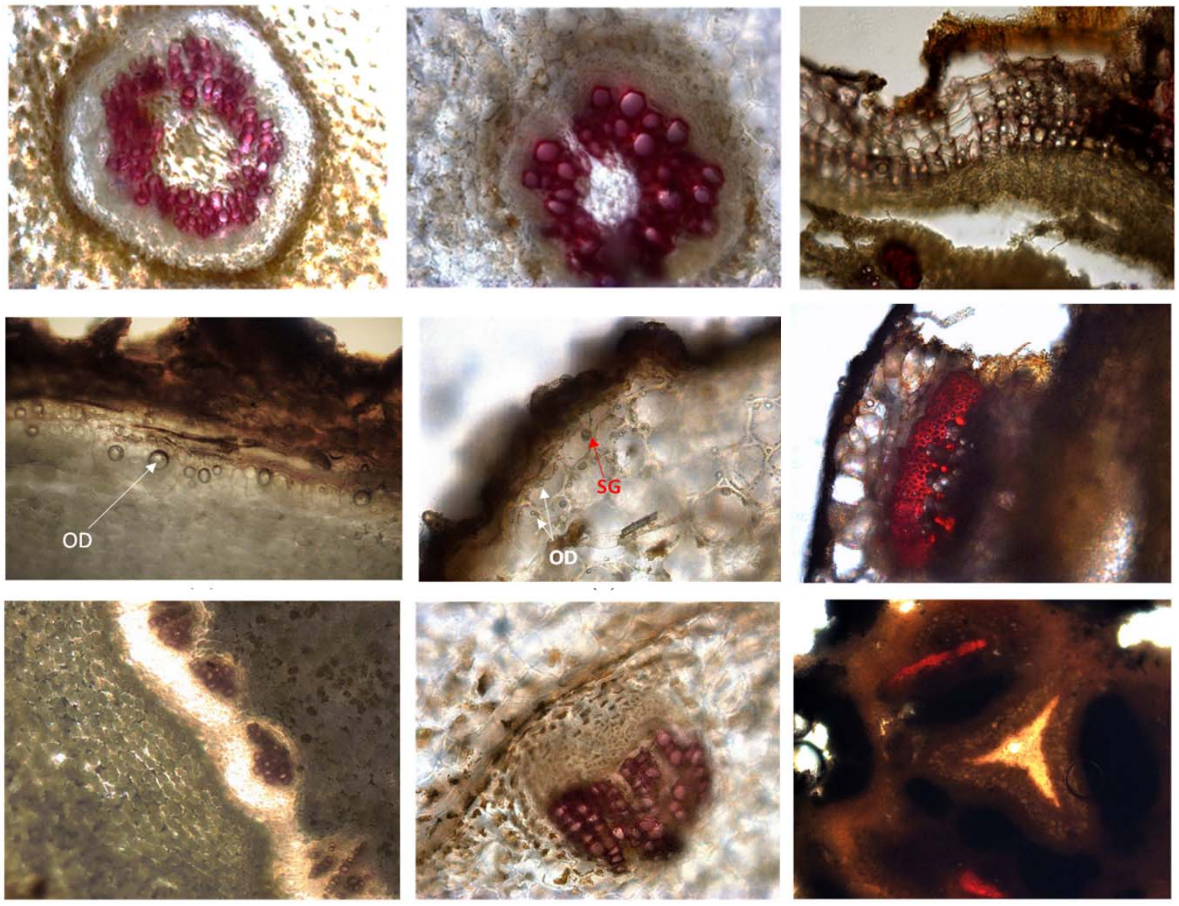
Starch grains (40×)

Cork cells (10×)

Fibers and small starch grains (40×)

Dried root/rhizome *Powdered material*

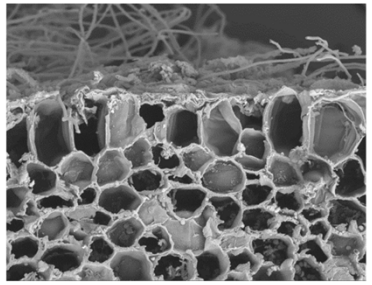
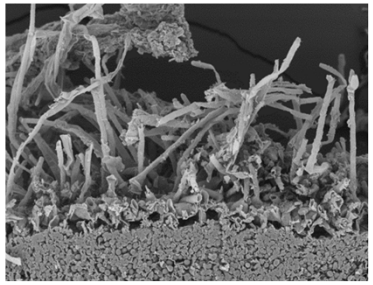
Light microscopy after phloroglucinol-HCl staining



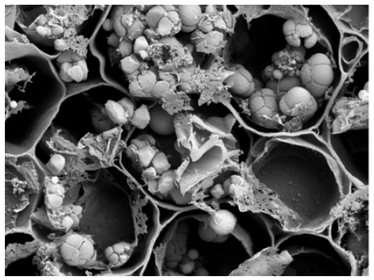
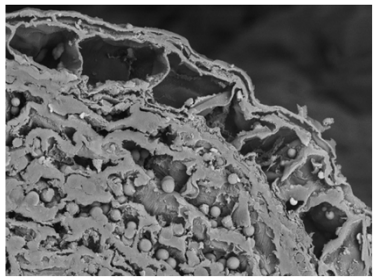
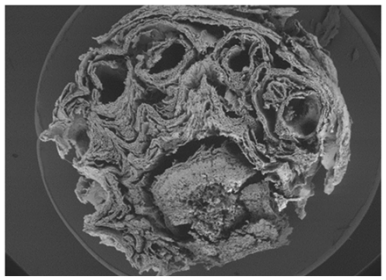
V. officinalis *V. jatamansi* *N. jatamansi*

- Cortex and stele in an older root
- Parenchymatous cortex contain oil globules
- Collateral vascular bundles circularly arranged
- Suberised cork cells containg many oil globules
- Bundles of sclerenchymatous fibres in red
- Parenchymatous pith showing a characteristic subtriangular-stellate shape, which is enclosed by cork rings

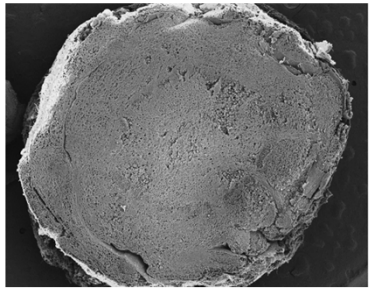
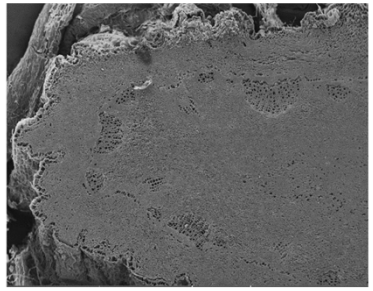
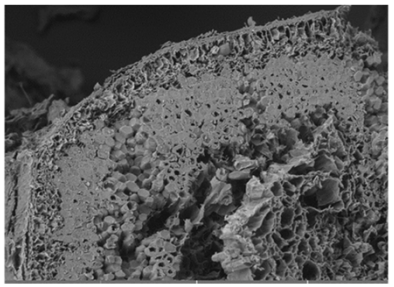
Scanning Electron Microscopy (SEM)

- Epidermis with root hairs and hypodermal layer of the cortex
- Single or composed starch grains (2-6 components)
- TS of rhizome showing vascular bundles circularly arranged
- Epidermis with many root hairs and parenchymatous cells filled with starch grains

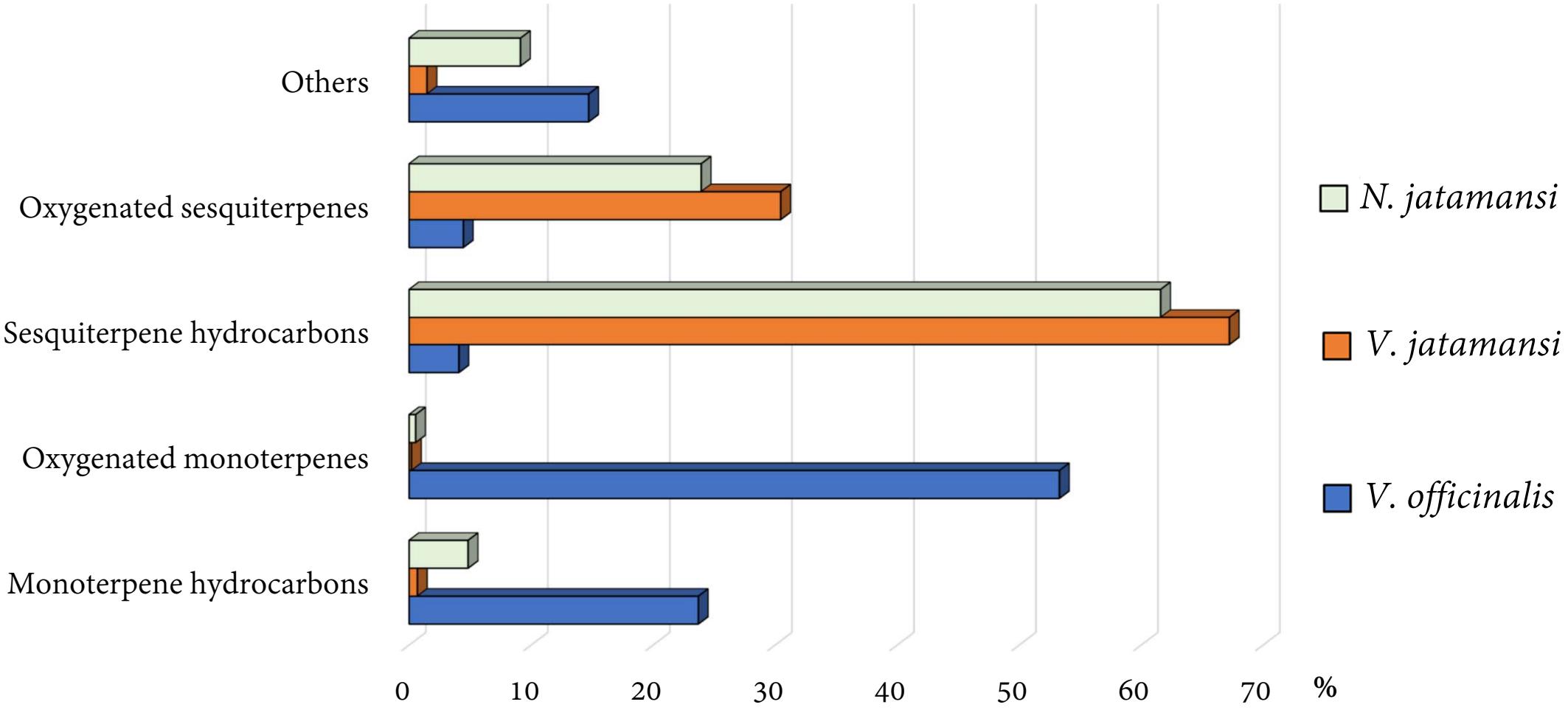
- Exoderm and parenchymatous cells filled with single starch or composed grains (2 components)
- TS of rhizome with many vascular bundles surrounding the central pith

- The rhizome is surrounded by many remains of the basal leaves petioles
- Particular of the TS of rhizome showing a multi-layered cork and large bundles of sclerenchymatous fibres

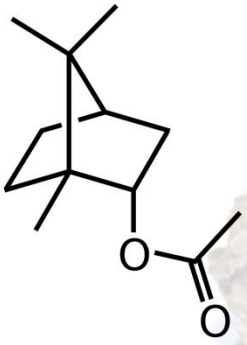
V. officinalis *V. jatamansi* *N. jatamansi*

Phytochemical profile of EOs by GC-MS analysis

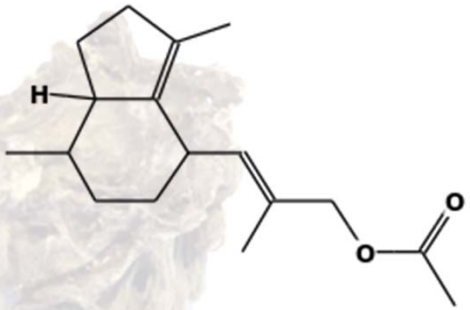


Phytochemical profile: the most representative compounds

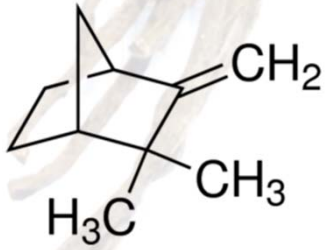
IC n. 39



Bornyl acetate
46.90%



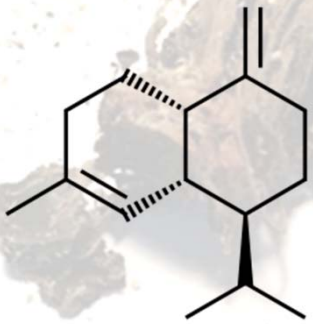
trans-Valerenyl acetate
13.18%



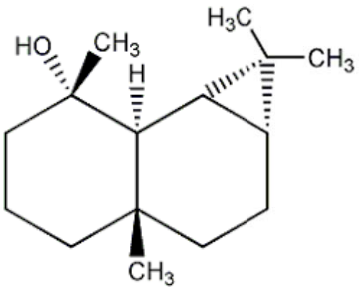
Camphene
13.85%

V. officinalis

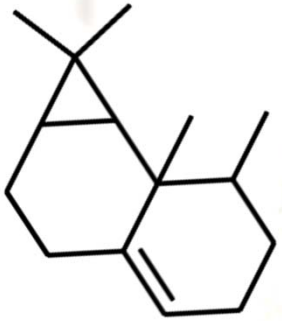
IC n. 42



γ-Gurjunene 11.88%



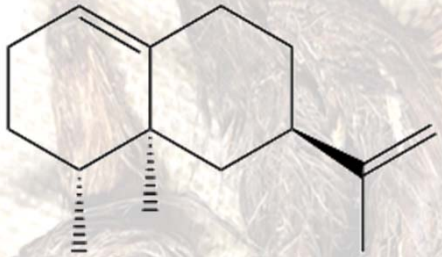
Maaliol
17.43%



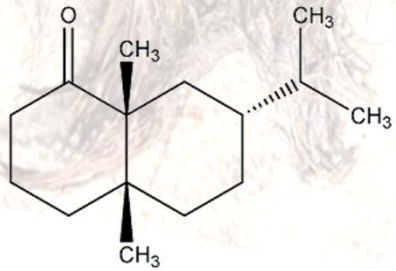
Calarene
10.57% Vj
8.22% Nj

V. jatamansi

IC n. 69



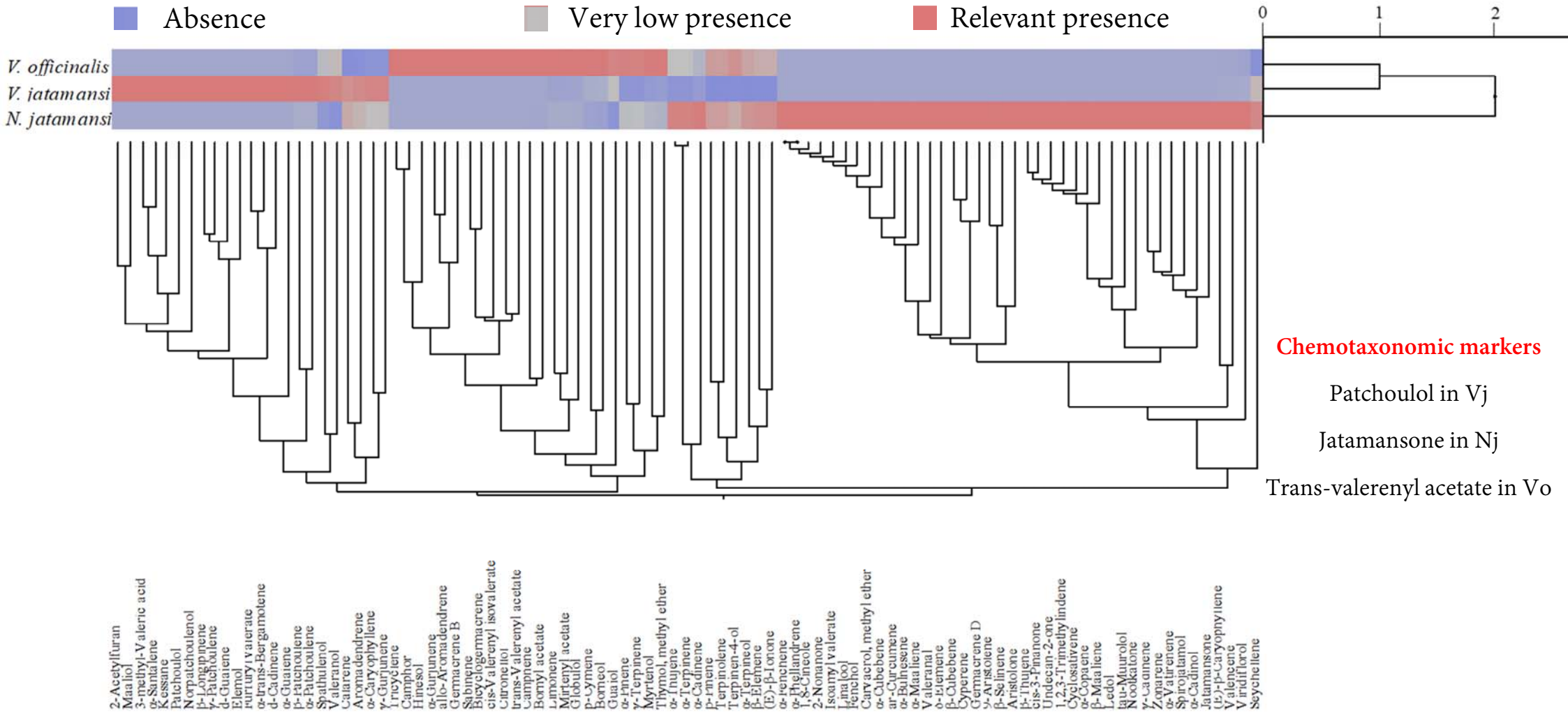
Valencene
8.05%



Jatamansone
13.96%

N. jatamansi

Agglomerative and two way-hierarchical clustering analyses



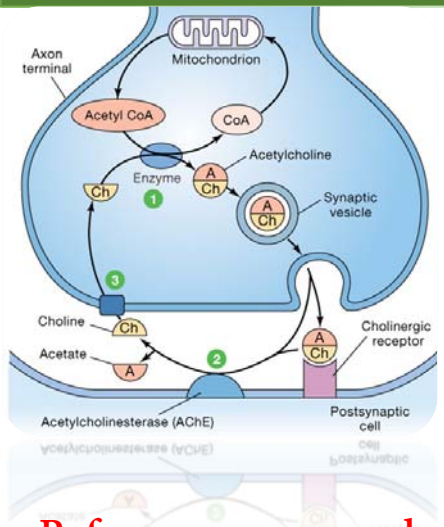
Chemotaxonomic markers

Patchoulol in Vj

Jatamansone in Nj

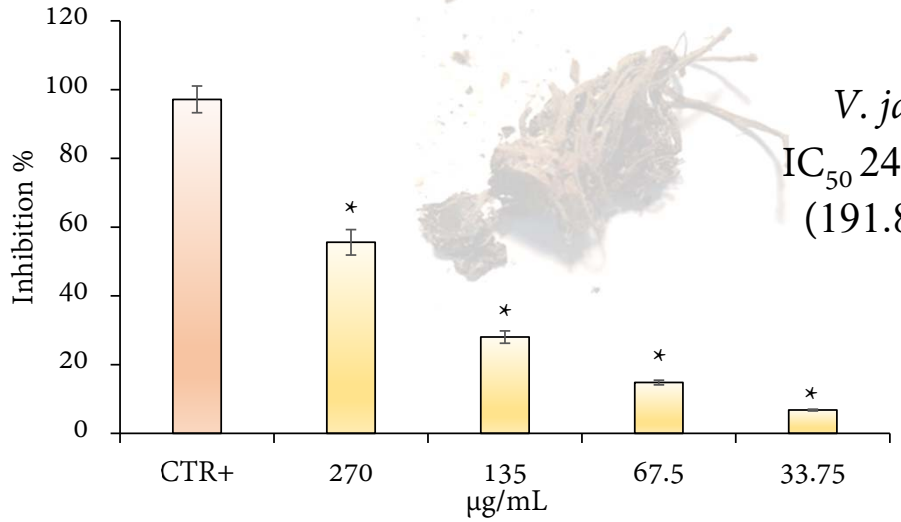
Trans-valerenyl acetate in Vo

Acetylcholinesterase-inhibition assay



Reference compound

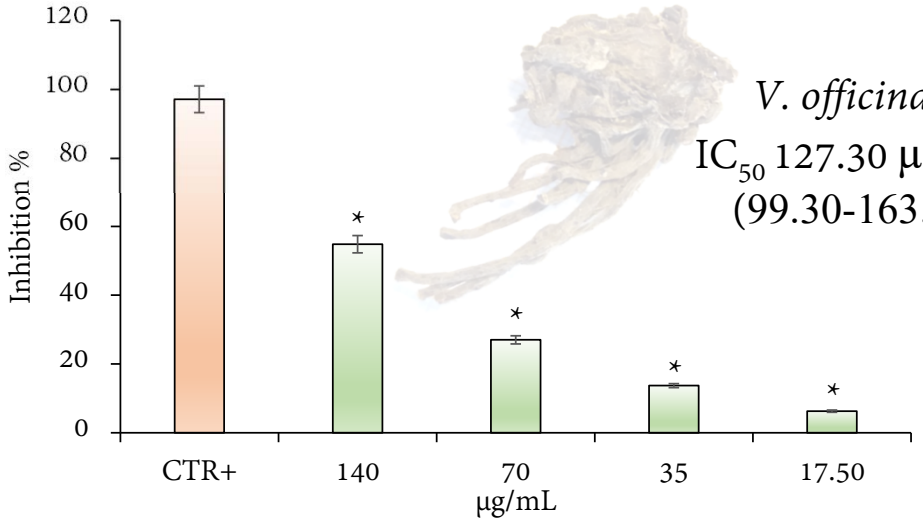
Galantamine 7 µg/mL



V. jatamansi

IC_{50} 246.84 µg/mL

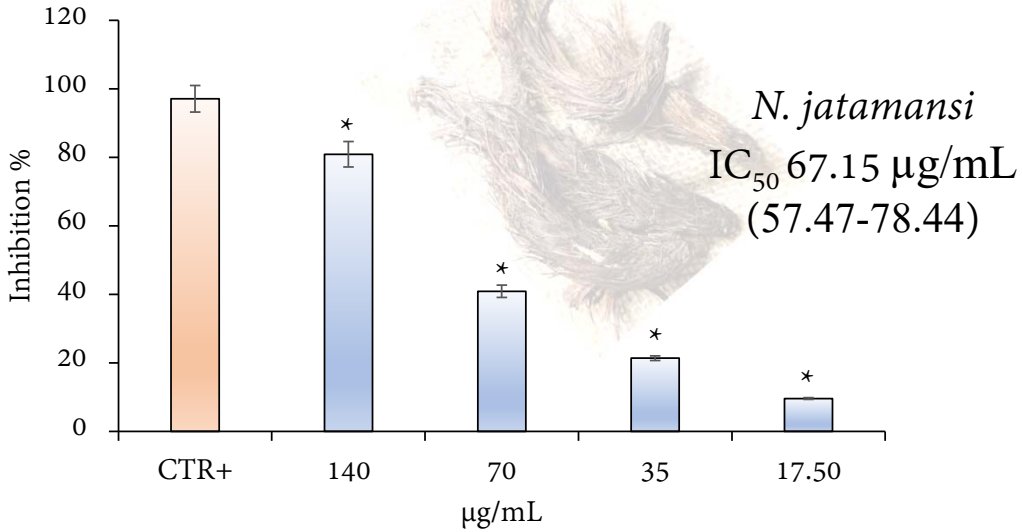
(191.86-317.58)



V. officinalis

IC_{50} 127.30 µg/mL

(99.30-163.20)



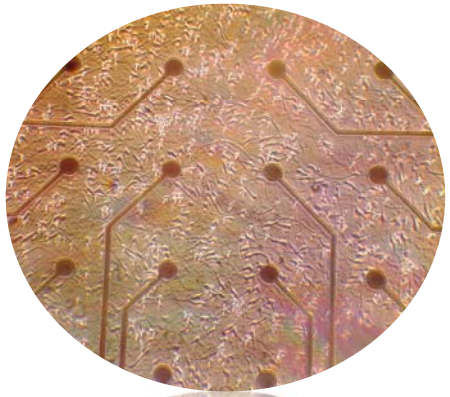
N. jatamansi

IC_{50} 67.15 µg/mL

(57.47-78.44)

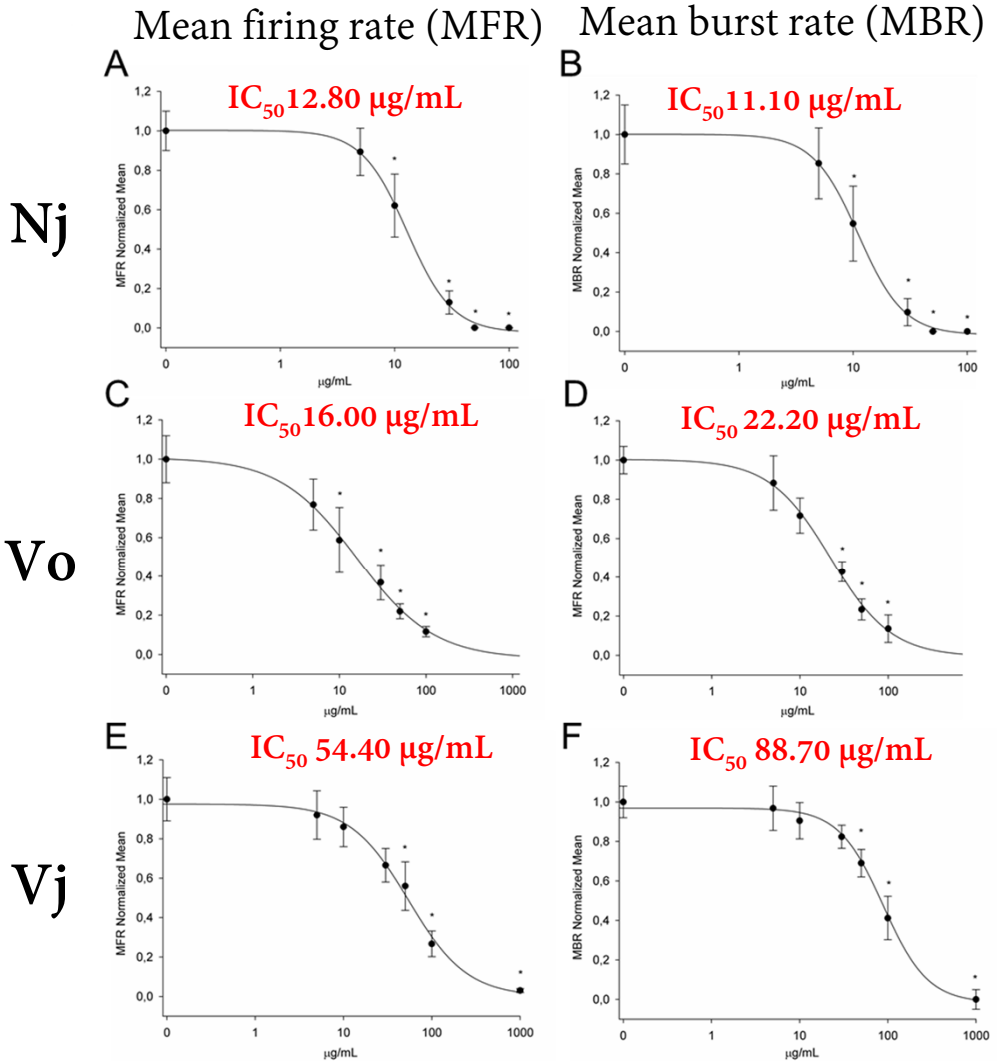
Neuroactive effects

Changes in the spontaneous electrical activity of *in vitro* cortical neuronal networks in response to Nj, Vj and Vo EOs were recorded by using multielectrode chips assay



Reference compound
Muscimol
IC_{50} 0.032 µg/mL (MFR)
IC_{50} 0.004 µg/mL (MFR)

- Concentration-dependent decrease in the electrical activity of neuronal networks until the complete loss of activity;
- Nj EO was the most potent in the inhibition of spontaneous activity;
- A slightly lower efficacy, without any statistically significant difference, was obtained with the administration of Vo EO;
- Vj EO induced a decrease of neuronal activity at higher concentrations (P<0.01 *vs* Vo and Nj).



*P < 0.05

Conclusions

- Microscopic and DNA barcoding analyses represent a rapid and valid approach for herbal drug identification, allowing discrimination of genera and species;
- These combined with the phytochemical fingerprinting of the EOs are important tools to avoid the adulteration of these herbal drugs and to discriminate between EOs of plants coming from different sites;
- This study demonstrates experimentally, for the first time, the effects on the central nervous system of *V. officinalis*, *V. jatamansi* and *N. jatamansi* EOs by both AChE inhibitory activity and a reconstituted murine neuronal network *in vitro;*
- This model reduces the number of animals used to a minimum, according to the principles of the 3Rs (Replacement, Reduction and Refinement) and represents an absolute innovation in the pharmacological/toxicological field to investigate the effects of plant complexes on the central nervous system;
- Therefore, our methods can be recommended for the correct identification of herbal drugs and for the evaluation of the effectiveness of EOs in the treatment of nervous disorders.

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Dr. Susanna Alloisio

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Dr. Govinda Ghimire

ehtpa European Herbal & Traditional Medicine Practitioners Association

Dr. Marco Valussi