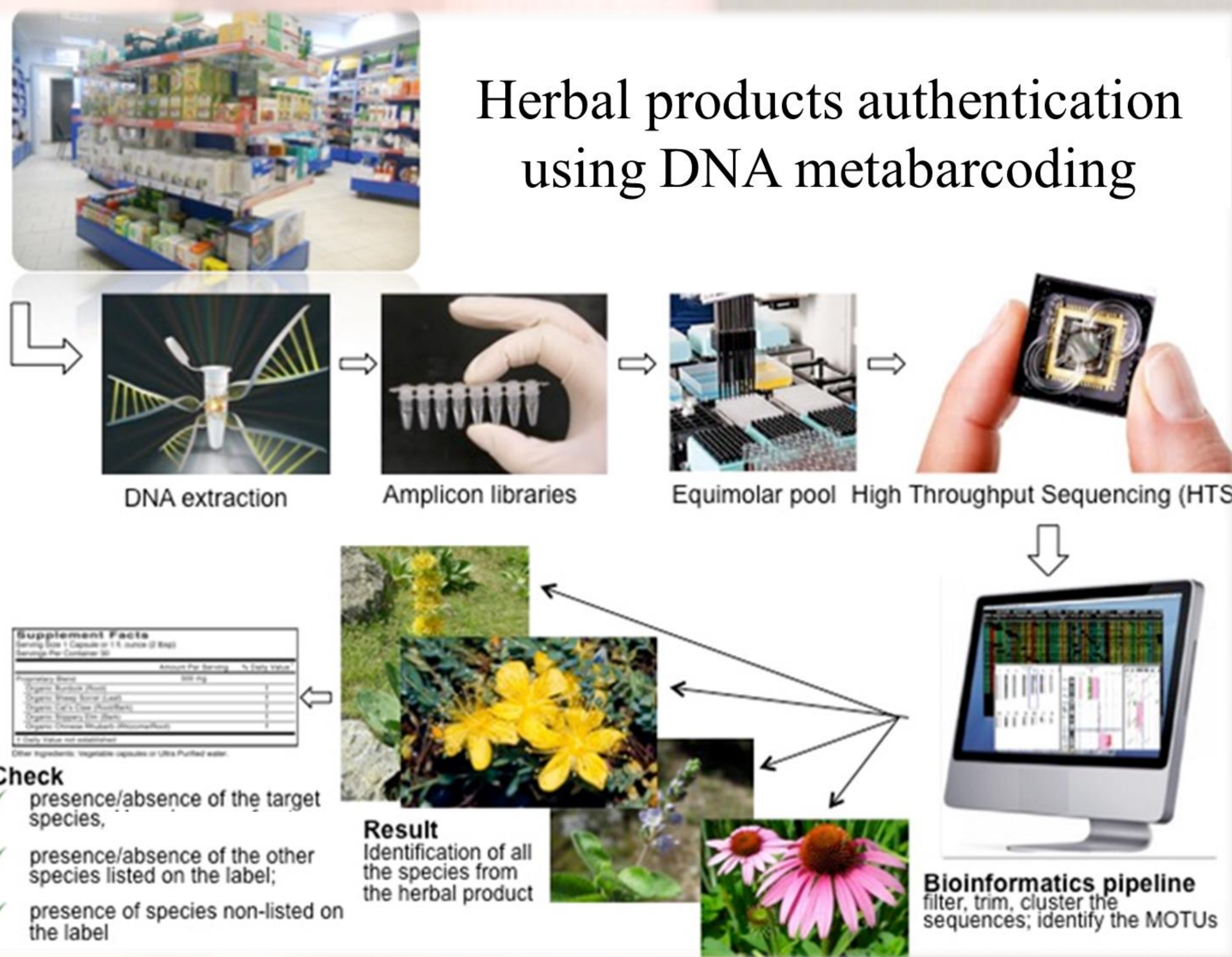


DNA metabarcoding authentication of Ayurvedic herbal products on the European market raises concerns of quality and fidelity

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Background

- Ayurveda is one among the traditional systems of medicine in the world, widely recognized as part of the complementary and alternative system of medicine.
- The growing commercial interest in Ayurvedic herbal products increases the incentive for adulteration and substitution in the medicinal plants market.
- Moreover lack of standardized methods for quality assessment and the highly competitive market of herbal products has also increased the incentive for using substitutes and unlabeled fillers.
- Adulteration is not necessarily intentional, and herbal products may be altered also due to accidental adulteration and misidentification, and confused nomenclature of ingredients of plant origin.
- Even though Ayurveda have a long history of use, there are rising concerns over these products efficacy, safety and quality in the wake of recent cases exposing discrepancies between labeling and constituents.

Aim

- To test the composition and fidelity of Ayurvedic products marketed in Europe using DNA metabarcoding
- To evaluate the ability of DNA metabarcoding to identify the presence of authentic species, any substitution and adulteration and/or presence of other off labeled plant species

Materials and method

Seventy nine herbal products marketed in the Europe as herbal food supplements and herbal drugs were randomly purchased via e-commerce (n=53) and pharmacies (n=26). The amplicon libraries for high throughput sequencing were prepared using fusion primers based on nuclear ribosomal internal transcribed spacers (nrITS1 and nrITS2) in PCR. The sequencing was performed in an Ion Torrent Personal Genome Machine (Life Technologies, Thermo-Fisher Scientific, USA).

	Product form	Mode of acquisition	Country of purchase
	Tablets	e-commerce	Norway
	Capsules		Sweden
	Powders	Pharmacies	Romania
	Extracts		26
Total	79		

Results

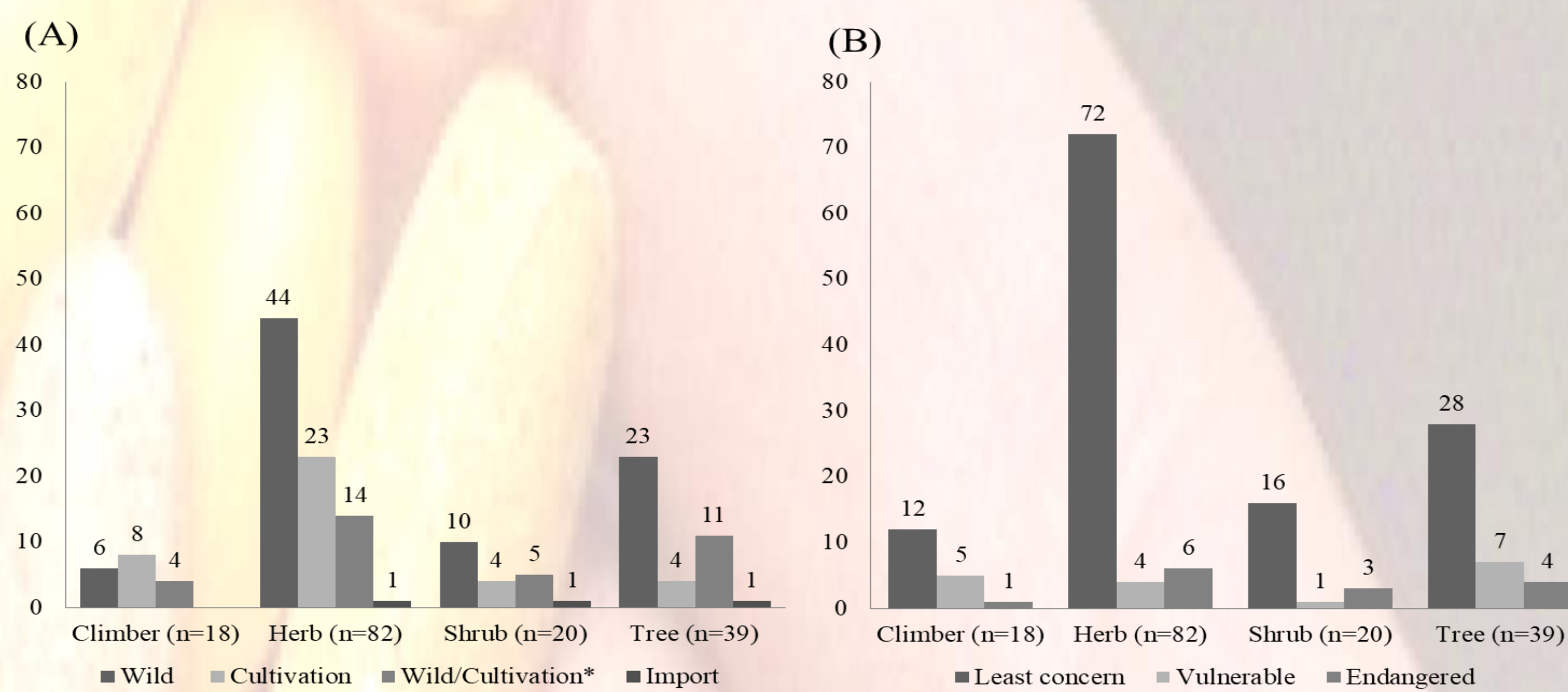


Fig.1: Source and conservation of species in Ayurvedic products. (A) Source of plants labeled as ingredients in the herbal products studied. (B) Threat status of plants labeled as ingredients in the herbal products studied. N = total number of species. *Wild/Cultivation denotes that the plants species are sourced both from wild and cultivation.

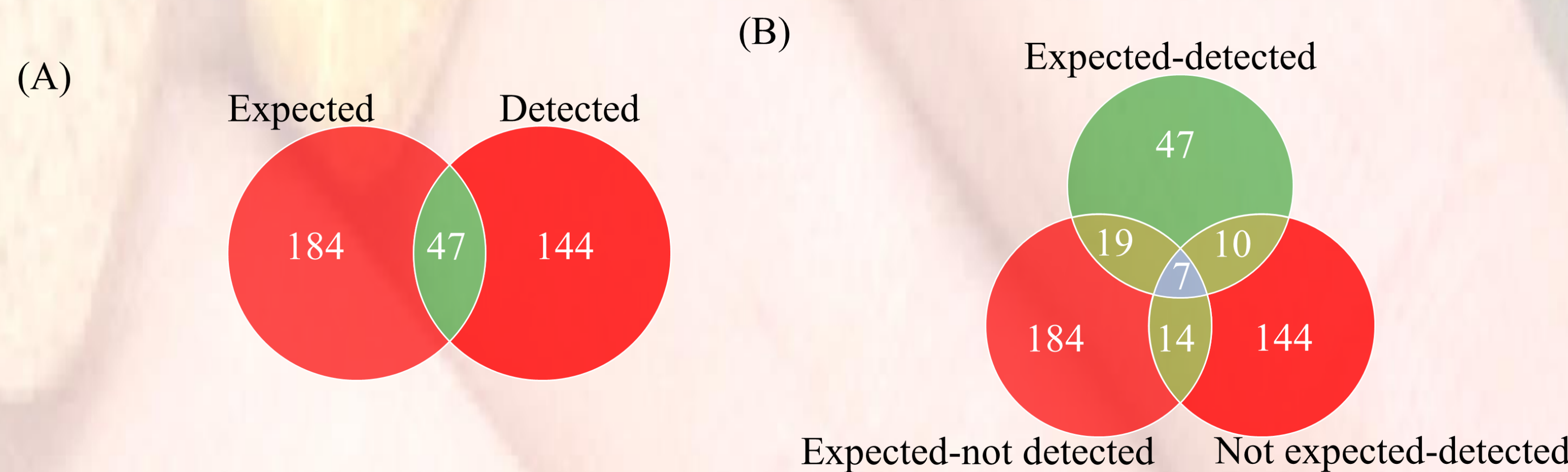


Fig.2: Discrepancies between listed species and detected species using DNA metabarcoding in Ayurvedic herbal products. (A) Total number of occurrences of expected species as labelled in the herbal products and detected species using DNA metabarcoding. (B) Total number of detected species occurred among expected species as labelled in herbal products (expected-detected), the number of undetected species among the expected species as labelled (expected-not detected), and the number of detected unexpected species (not expected-detected) found in herbal products using DNA metabarcoding. The overlapping numbers are the same species detected in herbal products as expected, detected and unexpected detected.

Discussion

The current standard identification approaches suggested by the European Pharmacopoeia and the European Medicine Agency, such as HPLC-MS and TLC, are accurate methods for authenticating presence of the target compounds, but have limited efficiency in detecting infrageneric substitution and do not yield any information on other plant ingredients in the products. Our results confirm that DNA metabarcoding is applicable to test for the presence of target species and simultaneously to detect substitution, adulteration and/or admixture of other species. If product safety relies on threshold levels of specific bioactive compounds, absence of toxins, allergens and admixed pharmaceuticals, then chemical analysis methods are more relevant than DNA based composition analysis, but if product fidelity, species substitution or adulteration is suspected then the latter method outperforms in terms of resolution.

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