**Table S2.** New Approach Methodologies used to assess the skin sensitization potential of botanicals.

| Testing Methodology | | Botanical Specifics | | | | | Conclusions | Reference |
|---|---|---|---|---|---|---|---|---|
| **NAM Test System** | **Paired *In Vivo*/Other Data** | **Plant Tested (Part of Plant Extracted)** | **Type of Extract Preparation** | **Chemical Characterization Method** | **Extracted Ingredient Identified with Sensitization Potential** | **Intended Use/Product Type** | | |
| KeratinoSens™ | NA | - Chamomile (flower)<br>- Green Tea (leaf)<br>- Gurana (seed)<br>- Papaya (fruit) | - Chamomile: flower solubilized in 27% propylene glycol and 53% water<br>- Green Tea: powder<br>- Gurana and Papaya: fruit and seed, respectively, solubilized in 2.5% water and 96.8% glycerol as carrier<br><br>Extracts were spiked with different doses of known sensitizers:<br>- Cinannmic aldehyde<br>- Citral<br>- Isoeugenol | NP | - Chamomile: 20% active plant matter consisted mostly in flavonoids<br>- Green Tea: 95% polyphenols and 50% catechin<br>- Gurana and Papaya: 0.7% of active plant content | Cosmetic product | - Based on LLNA data for the sensitizers used in the spiked extracts, citral is not expected to be detected when present at concentrations below 10% in mixtures, while cinnamic aldehyde and isoeugenol are not expected to be detected below 2% and 1.5%, respectively.<br>- The results indicate that a single moderate sensitizer can be detected when added to non-cytotoxic plant extracts that are otherwise negative in the KeratinoSens™ assay. Detection of isoeugenol at 5% and citral at 0.5% in the spiked chamomile extract highlights the sensitivity of this NAM. | [14] |
| - HaCaT IL-18<br>- mDPRA<br>- MUSST IL-8 | NA | *Eugenia dysenterica* (leaf) | Hydroalcoholic EDE (2.5-1,000 µg/mL) | HPLC | - Tannin (ellagic acid) | Cosmetic product | - mDPRA: EDE was correctly classified as a skin sensitizer in the high reactivity class. Cysteine depletion was particularly pronounced and showed | [15] |

| Testing Methodology | | Botanical Specifics | | | | | Conclusions | Reference |
|---|---|---|---|---|---|---|---|---|
| NAM Test System | Paired *In Vivo*/Other Data | Plant Tested (Part of Plant Extracted) | Type of Extract Preparation | Chemical Characterization Method | Extracted Ingredient Identified with Sensitization Potential | Intended Use/Product Type | | |
| | | | | | - Flavonoids (quercetin and gallic acid) | | a concentration-dependent pattern. Based on mean peptide depletion, EDE fell within the moderate to high reactivity category.<br>- HaCaT and IL-18 assay: EDE did not induce an increase in IL-18 levels in HaCaT cells at non-cytotoxic concentrations of 40, 80, and 160 µg/mL, indicating no sensitization potential for this endpoint.<br>- MUSST and IL-8 assay: EDE was classified as a non-sensitizer at non-cytotoxic concentrations ranging from 12.5 to 200 µg/mL. Tannins are known to form complexes with proteins through hydrogen bonding and hydrophobic interactions, which may partially interfere with the analytical detection of peptide depletion induced by EDE. | |

| Testing Methodology | | Botanical Specifics | | | | | Conclusions | Reference |
|---|---|---|---|---|---|---|---|---|
| **NAM Test System** | **Paired *In Vivo*/Other Data** | **Plant Tested (Part of Plant Extracted)** | **Type of Extract Preparation** | **Chemical Characterization Method** | **Extracted Ingredient Identified with Sensitization Potential** | **Intended Use/Product Type** | | |
| DCYA | NA | - *Calendula officinalis* (flower)<br>- *Cinnamonum verum* (bark)<br>- *Magnolia grandiflora* (leaf)<br>- *Rosa canina* (fruit) | - *Cinnamonum verum* and *Calendula officinalis* extracted in methanol<br>- *Magnolia grandiflora* was used to select the solvent from methanol, acetone, diethyl ether, ethyl acetate, cyclohexane and hexane<br>- *Rosa canina* extracted in methanol<br><br>The least DCYA-reactive extract (of *Rosa canina*) was spiked with the following sensitizers at various concentrations:<br>- Cinnamaldehyde<br>- Coumarin<br>- Parthenolide | NP | - *Calendula officinalis*: no sensitizing components<br>- *Cinnamonum verum*: cynnamaldehide<br>- *Magnolia grandiflora*: sesquiterpene lactones (parthenolide, costunolide)<br>- *Rosa canina*: no sensitizing components | NS | - The extract of *Rosa canina* was spiked with parthenolide, cinnamaldehyde, and coumarin, each prepared as a 20 mM stock. The final spiking concentrations were selected to range from 50% to 150% of the optimal concentration (5 mM) for pure compounds (3 concentrations were used).<br>- The methanol extract of *Rosa canina* spiked with parthenolide showed measurable DCYA depletion (TRI increase) at all tested concentrations. When the plant material was spiked with 1 equivalent of parthenolide, a concentration-dependent reactivity was evident between 5 and 20 mg/mL of total extract. A saturated response occurred when the parthenolide/DCYA ratio exceeded | [18] |

| Testing Methodology | | Botanical Specifics | | | | | Conclusions | Reference |
|---|---|---|---|---|---|---|---|---|
| **NAM Test System** | **Paired *In Vivo*/Other Data** | **Plant Tested (Part of Plant Extracted)** | **Type of Extract Preparation** | **Chemical Characterization Method** | **Extracted Ingredient Identified with Sensitization Potential** | **Intended Use/Product Type** | | |
| | | | | | | | 2:1, resulting in complete DCYA depletion at all tested extract concentrations.<br>- *Rosa canina* samples spiked with the moderate sensitizer, cinnamaldehyde, produced a concentration-dependent fluorescence response, although only partial DCYA reactivity was observed at any tested concentration.<br>- In contrast, no concentration-dependent reactivity was observed in the presence of coumarin, a non-reactive electrophile. | |
| - DPRA<br>- h-CLAT<br>- KeratinoSens™<br>- Sens-IS<br>- U-SENS™ | - LLNA<br>- Human | - *Jasmine absolute frandiflorum*<br>- *Jasmine absolute sambac*<br>- Oakmoss<br>- Treemoss | NP | NP | NA | NA | - Natural extracts present specific challenges, as their composition depends on the source material and extraction process. As a result, they cannot be precisely defined and are likely to vary over time. This variability compromises comparisons with | [54,63] |

| Testing Methodology | | Botanical Specifics | | | | | Conclusions | Reference |
|---|---|---|---|---|---|---|---|---|
| **NAM Test System** | **Paired *In Vivo*/Other Data** | **Plant Tested (Part of Plant Extracted)** | **Type of Extract Preparation** | **Chemical Characterization Method** | **Extracted Ingredient Identified with Sensitization Potential** | **Intended Use/Product Type** | | |
| | | - Tea leaf absolute<br>- Ylang-ylang | | | | | existing data, which often lack adequate documentation of extract composition.<br>- A comprehensive, harmonized database of 128 substances was generated and included results from five NAMs, LLNA and human reference data. This group included 6 natural extracts, providing diversity on the data set to be used as reference covering various domains such as complex mixtures. | |
| - h-CLAT<br>- KeratinoSens™ | LLNA | NA | NA | NA | NA | Cosmetics | - This study was based on the assumption that botanical extracts may contain skin sensitizers that yield positive results in *in vitro* test methods. The detection limits of these sensitizers in botanical extracts were compared between the LLNA and the in vitro assays KeratinoSens™ and h-CLAT. | [94] |

| Testing Methodology | | Botanical Specifics | | | | | Conclusions | Reference |
|---|---|---|---|---|---|---|---|---|
| **NAM Test System** | **Paired *In Vivo*/Other Data** | **Plant Tested (Part of Plant Extracted)** | **Type of Extract Preparation** | **Chemical Characterization Method** | **Extracted Ingredient Identified with Sensitization Potential** | **Intended Use/Product Type** | | |
| | | | | | | | - It was assumed that the botanical extracts were water-soluble, non-cytotoxic, and did not exhibit general masking effects that could interfere with the detection of skin sensitizers in their composition.<br>- A total of 146 chemicals were analyzed, of which 116 and 129 were positive in the KeratinoSens™ and h-CLAT assays, respectively.<br>- The results support the broad applicability of *in vitro* assays for detecting skin-sensitizing constituents in botanical extracts. However, the applicability of *in vitro* methods is limited for oil-based or cytotoxic botanical extracts.<br>- Some sensitizers showed higher detection limits on *in vitro* test methods than LLNA, suggesting that the botanical extract containing sensitizers | |

| Testing Methodology | | Botanical Specifics | | | | | Conclusions | Reference |
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| **NAM Test System** | **Paired *In Vivo*/Other Data** | **Plant Tested (Part of Plant Extracted)** | **Type of Extract Preparation** | **Chemical Characterization Method** | **Extracted Ingredient Identified with Sensitization Potential** | **Intended Use/Product Type** | | |
| | | | | | | | might be positive in LLNA but negative in the binary test battery. | |
| - h-CLAT<br>- h-CLAT adaptation in DC2.4 cells | LLNA | Brazilian Green Propolis (from *Baccharis dracunculifolia*) | 95% Ethanol/water extraction (55% BGP in ethanol extract was tested) | Provided by the extract supplier | Cinnamic acid derivatives | - Biopharmaceuticals<br>- Cosmetics<br>- External preparations<br>- Food supplements<br>- Shampoos<br>- Toothpaste<br>- Traditional medicine | - BGP was identified as a non-sensitizer in the standard h-CLAT assay.<br>- BGP was determined as positive in the DC2.4 adaptation of the h-CLAT assay, and was identified as a moderate skin sensitizer in the LLNA.<br>- The appropriate classification of BPG when using the DC2.4 adaptation of the h-CLAT assay indicates that the proposed model may have increased sensitivity for the detection of sensitizers that otherwise may lead to false negative results in the standard h-CLAT assay. | [4] |
| DPRA | NA | - *Lavandula angustifolia*<br>- *Melissa officinalis*<br>- *Mentha longifolia* | NP | GC/MS | NP | Cosmetics | - The DPRA was able to predict 4 out of 6 essential oils as skin sensitizers. These results appear to be in accordance with literature reports regarding the potential of these oils to cause skin sensitization. | [93] |

| Testing Methodology | | Botanical Specifics | | | | | Conclusions | Reference |
|---|---|---|---|---|---|---|---|---|
| **NAM Test System** | **Paired *In Vivo*/Other Data** | **Plant Tested (Part of Plant Extracted)** | **Type of Extract Preparation** | **Chemical Characterization Method** | **Extracted Ingredient Identified with Sensitization Potential** | **Intended Use/Product Type** | | |
| | | - *Rosmarinus officinalis*<br>- *Salvia officinalis*<br>- *Thymus vulgaris* | | | | | - Based on these results, the authors propose DPRA as an assay capable to identify the sensitization potential of essential oils. | |
| - B-PPRA<br>- Derek Nexus<br>- OASIS TIMES *in silico* model | NA | - *Arnica montana* (flower)<br>- Calendula (flower)<br>- Chamomile (flower)<br>- Feverfew (leaf)<br>- Propolis (bee glue)<br>- Rosemary (leaf) | The botanical extracts contained <2% active in a carrier composed of 15-25% water and either 75-85% glycerin or 75-85% propylene glycol.<br><br>Sensitivity of B-PPRA was evaluated using the Chamomile extract (85% propylene glycol and 15% water) spiked with: | Multiple as identified through literature search<br><br>(*see the Discussion section in the manuscript for more details*) | The most likely components with sensitization potential were identified based on literature search and by *in silico* determination<br><br>(*see the Discussion section in the manuscript for more details*) | Consumer products | - A modification of PPRA was explored in this study for screening of botanicals or natural substances. The assay incorporates an oxidation system (+HRP/P) for the activation of potential pre- and pro-haptens.<br>- Initial studies demonstrated that B-PPRA results were reproducible and the carrier's impact was low.<br>- The spiking experiments identified mixtures containing isoeugenol as sensitizers at concentrations as low as 0.05%.<br>- The presence of pro- or pro-hapten constituents contribute to the higher | [86] |

| Testing Methodology | | Botanical Specifics | | | | | Conclusions | Reference |
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| **NAM Test System** | **Paired *In Vivo*/Other Data** | **Plant Tested (Part of Plant Extracted)** | **Type of Extract Preparation** | **Chemical Characterization Method** | **Extracted Ingredient Identified with Sensitization Potential** | **Intended Use/Product Type** | | |
| | | | - Cinnamic aldehyde (moderate sensitizer that forms adducts and oxidizes Cys to form dimers)<br>- Citral (weak sensitizer that forms adducts in peptide reactivity assays)<br>- Isoeugenol (moderate sensitizer that forms adducts and oxidizes Cys to form dimers, is classified as a pre-hapten) | | | | peptide depletion observed for extracts of *Arnica montana*, Calendula, Propolis, and Rosemary and vice-versa for extracts of Feverfew and Chamomile which predicted to be non-sensitizers.<br>- B-PPRA may be considered as a screening assay and used as part of an integrated approach for skin sensitization risk assessment. | |
| - DPRA<br>- h-CLAT<br>- LuSens<br>- mMUSST<br>- Sens-IS | - LLNA<br>- GPMT | - *Bixa orellana*<br>- *Cariniana brasiliensis*<br>- *Cistus monspeliensis*<br>- *Diospyros mespliformis* | Commercial plant extracts tested as follows:<br><br>- DPRA: 8 extracts<br>- h-CLAT: 8 extracts<br>- LuSens: 4 extracts<br>- mMUSST: 2 extracts | NA | NA | - Nutrition products<br>- Personal care products | - The balanced accuracy was 50% for DPRA, h-CLAT, and mMUSST; 67% for LuSens, and 88% for Sens-IS.<br>- The 2o3 approach was also evaluated for some of the extracts with available DPRA, LuSens, and h-CLAT data, resulting in a balanced accuracy of 50%. | [17] |

| Testing Methodology | | Botanical Specifics | | | | | Conclusions | Reference |
|---|---|---|---|---|---|---|---|---|
| **NAM Test System** | **Paired *In Vivo*/Other Data** | **Plant Tested (Part of Plant Extracted)** | **Type of Extract Preparation** | **Chemical Characterization Method** | **Extracted Ingredient Identified with Sensitization Potential** | **Intended Use/Product Type** | | |
| | | - *Epilobium angustifolium*<br>- *Ferula hermonis*<br>- *Grifola frondosa*<br>- *Hippophae rhamnoides*<br>- *Lansium domesticum*<br>- *Manilkara multinervis*<br>- *Parkia biglosa*<br>- *Persicaria bistorta* | - Sens-IS: 12 extracts<br><br>(*see the manuscript for more details on the identity of extracts tested and using which assays)* | | | | - When mMUSST was used within the 2o3 approach instead of h-CLAT, turned one true-positive extract (*Parkia biglosa*) into a false-negative, while *Cariniana brasiliensis* remained a true-positive.<br>- The authors indicate that the 2o3 approach that includes DPRA, LuSens/KeratinoSens™, and h-CLAT is not recommended for botanical extracts.<br>- The results showed that some substance subgroups may not be within the applicability domains of the methods used. | |
| - DPRA<br>- h-CLAT<br>- Keratinosens™<br>- Sens-IS | - GPMT<br>- HMT<br>- HRIPT<br>- LLNA | - *Aloe barbadensis* (inner leaf gel)<br>- *Allium sativum* (bulb) | Varied depending on the plant, part of the plant, etc.<br><br>(*see details included in the manuscript for each of the botanicals used*) | Accomplished through literature search for each botanical extract | For each botanical, literature information on composition and drivers for | - Consumer products<br>- Cosmetics<br>- Household care products | - This study compiled the available information for 14 commonly used botanicals in consumer products, to classify their sensitization potential using a WoE approach.<br>- Based on available information, the botanicals were classified into: | [95] |

| Testing Methodology | | Botanical Specifics | | | | | Conclusions | Reference |
|---|---|---|---|---|---|---|---|---|
| **NAM Test System** | **Paired *In Vivo*/Other Data** | **Plant Tested (Part of Plant Extracted)** | **Type of Extract Preparation** | **Chemical Characterization Method** | **Extracted Ingredient Identified with Sensitization Potential** | **Intended Use/Product Type** | | |
| | | - *Anacardium occidentale* (nutshell)<br>- *Camellia sinensis* (leaf)<br>- *Centella asiatica* (whole plant)<br>- *Citrullus lanatus* (seed oil)<br>- *Cucumis sativus* (fruit)<br>- *Matricaria chamomila* (flower)<br>- *Rhodymenia palmata* (whole algae)<br>- *Rosmarinus officinalis* (leaf)<br>- *Silybum marianum* (fruit, seed) | | (*see the manuscript for specific details*) | sensitization effect is included<br><br>(*see the manuscript for specific details on each extract*) | | a. Strong evidence to cause sensitization<br>b. Some but limited evidence of causing sensitization<br>c. Strong evidence that they are non-sensitizers<br>- When available data include sufficient potency-related information, a sensitization potency assessment was also provided using a WoE approach, classifying the botanicals as strong, moderate, weak, or non-sensitizers..<br>- The proposed framework include the following sequential steps:<br>1. Determine if the exposure to the botanical is below the threshold of exposure; if not, proceed to step 2<br>2. Compile all available historical information; if the information is insufficient to classify the hazard of the | |

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|---|---|---|---|---|---|---|---|---|
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| | | - *Tanacetum parthenium* (leaf, petal)<br>- *Toxicodendron diversilobum* (leaf, stem, bark, fruit)<br>- *Toxicodendron radicans* (leaf, stem, fruit) | | | | | botanical, proceed to step 3;<br>3. Generate new data, such as NAMs data, then return to 2.<br>- The set of botanicals included in this analysis can be considered a reference set based on their "data rich" profile covering a range of sensitization potencies that can be used to evaluate existing test methods or support the development of new predictive models for skin sensitization. | |

2o3, 2 out of 3 approach; BGP, Brazilian Green Propolis; B-PPRA, Botanicals Peroxidase Peptide Reactivity Assay; Cys, Cysteine; DC, dendritic cells; DCYA, Dansyl Cysteamine Assay; DPRA, Direct Peptide Reactivity Assay; EDE, *Eugenia dysenterica* extract; DCYA, Dansyl Cystemine Assay; GC/MS, Gas Chromatography/Mass Spectrometry; GPMT, Guinea Pig Maximization Test; h-CLAT, human Cell Line Activation Test; HMT, Human Maximisation Test; HPLC, High-Performance Liquid Chromatography; HRIPT, Human Repeated Insult Patch Test; HRP/P, Horseradish Peroxidase/Hydrogen peroxide; IL, Interleukin; LLNA, Local Lymph Node Assay; mDPRA, micro or modified DPRA; mMUSST, modified Myeloid U937 Skin Sensitization Test; MW, Molecular Weight; NA, Not Applicable; NAM, New Approach Methodology; NP, Not Provided; NS, Not Specified; TIMES, Times Metabolism Stimulator for Skin Sensitization; TRI, Total Reactive Index; WoE, Weight of Evidence.

Note: The references are presented in chronological order and alphabetically within the same year (where applicable).