Skin sensitization potential of German chamomile – a case study for integrated testing approaches to the safety evaluation of botanical ingredients

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Abstract

German chamomile is one of the most prevalent botanical ingredients used in cosmetics based on preliminary market analysis. The objective of this study was to apply integrated non-animal testing strategies to the skin sensitization assessment of botanicals using German chamomile as a case study. A comprehensive list of chemical constituents (N=246) from chamomile was built from various database sources. These chemicals were first screened for their skin sensitization potentials using *in silico* methods that comprises commercially available two c-QSAR models (ADMET predictor and Case Ultra) and an expert knowledge-based system (Derek Nexus). Based on *in silico* screening results and other relevant considerations, 30 chemical constituents were selected for experimental testing using four non-animal testing methods (DPRA, HTS-DCYA, KeratinoSens, and hCLAT). A combination of *in silico* predictions for the chosen compounds set resulted in 15 compounds positive for skin sensitization. Experimental models were combined using an integrated "2 out of 3" strategy for sensitization, resulting in 12 compounds being capable of activating at least two key events. Forty-seven per cent (47%) agreement was found between predictive and experimental results. Seven compounds (umbelliferone, apigenin, kaempferol, isorhamnetin, nerol, α-terpinene, and carvone) were positive and seven compounds (caffeic acid, t-ferulic acid, cosemetin, hyperoside, α-terpineol, α-bisabolol, and chamazulene) were negative in both models, i.e., *in silico* and experimental settings. These results suggest that additional work is needed to assess these compounds for their ability to cause skin sensitization.

Introduction

German chamomile (*Matricaria chamomilla* L.) is one of the most popular botanical ingredients used in cosmetics and personal care products, as a skin conditioning agent, cosmetic biocide, fragrance, or flavoring agent. Although commonly reputed to be a gentle ingredient, allergic reactions to the whole plant and products containing chamomile have been observed on some occasions, without information on the probable causative agents. Both non-volatile and volatile organic components (VOCs) are used in cosmetic preparations, in the form of extracts or essential oils, respectively. The extracts obtained from German chamomile are typically enriched in flavonoid glucosides, non-glycosylated flavonoids, and organic acids.

Chamomile essential oils typically contain α-bisabolol (up to 41.45% of the oil), oxygenated bisaboloids, fatty acid methyl esters, and pre-azulenes among the main VOCs. The characteristic blue color of some of these essential oils is due to chamazulene formed upon degradation of matricin during the hydro-distillation process.

In the present work, five non-animal methods were applied to evaluate the skin sensitization ability of various phytochemical constituents found in *M. chamomilla*. The panel of chosen methods included *in silico* preliminary screening and a combination of four experimental methods. *In silico* screening of German chamomile was performed using two classification-quantitative structure-activity relationship (c-QSAR) models constructed under two different environments (e.g., ADMET Predictor™ version 9.5 and Case Ultra 1.8.0.2) and an expert knowledge-based system (Derek Nexus 6.0.1). The data generated from the three *in silico* predictions were combined using a simple majority rule (SMR). Further experimental research on the selected list of compounds was carried out using three OECD methods (DPRA, KeratinoSens and hCLAT) and the HTS-DCYA. Experimental results were then combined using an integrated "2 out of 3" (a weight of evidence) approach.

Materials and Methods

A comprehensive list of chamomile constituents (N = 283) was compiled from different sources, including SciFinder, Dictionary of Natural Products database, CIR reviews as well as data generated in-house. The simplified molecular-input line-entry system (SMILES) strings were then generated for each component. This list was further reduced to 246 based on *in silico* suitability.

***In silico* predictors:** Two c-QSAR models of skin sensitization (LLNA), constructed under different environments, were obtained from two different commercial software programs, *i.e.* ADMET Predictor™ (version 9.5, Simulations-Plus Inc.) and Case Ultra (version 1.8.0.2, Multicase Inc.). An expert knowledge-based system was obtained from Lhasa Limited (Derek Nexus 6.0.1). All three *in silico* predictors were used through a CFSAN Research Collaboration Agreement (RCA).

***In chemico* methods:** The Direct Peptide Reactivity Assay (DPRA) was performed as described by OECD guidelines 442C [1]. The high throughput assay with dansylated cysteamine (HTS-DCYA) method was performed as described by Avonto *et al.* [2].

***In vitro* methods:** Both KeratinoSens™ and human Cell Line Activation Test (hCLAT) were performed following OECD guidelines 442D and 442E, respectively [3,4].

Table 1. *In silico* skin sensitization (LLNA) prediction results using ADMET Predictor, Case Ultra, and Derek Nexus models.

SN	Ingredient Name	ADMET Predictor		Case Ultra		Derek Nexus		SMR
		Pred	Conf.	Pred.	PP	Pred.	Reasoning	
1	Umbelliferone	**0**	97%	1	57.8%	1	Plausible	Positive
2	Herniarin	Inc.	Inc.	0	30.5%	1	Plausible	Inc.
3	Hydroxytyrosol	1	64%	1	86.4%	1	Plausible	Positive
4	2,5-$(OH)_2$-benzoic acid	0	97%	Inc.	Inc.	1	Plausible	Inc.
5	Syringic Acid	0	97%	0	11.9%	0	NMUF	Negative
6	Caffeic acid	0	75%	1	80.3%	0	NMUF	Negative
7	*t*-ferulic acid	0	89%	1	73.2%	0	NMUF	Negative
8	Apigenin	0	97%	1	72.5%	1	Plausible	Positive
9	Luteolin	0	97%	1	87.8%	1	Plausible	Positive
10	Quercetin	0	82%	1	93.1%	1	Plausible	Positive
11	Kaempferol	0	97%	1	83.2%	1	Plausible	Positive
12	Isorhamnetin	0	93%	1	90%	1	Plausible	Positive
13	Epicatechin	Inc.	Inc.	Inc.	Inc.	1	Plausible	Inc.
14	Cosemetin	0	97%	0	2.1%	Inc.	Equivocal	Negative
15	Hyperoside	0	97%	0	32.2%	1	Plausible	Negative
16	Quercitrin	0	97%	1	64.4%	1	Plausible	Positive
17	Rutin	OAD	OAD	1	64.4%	1	Plausible	Positive
18	Myrcene	1	97%	Inc.	Inc.	Inc.	Equivocal	Inc.
19	Nerol	1	79%	1	56.6%	1	Certain	Positive
20	α-Pinene	1	93%	1	66.6%	Inc.	Equivocal	Positive
21	β-Pinene	1	88%	1	77.6%	0	NMUF	Positive
22	α-Terpinene	1	95%	0	23.9%	1	Certain	Positive
23	α-Terpineol	0	78%	0	24.3%	Inc.	Equivocal	Negative
24	Carvone	1	99%	1	87.1%	1	Plausible	Positive
25a	α- Farnesene	OAD	OAD	Inc.	Inc.	Inc.	Equivocal	Inc.
25b	β- Farnesene	1	99%	Inc.	Inc.	Inc.	Equivocal	Inc.
25	Farnesene*	Inc.	Inc.	Inc.	Inc.	Inc.	Equivocal	Inc.
26	Farnesol	1	83%	1	56.6%	1	Certain	Positive
27	α-Bisabolol	0	64%	0	38.1%	Inc.	Equivocal	Negative
28	Chamazulene	1	93%	0	35.6%	0	CUF	Negative
29	Guaiazulene	1	90%	0	35.6%	0	CUF	Negative
30	Isophytol	0	82%	1	61.9%	0	NMUF	Negative
	Positive/Negative/ Inconclusive/OAD	***11/16/3/2 (10/16/3/1)*****		***17/9/6/0 (17/9/4/0)*****		***17/7/8/0 (17/7/6/0)*****		***15/10/7/0 (15/10/5/0)*****

Where, 1 = Positive; 0 = Negative; OAD = Outside the applicability domain; Inc. = Inconclusive; Pred. = Prediction; Conf. = Confidence; PP = Probability of being Positive; SMR = Simple majority rule; NMUF = No misclassified or unclassified feature; CUF = Contains unclassified features; **In silico* prediction of 'Farnesene' is based on its components: α-Farnesene and β-Farnesene; **Data of α-Farnesene and β-Farnesene were excluded.

Results and Discussion

Based on market analyses (Mintel), the most prevalent use of German chamomile (*M. chamomilla*) as a cosmetic ingredient is in the form of either flower or whole plant extracts.

The *in silico* extrapolations of the preliminary comprehensive list of German chamomile constituents (N=246) were obtained with prediction confidence (quantitative or qualitative), and outside the applicability domain (OAD) flag (if any). Finally, 30 constituents of German chamomile were selected as test articles (Table 1), with the goal of covering all representative chemical classes.

Table 2. Experimental results for activation of Key Event (KE) 1 (DRPA, HTS-DCYA), KE 2 (KeratinoSens assay) and KE 3 (hCLAT)

	Compound	DPRA Pept. Depl. (%)	HTS-DCYA RI	KeratinoSens EC 1.5	hCLAT CD54/CD86
1	Umbelliferone	15.87	20.4	28.65	Positive/Negative
2	Herniarin	2.78	2.8	14.17	Negative/Negative
3	Hydroxytyrosol	n.q.	3.3	51.74	Negative/Negative
4	2,5-Dihydroxybenzoic acid	21.07	12.0	>2000	Negative/Negative
5	Syringic Acid	52.95	-1.6	987.76	Negative/Negative
6	Caffeic acid	36.68*	6.4	>2000	Negative/Negative
7	t-ferulic acid	26.53*	0.1	>2000	Negative/Negative
8	Apigenin	8.25	-6.3	1.79	Negative/Negative
9	Luteolin	99.24*	14.9	>2000	Negative/Negative
10	Quercetin	98.41*	61.3	>2000	Negative/Negative
11	Kaempferol	52.42*	1.4	4.72	Negative/Negative
12	Isorhamnetin	n.q.	41.1	1.62	Negative/Positive
13	Epicatechin	91.38*	3.9	8.76	Positive/Negative
14	Cosemetin	n.q.	-11.7	44.23	Negative/Negative
15	Hyperoside	n.q.	10.8	>2000	Negative/Negative
16	Quercitrin	37.15*	10.0	>2000	Negative/Negative
17	Rutin	n.q.	3.5	>2000	Positive/Negative
18	Myrcene	1.01	1.6	>2000	Negative/Negative
19	Nerol	1.05	-1.0	19.55	Positive/Negative
20	α-Pinene	4.4	1.0	>2000	Positive /Negative
21	β-Pinene	4.05	2.0	>2000	Negative/Negative
22	α-Terpinene	54.69	0.6	11.66	Positive /Positive
23	α-Terpineol	5.20	0.5	>2000	Positive /Negative
24	Carvone	11.95	12.8	72.86	Positive /Negative
25	Farnesene	62.72	8.9	1 ± 0	Negative/Negative
26	Farnesol	2.07	1.5	4.25	Negative/Negative
27	α-Bisabolol	4.94	4.1	>2000	Positive /Negative
28	Chamazulene	29.71	1.1	>2000	Negative/Negative
29	Guaiazulene	3.2	2.5	7.45	Positive/Negative
30	Isophytol	2.73	1.4	4.42	Positive/Negative

**Cys*-model applied

Thirty-three percent of the compounds were predicted positive from the ADMET predictor, while 56% were predicted positive from both Case Ultra and Derek Nexus (Table 1).

Fifteen compounds were positive in the DPRA (Table 2). Out of those resulting positive for peptides depletion, the majority (13/15) was more reactive toward the Cys-peptide than toward the Lys-peptide. Nine compounds were identified as reactive with DCYA. Fifty-three and forty percent of the compounds tested were able to trigger the activation of KE 2 and 3, respectively.

When combined in a "2 out of 3" approach (Table 3), only 12 compounds were classified as skin sensitizers, with carvone and umbelliferone being the two sole compounds positive across all the four methods used. Marker compounds α-bisabolol and chamazulene were considered non-sensitizers based on weight of evidence (WoE: "2 out of 3" approach), while farnesene was positive in all *in chemico* and *in vitro* assays except for h-CLAT.

Four flavonoids (luteolin, quercetin, quercitrin, and rutin) were positive in *in silico* predictions but negative in experimental WoE.

Table 3. Binary classification and WoE results from experimental methods.

	Ingredient Name	Experimental *In Chemico* Data		Experimental *In Vitro* Data		WoE* 2 Out of 3
		DCYA	DPRA	KS	h-CLAT	
1	Umbelliferone	1	1	1	1	Positive
2	Herniarin	0	0	1	0	Negative
3	Hydroxytyrosol	0	n.q.	1	0	Negative
4	2,5-$(OH)_2$-benzoic acid	1	1	0	0	Negative
5	Syringic Acid	0	1	1	0	Positive
6	Caffeic acid	0	1	0	0	Negative
7	t-ferulic acid	0	1	0	0	Negative
8	Apigenin	0	1	1	0	Positive
9	Luteolin	1	1	0	0	Negative
10	Quercetin	1	1	0	0	Negative
11	Kaempferol	0	1	1	0	Positive
12	Isorhamnetin	1	n.q.	1	1	Positive
13	Epicatechin	0	1	1	1	Positive
14	Cosemetin	0	n.q.	1	0	Negative
15	Hyperoside	1	n.q.	0	0	Negative
16	Quercitrin	1	1	0	0	Negative
17	Rutin	0	n.q.	0	1	Negative
18	Myrcene	0	0	0	0	Negative
19	Nerol	0	0	1	1	Positive
20	α-Pinene	0	0	0	1	Negative
21	β-Pinene	0	0	0	0	Negative
22	α-Terpinene	0	1	1	1	Positive
23	α-Terpineol	0	0	0	1	Negative
24	Carvone	1	1	1	1	Positive
25	Farnesene	1	1	1	0	Positive
26	Farnesol	0	0	1	0	Negative
27	α-Bisabolol	0	0	0	1	Negative
28	Chamazulene	0	1	0	0	Negative
29	Guaiazulene	0	0	1	1	Positive
30	Isophytol	0	0	1	1	Positive
	Positive/negative/inconclusive	**9/21/0**	**15/10/5**	**16/14/0**	**12/18/0**	**12/18/0**

*Experimental WoE based on one result for each KE. If DPRA quantification was not possible, DCYA results were used instead. n.q. = not quantifiable

Conclusion

Identical classification was found for 14 compounds upon comparison between *in silico* SMR classification and experimental methods. Seven compounds (umbelliferone, apigenin, kaempferol, isorhamnetin, nerol, α-terpinene, and carvone) were found to be sensitizers by both models, i.e., *in silico* and experimental settings. Seven other compounds (caffeic acid, t-ferulic acid, cosemetin, hyperoside, α-terpineol, α-bisabolol, and chamazulene) were found to be non-sensitizers instead.

Umbelliferone and farnesene are of particular concern because of their positive classification and significant concentration in German chamomile. Umbelliferone can be found in German chamomile extracts in concentration up to 290 ppm, while α- and β-farnesenes are the major constituents of German chamomile essential oils, with concentrations reaching up to 30% and 50%, respectively, depending on chemotypes. Due to their abundance in German chamomile, more studies are needed to characterize their potential bioavailability upon topical application in order to evaluate quantitative risk assessments.

REFERENCES

[1] OECD (2020) Test No. 442C: *In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA), OECD Publishing, Paris. [2] Avonto, C. *et al.* (2015) *Toxicol. Appl. Pharmacol.* 289, 177-184. [3] OECD (2018) Test No. 442D: *In Vitro* Skin Sensitisation: ARE-Nrf2 Luciferase Test Method, OECD Publishing, Paris. [4] OECD (201), Test No. 442E: *In Vitro* Skin Sensitisation: Human Cell Line Activation Test (h-CLAT), OECD Publishing, Paris.