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Research

In Silico Toxicity of Bioactive Compounds from Citronella (*Cymbopogon nardus*) and Neem (*Azadirachta indica*)

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ABSTRACT

Natural active-ingredient-based shampoos offer an effective and eco-friendly solution for maintaining the skin and hair health of companion animals. This study aimed to predict the toxicity of phytochemical compounds from citronella oil (*Cymbopogon nardus*) and neem oil (*Azadirachta indica*) using an in silico approach with the ProTox-II platform. The analyzed compounds included limonene, farnesene, eucalyptol, menthol, oleic acid, p-cymene, indole, (9Z)-9-octadecenamide, amylbenzene, p-cresol, camphor, and dieugenol, selected based on LC-MS/MS analysis. The evaluated toxicological parameters comprised oral acute toxicity (LD_{50}), hepatotoxicity, immunotoxicity, genotoxicity (cytotoxicity, mutagenicity, carcinogenicity), as well as nuclear receptor signaling and stress response pathways. The results revealed that p-cymene exhibited the highest toxicity (LD_{50} = 3 mg/kg, class I), followed by oleic acid (LD_{50} = 48 mg/kg, class II), whereas amylbenzene was classified as non-toxic (LD_{50} = 6430 mg/kg, class VI). All compounds were predicted to be non-hepatotoxic and non-immunotoxic; however, p-cymene, indole, and amylbenzene showed potential carcinogenicity. Limonene was predicted to be active against AhR, ER, MMP, and ATAD5 receptors, while dieugenol was active against nrf2/ARE, HSE, and MMP. These predictions suggest that most compounds are safe for shampoo applications, except for p-cymene, which requires further evaluation. This study provides preliminary insights for the development of safe and effective natural-based shampoos.

Keywords: *Azadirachta indica, Cymbopogon nardus*, in silico, phytochemical compounds, toxicity

ABSTRAK

Sampo berbahan aktif alami menjadi solusi efektif dan ramah lingkungan untuk perawatan kesehatan kulit dan rambut hewan kesayangan. Penelitian ini bertujuan untuk memprediksi toksisitas senyawa fitokimia dari ekstrak minyak serai wangi (*Cymbopogon nardus*) dan minyak mimba (*Azadirachta indica*) menggunakan pendekatan in silico dengan platform ProTox-II. Senyawa yang dianalisis meliputi limonene, farnesene, eucalyptol, menthol, oleic acid, p-cymene, indole, (9Z)-9-Octadecenamide, amylbenzene, p-cresol, camphor, dan dieugenol, yang dipilih berdasarkan analisis LC-MS/MS. Parameter toksikologi yang dievaluasi mencakup toksisitas akut oral (LD_{50}), hepatotoksisitas, imunotoksisitas, genotoksisitas (sitotoksisitas, mutagenisitas, karsinogenisitas), serta jalur sinyal reseptor nuklir dan respons stres. Hasil menunjukkan bahwa p-cymene memiliki toksisitas tertinggi (LD_{50} = 3 mg/kg, kelas I), diikuti oleic acid (LD_{50} = 48 mg/kg, kelas II), sedangkan amylbenzene tergolong tidak beracun (LD_{50} = 6430 mg/kg, kelas VI). Semua senyawa non-hepatotoksik dan non-imunotoksik, tetapi p-cymene, indole, dan amylbenzene berpotensi karsinogenik. Limonene aktif terhadap reseptor AhR, ER, MMP, dan ATAD5, sedangkan dieugenol aktif terhadap nrf2/ARE, HSE, dan MMP. Prediksi ini menunjukkan bahwa sebagian besar senyawa aman untuk aplikasi sampo, kecuali p-cymene yang memerlukan evaluasi lebih lanjut. Studi ini memberikan wawasan awal untuk pengembangan sampo berbahan alami yang aman dan efektif.

Kata kunci: *Azadirachta indica, Cymbopogon nardus*, in silico, senyawa fitokimia, toksisitas

INTRODUCTION

Maintaining the health of companion animals' skin and hair requires effective, safe, and easy-to-apply products, predominantly in the form of shampoos. Shampoos are liquid preparations containing active ingredients capable of caring for and protecting the skin from various diseases (Budreckiene *et al*. 2016). The use of alternative ingredients derived from environmentally friendly natural sources by shampoo manufacturers has increased by 25-30% (Bezerra *et al*. 2023). Natural ingredients that can enhance the effectiveness and efficacy of shampoos in protecting skin and hair health are citronella oil (*Cymbopogon nardus*) and neem oil (*Azadirachta indica*). Citronella oil has antifungal, antiparasitic, effective insect repellent, and antibacterial properties (Mahmud *et al*. 2022). Similarly, neem is recognized for its various medicinal properties, including the treatment of infections, skin problems, pain, and diseases caused by oxidative stress (Sidat *et al*. 2023).

In toxicology studies, in silico methods have become an increasingly popular approach due to their ability to accelerate the toxicity evaluation process, reduce research costs, and minimize the need for animal testing. One computational tool used in toxicity analysis is ProTox-II, a webserver that can predict various toxicological parameters of chemical compounds, including acute oral toxicity, organ toxicity (particularly hepatotoxicity), immunotoxicity, genetic toxicity (mutagenicity, cytotoxicity, and carcinogenicity), as well as nuclear receptor signaling pathways and cellular stress response pathways (Banerjee 2018).

This study aims to predict the toxicity of various phytochemical compounds derived from *C. nardus* and *A. indica* extracts using the ProTox-II. The results of this study are expected to provide preliminary insights into the safety and suitability of utilizing phytochemicals from citronella and neem as active ingredients in shampoo formulations.

MATERIALS AND METHOD

Compound Selection

Twelve phytochemical compounds from *Cymbopogon nardus* and *Azadirachta indica* extracts, identified through LC-MS/MS analysis, were selected based on high abundance in the extracts based on LC-MS/MS quantification (compounds with composition >1% were prioritized) and relevant biological activities for shampoo applications (antimicrobial, antioxidant, and anti-inflammatory properties). The list of compounds is presented in Table 1.

In Silico Study

The in silico study was conducted using the ProTox-II webserver (Drwal 2014). The results from ProTox-II provide preliminary predictions. In silico studies have several limitations, as they do not account for *in vivo* factors such as bioavailability and synergistic interactions among compounds in the extract. Predictions for the 12 identified phytochemical compounds were performed following the protocol developed by Banerjee (Banerjee 2016).

The parameters analyzed using ProTox-II included acute oral toxicity in rats, with specific reference to the median lethal dose (LD_{50}) in mg/kg, organ-specific toxicity endpoints focusing on hepatotox-

Table 1. Phytochemical compounds identified from *C. nardus* and *A. indica* extracts.

No	Compound Name	Composition (%)	Molecular Weight (m/z) (Da)	Biological Activity
1	(E,E)-α- Farnesene	8.51	204,19	antioxidant
2	Limonene	6.55	136,13	antimicrobial
3	Oleic acid	3,49	282,26	anti-inflammatory
4	Eucalyptol	3,47	154,13	antimicrobial
5	Amylbenzene	3,31	148,13	Stability
6	Indole	2,22	117,05	antimicrobial
7	(9Z)-9-Octadecenamide	2	281,27	antioxidant
8	p-Cresol	1,93	108,05	antimicrobial
9	p-cymene	1,79	134,11	antimicrobial, anti-inflammatory
10	Dieugenol	1,54	326,15	anti-inflammatory
11	Menthol	1,34	156,15	anti-inflammatory
12	Camphor	1,19	152,12	antimicrobial

city, immunotoxicity, and genotoxicity (encompassing cytotoxicity, mutagenicity, and carcinogenicity). Furthermore, the analysis covered nuclear receptor signaling pathways (AhR, AR, AR-LBD, ER, ER-LBD, and PPARγ) and stress response pathways (nrf2/ARE, HSE, MMP, p53, and ATAD5)

RESULTS

Acute Oral Toxicity

The predicted median lethal dose (LD_{50}) values for acute oral toxicity, predictions of various toxicity classes (I-VI), and prediction accuracy in percent for various phytochemical compounds from *Cymbopogon nardus* and *Azadirachta indica* were determined through in silico modeling (Table 2).

According to Drwal *et al*. (2014), the toxicity classification is as follows: Class I: Fatal if swallowed ($LD_{50} \leq 5$ mg/kg). Class II: Fatal if swallowed ($5 < LD_{50} \leq 50$ mg/kg). Class III: Toxic if swallowed ($50 < LD_{50} \leq 300$ mg/kg). Class IV: Harmful if swallowed ($300 < LD_{50} \leq 2000$ mg/kg). Class V: May be harmful if swallowed ($2000 < LD_{50} \leq 5000$ mg/kg). Class VI: Non-toxic ($LD_{50} > 5000$ mg/kg).

The predicted median lethal dose (LD_{50}) values for acute oral toxicity of compounds identified from *Cymbopogon nardus* and *Azadirachta indica* oils, determined through in silico modeling (Table 2), were systematically classified according to the Organisation for Economic Co-operation and Development (OECD) guidelines for acute systemic toxicity (OECD Test Guideline 423 and Globally Harmonized System [GHS] criteria), as indicated by the prediction accuracy percentages (OECD, 2001). Discrepancies between predicted and experimental OECD values highlight the limitations of in silico tools (Banerjee *et al*., 2018)

Based on Table 2, p-cymene has the lowest LD_{50} of 3 mg/kg (Class I), categorized as fatal if swallowed ($LD_{50} \leq 5$ mg/kg), with a high prediction accuracy of 100%. Oleic acid shows a toxicity level of LD_{50} of 48 mg/kg (Class II), categorized as fatal if swallowed ($5 < LD_{50} \leq 50$), also with a prediction accuracy of 100%.

Similar LD_{50} values were obtained for p-cresol and 3-tropanol, namely 160 mg/kg and 227 mg/kg, which fall into Class III (toxic if swallowed, $50 < LD_{50} \leq 300$) with prediction accuracies of 100% for p-cresol. The compounds indole, (9Z)-9-octadecenamide, camphor, menthol, limonene, and dieugenol have LD_{50} values of 360 mg/kg, 750 mg/kg, 775 mg/kg, 940 mg/kg, 1900 mg/kg, and 1930 mg/kg, respectively, falling into Class IV (harmful if swallowed, $300 < LD_{50} \leq 2000$) with prediction accuracies of 72.9%, 69.26%, 100%, 100%, 100%, and 68.07%, respectively.

The compounds eucalyptol, and farnesene, have LD_{50} values of 2480 mg/kg, and 3650 mg/kg, were categorized in Class V (may be harmful if swallowed, $2000 < LD_{50} \leq 5000$) with prediction accuracies of 100% except for farnesene at 69.26%. Finally, amylbenzene exhibited the lowest toxicity with LD_{50} of 6430 mg/kg, categorized in Class VI (non-toxic, $LD_{50} > 5000$ mg/kg) with a prediction accuracy of 70.97%.

Hepatotoxicity and Immunotoxicity

All compounds are non-hepatotoxic and non-immunotoxic (Table 3), with probabilities of 0.55–0.93 (hepatotoxicity) and 0.75–0.99 (immunotoxicity).

Table 2. Predictions of acute oral toxicity, class, and accuracy from *C. nardus* and *A. indica* compounds.

No	Compound Name	Predicted Oral LD_{50} (mg/Kg)	*Predicted Toxicity Class*	*Prediction accuracy* (%) (OECD/GHS)
1	p-cymene	3	I	100
2	Oleic acid	48	II	100
3	p-Cresol	160	III	100
4	Indole	360	IV	72,9
5	(9Z)-9-Octadecenamide	750	IV	69,26
6	Camphor	775	IV	100
7	Menthol	940	IV	100
8	Limonene	1900	IV	100
9	Dieugenol	1930	IV	68,07
10	Eucalyptol	2480	V	100
11	Farnesene	3650	V	69,26
12	Amylbenzene	6430	VI	70,97

Genotoxicity

Regarding genotoxicity (Table 4), all compounds were predicted to be non-cytotoxic and non-mutagenic. However, the predictions of genetic toxicity with the carcinogenicity parameter show that p-cymene, indole, and amylbenzene exhibit carcinogenic properties with probability scores of 0,67, 0,53, and 0,53, respectively.

Nuclear Receptor Signaling Pathways

The prediction results for Nuclear Receptor Signaling Pathways in Table 5, showed specific activity, limonene is active against the Aryl hydrocarbon Receptor (AhR) with a probability score of 0.93. The other 14 compounds are inactive against AhR. The Androgen Receptor (AR) parameter shows that all predicted compounds are inactive against AR. The Androgen Receptor Ligand Binding Domain (AR-LBD) parameter shows that p-cymene and indole are active against AR-LBD with probability scores of 1,0 and 0,99. The Aromatase (Aro) parameter shows that all compounds are inactive. Predictions for limonene show activity against the Estrogen Receptor Alpha (ER) parameter. The other compounds show inactivity against ER. The Estrogen Receptor Ligand Binding Domain (ER-LBD) and Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma) parameters show that all compounds are inactive.

Stress Response Pathways

Based on Table 6, the Tox21 stress pathway predictions provided insights into the potential toxicity and biological effects of compounds, whether protective, neutral, or harmful to cells and body defense mechanisms. The predictions show that potentially active compounds are limonene for MMP and ATAD5 parameters with probability scores of 0,90 and 0,93. Farnesene and oleic acid for nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE) and Heat shock factor response element (HSE) with probability scores of 1.0 and 0.91, respectively. Dieugenol show predicted activity agains nrf2/ARE, HSE, and MMP with probability scores of 0,51, 0,51, and 0,77. All other compounds show inactive properties.

Based on Table 6, farnesene, oleic acid, and dieugenol show predicted activity against the nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE) and Heat shock factor response element (HSE). These processes are predicted to trigger the formation of reactive oxygen species (ROS) causing oxidative stress in cells. In the first case, the antioxidant response element (ARE) serves as a protective mechanism (Simmons *et al*., 2009). In the second case, another stress response pathway, the heat shock factor response element (HSE), increases transcription of genes producing heat shock proteins, which can cause protein denaturation due to chemical exposure (Voellmy and Boellmann, 2007).

Table 3. Predictions of Organ Toxicity and Immunotoxicity Endpoints from the Compounds

No	Compound Name	Hepatotoxicity	Probability	Immunotoxicity	Probability
1	Limonene	I	0,76	I	0,95
2	Farnesene	I	0,79	I	0,99
3	Eucalyptol	I	0,86	I	0,99
4	Menthol	I	0,77	I	0,99
5	Oleic acid	I	0,55	I	0,99
6	p-cymene	I	0,87	I	0,99
7	Indole	I	0,55	I	0,99
8	(9Z)-9-Octadecenamide	I	0,82	I	0,92
9	Amylbenzene	I	0,82	I	0,97
10	p-Cresol	I	0,79	I	0,99
11	Camphor	I	0,72	I	0,96
12	Dieugenol	I	0,67	I	0,75

I = Inactive; and A = Active

Table 4. Predictions of Genetic Toxicity Parameters from the Studied Compounds

No	Compound Name	Cytotoxicity	P	Mutagenicity	P	Carcinogenicity	P
1	Limonene	0,91	I	0,99	I	0,68	I
2	Farnesene	0,81	I	0,98	I	0,73	I
3	Eucalyptol	0,75	I	0,96	I	0,68	I
4	Menthol	0,88	I	0,73	I	0,89	I
5	Oleic acid	0,71	I	1,0	I	0,64	I
6	p-cymene	0,89	I	0,98	I	0,67	A
7	Indole	0,90	I	0,82	I	0,53	A
8	(9Z)-9-Octadecenamide	0,72	I	0,93	I	0,60	I
9	Amylbenzene	0,84	I	0,98	I	0,53	A
10	p-Cresol	0,88	I	0,99	I	0,68	I
11	Camphor	0,61	I	0,94	I	0,68	I
12	Dieugenol	0,99	I	0,85	I	0,66	I

I = Inactive; A = Active and P = Probability

Table 5. Predictions of Tox21 Nuclear Receptor Signaling Pathways

No	Compound Name	AhR	P	AR	P	AR-LBD	P	Aro	P	ER	P	ER-LBD	P	PPAR-Gamma	P
1	Limonene	0,93	A	0,99	I	0,99	I	0,98	I	0,94	A	1,0	I	0,99	I
2	Farnesene	1,0	I	0,99	I	0,99	I	1,0	I	0,98	I	0,99	I	1,0	I
3	Eucalyptol	0,98	I	0,99	I	1,0	I	0,98	I	0,96	I	0,97	I	0,99	I
4	Menthol	0,99	I	1,0	I	1,0	I	1,0	I	0,98	I	1,0	I	0,99	I
5	Oleic acid	1,0	I	1,0	I	1,0	I	1,0	I	1,0	I	1,0	I	0,90	I
6	p-cymene	1,0	I	1,0	I	1,0	A	1,0	I	1,0	I	1,0	I	1,0	I
7	Indole	0,77	I	0,99	I	0,99	A	0,98	I	0,98	I	0,99	I	0,99	I
8	(9Z)-9-Octadecenamide	0,99	I	1,0	I	1,0	I	1,0	I	0,98	I	0,99	I	0,99	I
9	Amylbenzene	0,99	I	1,0	I	1,0	I	0,98	I	0,99	I	1,0	I	0,99	I
10	p-Cresol	0,99	I	1,0	I	1,0	I	1,0	I	0,99	I	0,99	I	1,0	I
11	Camphor	1,0	I	0,99	I	1,0	I	0,99	I	0,99	I	0,99	I	1,0	I
12	Dieugenol	0,62	I	0,97	I	0,98	I	0,84	I	0,59	A	0,57	I	0,75	I

AhR = Aryl hydrocarbon Receptor; AR = Androgen receptor; AR-LBD = Androgen Receptor Ligand Binding Domain; Aro = Aromatase; ER = Estrogen Receptor Alpha; ER-LBD = Estrogen Receptor Ligand Binding Domain; PPAR-Gamma = Peroxisome Proliferator Activated Receptor Gamma; I = Inactive; A = Active and P = Probability

Table 6. Predictions of Tox21 Stress Response Pathways

No	Compound Name	nrf2/ARE	P	HSE	P	MMP	P	p53	P	ATAD5	P
1	Limonene	0,98	I	0,98	I	0,90	A	1,0	I	0,93	A
2	Farnesene	1,0	A	1,0	A	0,99	I	1,0	I	1,0	I
3	Eucalyptol	0,99	I	0,99	I	0,89	I	0,99	I	0,99	I
4	Menthol	1,0	I	1,0	I	0,99	I	0,99	I	0,99	I
5	Oleic acid	0,91	A	0,91	A	1,0	I	1,0	I	1,0	I
6	p-cymene	1,0	I	1,0	I	1,0	I	1,0	I	1,0	I
7	Indole	0,99	I	0,99	I	0,97	I	0,98	I	0,99	I
8	(9Z)-9-Octadecenamide	0,98	I	0,98	I	0,96	I	0,98	I	1,0	I
9	Amylbenzene	0,99	I	0,99	I	0,98	I	1,0	I	1,0	I
10	p-Cresol	1,0	I	1,0	I	0,98	I	0,99	I	1,0	I
11	Camphor	0,98	I	0,98	I	0,98	I	0,99	I	1,0	I
12	Dieugenol	0,51	A	0,51	A	0,77	A	0,50	I	0,82	I

nrf2/ARE = Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element; HSE = Heat shock factor response element; MMP = Mitochondrial Membrane Potential; p53 = Phosphoprotein (tumour supressor); ATAD5 = ATPase family AAA domain-containing protein 5; I = Inactive; A = Active and P = Probability

DISCUSSION

Twelve phytochemical compounds from *Cymbopogon nardus* and *Azadirachta indica* were selected based on their high abundance in the extracts via LC-MS/MS analysis, relevant biological activities for shampoo applications (antimicrobial, antioxidant, and anti-inflammatory properties), clear chemical structures, and potential toxicity risks (Banerjee *et al*. 2018). Compounds such as limonene, eucalyptol, and menthol (*C. nardus*), and oleic acid and (9Z)-9-octadecenamide (*A. indica*) reflect the characteristic chemical profiles of these plants, including terpenoids and fatty acids. The novelty of this study lies in the preliminary safety evaluation of natural ingredients for pet shampoos, an area with limited prior research, with recommendations for *in vivo* testing to optimize formulations (Kolodkin *et al*., 2010).

Acute Oral Toxicity

Based on acute oral toxicity data in rats (LD_{50}) in mg/kg (Table 2), 75% (n=9) of the compounds fall within Categories IV–VI, indicating low to negligible acute oral toxicity. This making their potential safety for topical application in pet shampoo formulations at controlled doses (Drawl *et al*., 2014). In silico toxicity predictions using ProTox-II revealed significant variability, with practical implications for developing pet shampoos. Although oral LD_{50} does not directly reflect topical risks, its relevance is significant due to the high potential for accidental ingestion through grooming behavior in animals (Budreckiene *et al*., 2016).

In silico predictions using ProTox-II revealed significant variability, with compounds like p-Cymene (LD_{50} = 3 mg/kg, Class I) and oleic acid (LD_{50} = 48 mg/kg, Class II) exhibit high predicted toxicity, while amylbenzene (LD_{50} = 6430 mg/kg, Class VI) is predicted to be non-toxic (Banerjee 2018). p-Cymene, an aromatic monoterpenoid, displays high toxicity due to its volatile properties, which affect the nervous system (Mahmud *et al*., 2022). Oleic acid poses risks at high doses (Sidat *et al*., 2022). Compounds in Class IV (limonene, menthol) and Class V (eucalyptol, farnesene) are safe for topical application in controlled doses (Bezerra *et al*., 2023). Compounds such as limonene, eucalyptol, and menthol, can be used in pet shampoos at concentrations of 0.5–1%, supporting environmentally friendly alternatives (Sidat *et al.,* 2023)

Hepatotoxicity and Immunotoxicity

All compounds were predicted to be non-hepatotoxic and non-immunotoxic, supporting the overall safety of Category IV–VI compounds for topical applications (Drwal *et al*., 2014). Terpenoid structures, such as limonene, are safer compared to long-chain fatty acids. This profile confirms the safe-

ty of menthol for use in pet shampoos (Bezerra *et al*. 2023)

Genotoxicity

All compounds are non-cytotoxic and non-mutagenic. However, p-cymene, indole, and amylbenzene showed potential carcinogenicity. The carcinogenicity of p-cymene is associated with its reactive metabolites (Mahmud *et al*. 2022), while indole poses risks due to its heterocyclic nature (Banerjee *et al*. 2018). Limonene and eucalyptol are non-carcinogenic, consistent with their use in cosmetics (Bezerra *et al*. 2023).

Nuclear Receptor Signaling Pathways

Based on the prediction results, limonene is active against AhR and ER, dieugenol is active against ER. p-Cymene and indole are active against AR-LBD. The other compounds show inactivity against AhR, AR, AR-LBD, Aro, ER, ER-LBD, and PPAR-gamma. According to Kolodkin (2010), nuclear receptor signaling plays a role in maintaining cell growth and development, inflammation, and metabolism, with dynamic ligand distribution. Some nuclear receptors are more abundant in the cell nucleus (such as pregnane X receptor and peroxisome proliferator-activated receptor gamma), while others are in both compartments (vitamin D receptor and mineralocorticoid receptor) or more in the cytoplasm (glucocorticoid receptor and androgen receptor).

Stress Response Pathways

Another stress response pathway, mitochondrial membrane potential (MMP) shows limonene and dieugenol as active. Mitochondria have double membranes that provide energy to cells through oxidative phosphorylation and prevent apoptosis (Hill *et al*., 2018). Mitochondrial stress caused by toxins can cause various diseases (Meyer *et al*., 2018). According to Richter *et al*. (2019), toxins inhibit mitochondrial protein synthesis and block stress responses.

Limonene exhibits activity toward ATPase family AAA domain-containing protein 5 (ATAD5) with a probability score of 0.93 (approaching 1.0), indicating high confidence in the in silico model's prediction of limonene's interaction with or influence on ATAD5 activity (Banerjee *et al*., 2018). ATAD5, a member of the ATPase protein family, plays a critical role in the DNA damage response, functioning as part of cellular protection mechanisms against genotoxic stress.

Limonene maintains MMP by preventing depolarization caused by reactive oxygen species (ROS), reducing apoptosis through the stabilization of ion channels and antioxidant enzymes (Kim *et al*., 2014). Furthermore, limonene mitigates DNA damage from oxidative stress via the mitochondrial unfolded protein response (UPR_{mt}) pathway. This supports the safety of limonene for use in pet shampoos at low concentrations (<1%), though high doses may risk inducing excessive cellular stress. Dieugenol activates nrf2/ARE, HSE, and MMP due to its phenolic structure, inducing protective heat shock proteins and antioxidant responses, but potentially causing oxidative stress at high doses (Kim *et al*., 2014).

These predictions indicate that most compounds such as limonene, eucalyptol, and menthol (Categories IV-V), are safe for shampoo applications, being non-genotoxic and suitable at 0.5-1% concentrations. However, p-cymene, requires avoided or used cautiously due to its high acute toxicity and carcinogenicity potential.

Oleic acid and indole which requires further testing due to moderate toxicity or carcinogenicity risks. This study provides preliminary insights for safe and effective natural-based shampoos the development, with recommendations include dose optimization and *in vivo* dermal studies to validate these computational findings.

"*The authors declare that there is no conflict of interest with any parties involved in this research*."

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